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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
07 November 2019 (07.11.2019)

WIPO | PCT

(10) International Publication Number
WO 2019/211369 A1

(51) International Patent Classification:
A61K 39/395 (2006.01) *A61P 35/00* (2006.01)

(21) International Application Number:
PCT/EP2019/061227

(22) International Filing Date:
02 May 2019 (02.05.2019)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
18305555.7 03 May 2018 (03.05.2018) EP

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

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

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

(54) **Title:** METHODS AND PHARMACEUTICAL COMPOSITIONS FOR TREATING CANCER

(57) **Abstract:** The present invention relates to methods and pharmaceutical compositions for treating cancer in a subject in need thereof. The inventors investigated for the first time BTN3A2 protein expression using a specific BTN3A2 monoclonal antibody. Thus, the inventors postulated that BTN3A2 overexpression might sensitize AML blasts to Vy9V02 T-cells-mediated lysis. The inventors investigated the role of BTN3A2 in the recognition mechanism of AML blasts by Vy9V52 T-cells. First, the inventors confirmed using co-immunoprecipitation, FRET and PLA experiments that BTN3A2 interacted with BTN3A1 and to a lesser extent with BTN3A3. Next, the inventors generated BTN3A2 knock-out HL-60 cells, as well as a BTN3A1/A3 double KO using CRISPR/Cas9-gene editing. Vy9V52 T-cells were challenged against WT and KO HL- 60 cells, in presence of natural BTN3 ligand (Aminobisphosphonates) or using BTN3 specific agonist mAh. Surprisingly in leukemias lacking BTN3A1 expression, BTN3A2 was still, although to a lesser extent, able to induce AML killing using agonist BTN3A mAh. Thus, the present invention relates to a compound selected from the group consisting of BTN3A2 agonist or BTN3A2 expression activator for use in the treatment of cancer in a subject in need thereof.

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METHODS AND PHARMACEUTICAL COMPOSITIONS FOR TREATING CANCER

FIELD OF THE INVENTION:

The present invention relates to methods and pharmaceutical compositions for treating cancer in a subject in need thereof.

BACKGROUND OF THE INVENTION:

White blood cells are cells of the immune system involved in defending the body against pathogens. Among these cells, lymphocytes, monocytes, and dendritic cells can be cited. Monocytes may migrate from the bloodstream to other tissues and differentiate into tissue resident macrophages or dendritic cells. Dendritic cells play a role as antigen presenting cells (APC) that activate lymphocytes. Among lymphocytes, T cells can be divided into γδ T cells and αβ T cells.

Vγ9/Vδ2 T cells are important effectors of the immune defence and are the main peripheral blood γδT-cell subpopulation. This T-cell subset stands out as having the capacity to "sense" infected and malignant cells. They lyse directly pathogen infected or abnormal cells. In addition, they regulate immune responses by inducing dendritic cell (DC) maturation as well as the isotypic switching and immunoglobulin production. This important cell platform of the immune system is strictly regulated by surface receptors, chemokines and cytokines. Most primate Vγ9Vδ2 T cells are specifically activated by small organic non-peptidic pyrophosphate molecules, also called phosphoantigens (PAg). Among them, isopentenyl pyrophosphate (IPP) tends to accumulate in p53-null cancers. BTN3A/CD277 molecules expression on target cells has been shown to trigger the PAg-induced activation of human Vγ9Vδ2 T cells.

BTN3A molecules are members of the human butyrophilin (BTN), a type I transmembrane glycoproteins superfamily sharing strong homologies with the costimulatory molecules of the B7 family (Henry et al., 1999). The BTN3A cluster is composed of 3 isoforms (BTN3A1, BTN3A2, and BTN3A3) exhibiting 95% identity and mainly distinguished by their intracellular part (e.g., presence or absence of a B30.2/SPRY domain) (Williams and Barclay, 1988; Ruddy et al., 1997; Rhodes et al., 2001; Bensussan and Olive, 2005). PAg binding on B30.2 domain of BTN3A1 isoform has been shown to specifically trigger Vγ9Vδ2 T-cells activation. If BTN3A2 isoform, lacking the B30.2 intracellular domain, cannot trigger PAg-mediated Vγ9Vδ2 T-cells activation, it appears to be necessary to fully activate Vγ9Vδ2 T-cells against their targets.

SUMMARY OF THE INVENTION:

The present invention relates to methods and pharmaceutical compositions for treating cancer in a subject in need thereof.

The present invention also relates to a compound selected from the group consisting of BTN3A2 agonist or BTN3A2 expression activator for use in the treatment of cancer in a subject in need thereof.

DETAILED DESCRIPTION OF THE INVENTION:

For the first time, the inventors demonstrated that BTN3A2 protein expression using a specific BTN3A2 monoclonal antibody and showed that BTN3A2 overexpression sensitizes AML blasts to Vγ9Vδ2 T-cells–mediated lysis.

Thereby, the main goal was to elucidate the role of BTN3A2 in the recognition mechanism of AML blasts by Vγ9Vδ2 T-cells. First, the inventors confirmed using co-immunoprecipitation, FRET and PLA experiments that BTN3A2 interacted with BTN3A1 and to a lesser extent with BTN3A3. Next, the inventors generated BTN3A2 knock-out HL-60 cells, as well as a BTN3A1/A3 double KO using CRISPR/Cas9-gene editing. Vγ9Vδ2 T-cells were challenged against WT and KO HL-60 cells, in presence of natural BTN3 ligand (Aminobisphosphonates) or using BTN3 specific agonist mAb. Surprisingly in leukemias lacking BTN3A1 expression, BTN3A2 was still, although to a lesser extent, able to induce AML killing using agonist BTN3A mAb. This finding could unmask new therapeutic opportunities to treat AML patients with Vγ9Vδ2 T-cells immunotherapy.

Accordingly, the present invention relates to a compound selected from the group consisting of BTN3A2 agonist or BTN3A2 expression activator for use in the treatment of cancer in a subject in need thereof.

In some embodiments, the compound of the present invention is particularly suitable for Vγ9Vδ2 T-cells activation.

In some embodiments, the compound of the present invention is particularly suitable for Vγ9Vδ2 T-cells–mediated lysis activation.

As used herein, the term "subject" denotes a mammal. Typically, a subject according to the invention refers to any subject (preferably human) afflicted or at risk to be afflicted with cancer. In a particular embodiment, the term "subject" refers to a subject afflicted or at risk to be afflicted with acute myeloid leukemia (AML). In a particular embodiment, the term "subject" refers to a subject afflicted or at risk to be afflicted with pancreatic cancer. In a particular embodiment, the term "subject" refers to a subject afflicted or at risk to be afflicted with Pancreatic ductal adenocarcinoma (PDAC).

As used herein, the term "treatment" or "treat" refer to both prophylactic or preventive treatment as well as curative or disease modifying treatment, including treatment of subjects at risk of contracting the disease or suspected to have contracted the disease as well as subjects who are ill or have been diagnosed as suffering from a disease or medical condition, and includes suppression of clinical relapse. The treatment may be administered to a subject having a medical disorder or who ultimately may acquire the disorder, in order to prevent, cure, delay the onset of, reduce the severity of, or ameliorate one or more symptoms of a disorder or recurring disorder, or in order to prolong the survival of a subject beyond that expected in the absence of such treatment. By "therapeutic regimen" is meant the pattern of treatment of an illness, e.g., the pattern of dosing used during therapy. A therapeutic regimen may include an induction regimen and a maintenance regimen. The phrase "induction regimen" or "induction period" refers to a therapeutic regimen (or the portion of a therapeutic regimen) that is used for the initial treatment of a disease. The general goal of an induction regimen is to provide a high level of drug to a subject during the initial period of a treatment regimen. An induction regimen may employ (in part or in whole) a "loading regimen", which may include administering a greater dose of the drug than a physician would employ during a maintenance regimen, administering a drug more frequently than a physician would administer the drug during a maintenance regimen, or both. The phrase "maintenance regimen" or "maintenance period" refers to a therapeutic regimen (or the portion of a therapeutic regimen) that is used for the maintenance of a subject during treatment of an illness, e.g., to keep the subject in remission for long periods of time (months or years). A maintenance regimen may employ continuous therapy (e.g., administering a drug at a regular intervals, e.g., weekly, monthly, yearly, etc.) or intermittent therapy (e.g., interrupted treatment, intermittent treatment, treatment at relapse, or treatment upon achievement of a particular predetermined criteria [e.g., disease manifestation, etc.]).

As used herein, the term "cancer" has its general meaning in the art and includes, but is not limited to, solid tumors and blood borne tumors. The term cancer includes diseases of the skin, tissues, organs, bone, cartilage, blood and vessels. The term "cancer" further encompasses both primary and metastatic cancers. Examples of cancers that may be treated by methods and compositions of the present invention include, but are not limited to, cancer cells from the bladder, blood, bone, bone marrow, brain, breast, colon, esophagus, gastrointestine, gum, head, kidney, liver, lung, nasopharynx, neck, ovary, prostate, skin, stomach, testis, tongue, or uterus. In addition, the cancer may specifically be of the following histological type, though it is not limited to these: neoplasm, malignant; carcinoma; carcinoma, undifferentiated; giant and

spindle cell carcinoma; small cell carcinoma; papillary carcinoma; squamous cell carcinoma; lymphoepithelial carcinoma; basal cell carcinoma; pilomatrix carcinoma; transitional cell carcinoma; papillary transitional cell carcinoma; adenocarcinoma; gastrinoma, malignant; cholangiocarcinoma; hepatocellular carcinoma; combined hepatocellular carcinoma and cholangiocarcinoma; trabecular adenocarcinoma; adenoid cystic carcinoma; adenocarcinoma in adenomatous polyp; adenocarcinoma, familial polyposis coli; solid carcinoma; carcinoid tumor, malignant; branchiolo-alveolar adenocarcinoma; papillary adenocarcinoma; chromophobe carcinoma; acidophil carcinoma; oxyphilic adenocarcinoma; basophil carcinoma; clear cell adenocarcinoma; granular cell carcinoma; follicular adenocarcinoma; papillary and follicular adenocarcinoma; nonencapsulating sclerosing carcinoma; adrenal cortical carcinoma; endometroid carcinoma; skin appendage carcinoma; apocrine adenocarcinoma; sebaceous adenocarcinoma; ceruminous; adenocarcinoma; mucoepidermoid carcinoma; cystadenocarcinoma; papillary cystadenocarcinoma; papillary serous cystadenocarcinoma; mucinous cystadenocarcinoma; mucinous adenocarcinoma; signet ring cell carcinoma; infiltrating duct carcinoma; medullary carcinoma; lobular carcinoma; inflammatory carcinoma; paget's disease, mammary; acinar cell carcinoma; adenosquamous carcinoma; adenocarcinoma w/squamous metaplasia; thymoma, malignant; ovarian stromal tumor, malignant; thecoma, malignant; granulosa cell tumor, malignant; and roblastoma, malignant; Sertoli cell carcinoma; leydig cell tumor, malignant; lipid cell tumor, malignant; paraganglioma, malignant; extra-mammary paraganglioma, malignant; pheochromocytoma; glomangiosarcoma; malignant melanoma; amelanotic melanoma; superficial spreading melanoma; malig melanoma in giant pigmented nevus; epithelioid cell melanoma; blue nevus, malignant; sarcoma; fibrosarcoma; fibrous histiocytoma, malignant; myxosarcoma; liposarcoma; leiomyosarcoma; rhabdomyosarcoma; embryonal rhabdomyosarcoma; alveolar rhabdomyosarcoma; stromal sarcoma; mixed tumor, malignant; mullerian mixed tumor; nephroblastoma; hepatoblastoma; carcinosarcoma; mesenchymoma, malignant; brenner tumor, malignant; phyllodes tumor, malignant; synovial sarcoma; mesothelioma, malignant; dysgerminoma; embryonal carcinoma; teratoma, malignant; struma ovarii, malignant; choriocarcinoma; mesonephroma, malignant; hemangiosarcoma; hemangioendothelioma, malignant; kaposi's sarcoma; hemangiopericytoma, malignant; lymphangiosarcoma; osteosarcoma; juxtacortical osteosarcoma; chondrosarcoma; chondroblastoma, malignant; mesenchymal chondrosarcoma; giant cell tumor of bone; ewing's sarcoma; odontogenic tumor, malignant; ameloblastic odontosarcoma; ameloblastoma, malignant; ameloblastic fibrosarcoma; pinealoma, malignant; chordoma; glioma, malignant; ependymoma;

astrocytoma; protoplasmic astrocytoma; fibrillary astrocytoma; astroblastoma; glioblastoma; oligodendroglioma; oligodendroblastoma; primitive neuroectodermal; cerebellar sarcoma; ganglioneuroblastoma; neuroblastoma; retinoblastoma; olfactory neurogenic tumor; meningioma, malignant; neurofibrosarcoma; neurilemmoma, malignant; granular cell tumor, malignant; malignant lymphoma; Hodgkin's disease; Hodgkin's lymphoma; paragranuloma; malignant lymphoma, small lymphocytic; malignant lymphoma, large cell, diffuse; malignant lymphoma, follicular; mycosis fungoides; other specified non-Hodgkin's lymphomas; malignant histiocytosis; multiple myeloma; mast cell sarcoma; immunoproliferative small intestinal disease; leukemia; lymphoid leukemia; plasma cell leukemia; erythroleukemia; lymphosarcoma cell leukemia; myeloid leukemia; basophilic leukemia; eosinophilic leukemia; monocytic leukemia; mast cell leukemia; megakaryoblastic leukemia; myeloid sarcoma; and hairy cell leukemia.

In some embodiments, the subject suffers from a cancer selected from the group consisting of acute myeloid leukemia (AML), pancreatic cancer, colon cancer, rectal cancer, breast cancer, lung cancer, prostate cancer, testicular cancer, brain cancer, skin cancer, gastric cancer, esophageal cancer, sarcomas, tracheal cancer, head and neck cancer, liver cancer, ovarian cancer, lymphoid cancer, cervical cancer, vulvar cancer, melanoma, mesothelioma, renal cancer, bladder cancer, thyroid cancer, bone cancers, carcinomas, sarcomas, and soft tissue cancers.

In some embodiments, the subject suffers from cancer resistant to anti-cancer treatment.

The term “Acute myeloid leukemia” or “AML” has its general meaning in the art and refers to Acute myeloid leukemia such as revised in the World Health Organisation Classification C92. The term “Acute myeloid leukemia” is also known under the synonyms “acute myelocytic leukemia”, “acute myelogenous leukemia”, “acute granulocytic leukemia” or “acute non-lymphocytic leukemia” and is characterized by the accumulation of large numbers of abnormal cells that fail to differentiate into granulocytes or monocytes. Acute myeloid leukemia leads to the replacement of normal bone marrow with leukemic cells causing a drop in red blood cells, platelets, and normal white blood cells.

The term “pancreatic cancer” has its general meaning in the art and refers to pancreatic cancer such as revised in the World Health Organisation Classification C25. The term “pancreatic cancer” also refers to Pancreatic ductal adenocarcinoma (PDAC) (31-35). The term “pancreatic cancer” also refers to metastatic pancreatic cancer, exocrine pancreatic cancer and locally advanced PDAC.

The term "BTN3A2" has its general meaning in the art and refers to butyrophilin (BT) belonging to the BT3 family (Williams and Barclay, 1988; Ruddy et al., 1997; Rhodes et al., 2001). The term "BTN3A2" also refers to BT3.2, also called BTF4 (Rhodes et al., 2001). The reference coding sequence was the NM_001197246

The term "expression" when used in the context of expression of a gene or nucleic acid refers to the conversion of the information, contained in a gene, into a gene product. A gene product can be the direct transcriptional product of a gene (e.g., mRNA, tRNA, rRNA, antisense RNA, ribozyme, structural RNA or any other type of RNA) or a protein produced by translation of a mRNA. Gene products also include messenger RNAs, which are modified, by processes such as capping, polyadenylation, methylation, and editing, and proteins modified by, for example, methylation, acetylation, phosphorylation, ubiquitination, SUMOylation, ADP-ribosylation, myristilation, and glycosylation.

An "activator of expression" refers to a natural or synthetic compound that has a biological effect to activate the expression of a gene.

The term "BTN3A2 agonist" has its general meaning in the art and refers to a compound that selectively activates the BTN3A2. The term "BTN3A2 agonist" refers to any compound that can directly or indirectly stimulate the signal transduction cascade related to the BTN3A2. As used herein, the term "selectively activates" refers to a compound that preferentially binds to and activates BTN3A2 with a greater affinity and potency, respectively, than its interaction with the other sub-types or isoforms of the BTN3A family (BTN3A1 and BTN3A3). Compounds that prefer BTN3A2, but that may also activate other BTN3A sub-types, as partial or full agonists, and thus that may have multiple BTN3A activities, are contemplated. Typically, a BTN3A2 agonist is a small organic molecule, an antibody or a polypeptide.

Tests and assays for determining whether a compound is a BTN3A2 agonist are well known by the skilled person in the art such as described in Williams and Barclay, 1988; Ruddy et al., 1997; Rhodes et al., 2001.

In another embodiment, the compound of the invention is an antibody (the term including "antibody portion") directed against the target.

In one embodiment of the antibodies or portions thereof described herein, the antibody is a monoclonal antibody. In one embodiment of the antibodies or portions thereof described herein, the antibody is a polyclonal antibody. In one embodiment of the antibodies or portions thereof described herein, the antibody is a humanized antibody. In one embodiment of the antibodies or portions thereof described herein, the antibody is a chimeric antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody

comprises a light chain of the antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises a heavy chain of the antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises a Fab portion of the antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises a F(ab')2 portion of the antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises a Fc portion of the antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises a Fv portion of the antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises a variable domain of the antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises one or more CDR domains of the antibody.

As used herein, "antibody" includes both naturally occurring and non-naturally occurring antibodies. Specifically, "antibody" includes polyclonal and monoclonal antibodies, and monovalent and divalent fragments thereof. Furthermore, "antibody" includes chimeric antibodies, wholly synthetic antibodies, single chain antibodies, and fragments thereof. The antibody may be a human or nonhuman antibody. A nonhuman antibody may be humanized by recombinant methods to reduce its immunogenicity in man.

Antibodies are prepared according to conventional methodology. Monoclonal antibodies may be generated using the method of Kohler and Milstein (Nature, 256:495, 1975). To prepare monoclonal antibodies useful in the invention, a mouse or other appropriate host animal is immunized at suitable intervals (e.g., twice-weekly, weekly, twice-monthly or monthly) with antigenic forms of the target. The animal may be administered a final "boost" of antigen within one week of sacrifice. It is often desirable to use an immunologic adjuvant during immunization. Suitable immunologic adjuvants include Freund's complete adjuvant, Freund's incomplete adjuvant, alum, Ribi adjuvant, Hunter's Titermax, saponin adjuvants such as QS21 or Quil A, or CpG-containing immunostimulatory oligonucleotides. Other suitable adjuvants are well-known in the field. The animals may be immunized by subcutaneous, intraperitoneal, intramuscular, intravenous, intranasal or other routes. A given animal may be immunized with multiple forms of the antigen by multiple routes.

Briefly, the antigen may be provided as synthetic peptides corresponding to antigenic regions of interest in the target. Following the immunization regimen, lymphocytes are isolated from the spleen, lymph node or other organ of the animal and fused with a suitable myeloma cell line using an agent such as polyethylene glycol to form a hydridoma. Following fusion,

cells are placed in media permissive for growth of hybridomas but not the fusion partners using standard methods, as described (Coding, Monoclonal Antibodies: Principles and Practice: Production and Application of Monoclonal Antibodies in Cell Biology, Biochemistry and Immunology, 3rd edition, Academic Press, New York, 1996). Following culture of the hybridomas, cell supernatants are analyzed for the presence of antibodies of the desired specificity, i.e., that selectively bind the antigen. Suitable analytical techniques include ELISA, flow cytometry, immunoprecipitation, and western blotting. Other screening techniques are well-known in the field. Preferred techniques are those that confirm binding of antibodies to conformationally intact, natively folded antigen, such as non-denaturing ELISA, flow cytometry, and immunoprecipitation.

Significantly, as is well-known in the art, only a small portion of an antibody molecule, the paratope, is involved in the binding of the antibody to its epitope (see, in general, Clark, W. R. (1986) The Experimental Foundations of Modern Immunology Wiley & Sons, Inc., New York; Roitt, I. (1991) Essential Immunology, 7th Ed., Blackwell Scientific Publications, Oxford). The Fc' and Fc regions, for example, are effectors of the complement cascade but are not involved in antigen binding. An antibody from which the pFc' region has been enzymatically cleaved, or which has been produced without the pFc' region, designated an F(ab')2 fragment, retains both of the antigen binding sites of an intact antibody. Similarly, an antibody from which the Fc region has been enzymatically cleaved, or which has been produced without the Fc region, designated an Fab fragment, retains one of the antigen binding sites of an intact antibody molecule. Proceeding further, Fab fragments consist of a covalently bound antibody light chain and a portion of the antibody heavy chain denoted Fd. The Fd fragments are the major determinant of antibody specificity (a single Fd fragment may be associated with up to ten different light chains without altering antibody specificity) and Fd fragments retain epitope-binding ability in isolation.

Within the antigen-binding portion of an antibody, as is well-known in the art, there are complementarity determining regions (CDRs), which directly interact with the epitope of the antigen, and framework regions (FRs), which maintain the tertiary structure of the paratope (see, in general, Clark, 1986; Roitt, 1991). In both the heavy chain Fd fragment and the light chain of IgG immunoglobulins, there are four framework regions (FR1 through FR4) separated respectively by three complementarity determining regions (CDR1 through CDRS). The CDRs, and in particular the CDRS regions, and more particularly the heavy chain CDRS, are largely responsible for antibody specificity.

It is now well-established in the art that the non CDR regions of a mammalian antibody may be replaced with similar regions of conspecific or heterospecific antibodies while retaining the epitopic specificity of the original antibody. This is most clearly manifested in the development and use of "humanized" antibodies in which non-human CDRs are covalently joined to human FR and/or Fc/pFc' regions to produce a functional antibody.

This invention provides in certain embodiments compositions and methods that include humanized forms of antibodies. As used herein, "humanized" describes antibodies wherein some, most or all of the amino acids outside the CDR regions are replaced with corresponding amino acids derived from human immunoglobulin molecules. Methods of humanization include, but are not limited to, those described in U.S. Pat. Nos. 4,816,567, 5,225,539, 5,585,089, 5,693,761, 5,693,762 and 5,859,205, which are hereby incorporated by reference. The above U.S. Pat. Nos. 5,585,089 and 5,693,761, and WO 90/07861 also propose four possible criteria which may used in designing the humanized antibodies. The first proposal was that for an acceptor, use a framework from a particular human immunoglobulin that is unusually homologous to the donor immunoglobulin to be humanized, or use a consensus framework from many human antibodies. The second proposal was that if an amino acid in the framework of the human immunoglobulin is unusual and the donor amino acid at that position is typical for human sequences, then the donor amino acid rather than the acceptor may be selected. The third proposal was that in the positions immediately adjacent to the 3 CDRs in the humanized immunoglobulin chain, the donor amino acid rather than the acceptor amino acid may be selected. The fourth proposal was to use the donor amino acid reside at the framework positions at which the amino acid is predicted to have a side chain atom within 3A of the CDRs in a three dimensional model of the antibody and is predicted to be capable of interacting with the CDRs. The above methods are merely illustrative of some of the methods that one skilled in the art could employ to make humanized antibodies. One of ordinary skill in the art will be familiar with other methods for antibody humanization.

In one embodiment of the humanized forms of the antibodies, some, most or all of the amino acids outside the CDR regions have been replaced with amino acids from human immunoglobulin molecules but where some, most or all amino acids within one or more CDR regions are unchanged. Small additions, deletions, insertions, substitutions or modifications of amino acids are permissible as long as they would not abrogate the ability of the antibody to bind a given antigen. Suitable human immunoglobulin molecules would include IgGl, IgG2, IgG3, IgG4, IgA and IgM molecules. A "humanized" antibody retains a similar antigenic specificity as the original antibody. However, using certain methods of humanization, the

affinity and/or specificity of binding of the antibody may be increased using methods of "directed evolution", as described by Wu et al., /. Mol. Biol. 294:151, 1999, the contents of which are incorporated herein by reference.

Fully human monoclonal antibodies also can be prepared by immunizing mice transgenic for large portions of human immunoglobulin heavy and light chain loci. See, e.g., U.S. Pat. Nos. 5,591,669, 5,598,369, 5,545,806, 5,545,807, 6,150,584, and references cited therein, the contents of which are incorporated herein by reference. These animals have been genetically modified such that there is a functional deletion in the production of endogenous (e.g., murine) antibodies. The animals are further modified to contain all or a portion of the human germ-line immunoglobulin gene locus such that immunization of these animals will result in the production of fully human antibodies to the antigen of interest. Following immunization of these mice (e.g., XenoMouse (Abgenix), HuMAb mice (Medarex/GenPharm)), monoclonal antibodies can be prepared according to standard hybridoma technology. These monoclonal antibodies will have human immunoglobulin amino acid sequences and therefore will not provoke human anti-mouse antibody (KAMA) responses when administered to humans.

In vitro methods also exist for producing human antibodies. These include phage display technology (U.S. Pat. Nos. 5,565,332 and 5,573,905) and in vitro stimulation of human B cells (U.S. Pat. Nos. 5,229,275 and 5,567,610). The contents of these patents are incorporated herein by reference.

Thus, as will be apparent to one of ordinary skill in the art, the present invention also provides for F(ab') 2 Fab, Fv and Fd fragments; chimeric antibodies in which the Fc and/or FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; chimeric F(ab')2 fragment antibodies in which the FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; chimeric Fab fragment antibodies in which the FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; and chimeric Fd fragment antibodies in which the FR and/or CDR1 and/or CDR2 regions have been replaced by homologous human or non-human sequences. The present invention also includes so-called single chain antibodies.

The various antibody molecules and fragments may derive from any of the commonly known immunoglobulin classes, including but not limited to IgA, secretory IgA, IgE, IgG and IgM. IgG subclasses are also well known to those in the art and include but are not limited to

human IgGl, IgG2, IgG3 and IgG4. In a preferred embodiment, the compound of the invention is a Human IgG4.

In another embodiment, the antibody according to the invention is a single domain antibody. The term “single domain antibody” (sdAb) or "VHH" refers to the single heavy chain variable domain of antibodies of the type that can be found in Camelid mammals which are naturally devoid of light chains. Such VHH are also called “nanobody®”. According to the invention, sdAb can particularly be llama sdAb. The term “VHH” refers to the single heavy chain having 3 complementarity determining regions (CDRs): CDR1, CDR2 and CDR3. The term “complementarity determining region” or “CDR” refers to the hypervariable amino acid sequences which define the binding affinity and specificity of the VHH.

The VHH according to the invention can readily be prepared by an ordinarily skilled artisan using routine experimentation. The VHH variants and modified form thereof may be produced under any known technique in the art such as in-vitro maturation.

VHHs or sdAbs are usually generated by PCR cloning of the V-domain repertoire from blood, lymph node, or spleen cDNA obtained from immunized animals into a phage display vector, such as pHEN2. Antigen-specific VHHs are commonly selected by panning phage libraries on immobilized antigen, e.g., antigen coated onto the plastic surface of a test tube, biotinylated antigens immobilized on streptavidin beads, or membrane proteins expressed on the surface of cells. However, such VHHs often show lower affinities for their antigen than VHHs derived from animals that have received several immunizations. The high affinity of VHHs from immune libraries is attributed to the natural selection of variant VHHs during clonal expansion of B-cells in the lymphoid organs of immunized animals. The affinity of VHHs from non-immune libraries can often be improved by mimicking this strategy in vitro, i.e., by site directed mutagenesis of the CDR regions and further rounds of panning on immobilized antigen under conditions of increased stringency (higher temperature, high or low salt concentration, high or low pH, and low antigen concentrations). VHHs derived from camelid are readily expressed in and purified from the E. coli periplasm at much higher levels than the corresponding domains of conventional antibodies. VHHs generally display high solubility and stability and can also be readily produced in yeast, plant, and mammalian cells. For example, the “Hamers patents” describe methods and techniques for generating VHH against any desired target (see for example US 5,800,988; US 5,874, 541 and US 6,015,695). The “Hamers patents” more particularly describe production of VHHs in bacterial hosts such as E. coli (see for example US 6,765,087) and in lower eukaryotic hosts such as moulds (for example Aspergillus

or Trichoderma) or in yeast (for example Saccharomyces, Kluyveromyces, Hansenula or Pichia) (see for example US 6,838,254).

In some embodiments, the compound of the present invention is administered sequentially or concomitantly with one or more therapeutic active agent such as to anti-cancer compound, chemotherapeutic or radiotherapeutic.

The term "anti-cancer compound" has its general meaning in the art and refers to anti-cancer compounds used in anti-cancer therapy such as tyrosine kinase inhibitors, tyrosine kinase receptor (TKR) inhibitors, EGFR tyrosine kinase inhibitors, anti-EGFR compounds, anti-HER2 compounds, Vascular Endothelial Growth Factor Receptors (VEGFRs) pathway inhibitors, interferon therapy, alkylating agents, anti-metabolites, immunotherapeutic agents, Interferons (IFNs), Interleukins, and chemotherapeutic agents such as described below.

In a particular embodiment the compound of the invention (agonist of BTN3A2) is administered in combination with a phosphoantigens (PAg).

The term "tyrosine kinase inhibitor" or "TKI" has its general meaning in the art and refers to any of a variety of therapeutic agents or drugs such as compounds inhibiting tyrosine kinase, tyrosine kinase receptor inhibitors (TKRI), EGFR tyrosine kinase inhibitors, EGFR antagonists. The term "tyrosine kinase inhibitor" or "TKI" has its general meaning in the art and refers to any of a variety of therapeutic agents or drugs that act as selective or non-selective inhibitors of receptor and/or non-receptor tyrosine kinases. Tyrosine kinase inhibitors and related compounds are well known in the art and described in U.S Patent Publication 2007/0254295, which is incorporated by reference herein in its entirety. It will be appreciated by one of skill in the art that a compound related to a tyrosine kinase inhibitor will recapitulate the effect of the tyrosine kinase inhibitor, e.g., the related compound will act on a different member of the tyrosine kinase signaling pathway to produce the same effect as would a tyrosine kinase inhibitor of that tyrosine kinase. Examples of tyrosine kinase inhibitors and related compounds suitable for use in methods of embodiments of the present invention include, but are not limited to Erlotinib, sunitinib (Sutent; SU11248), dasatinib (BMS-354825), PP2, BEZ235, saracatinib, gefitinib (Iressa), erlotinib (Tarceva; OSI-1774), lapatinib (GW572016; GW2016), canertinib (CI 1033), semaxinib (SU5416), vatalanib (PTK787/ZK222584), sorafenib (BAY 43-9006), imatinib (Gleevec; STI571), leflunomide (SU101), vandetanib (Zactima; ZD6474), MK-2206 (8-[4-aminocyclobutyl)phenyl]-9-phenyl-1,2,4-triazolo[3,4-f][1,6]naphthyridin-3(2H)-one hydrochloride) derivatives thereof, analogs thereof, and combinations thereof. Additional tyrosine kinase inhibitors and related compounds suitable for use in the present invention are described in, for example, U.S Patent Publication

2007/0254295, U.S. Pat. Nos. 5,618,829, 5,639,757, 5,728,868, 5,804,396, 6,100,254, 6,127,374, 6,245,759, 6,306,874, 6,313,138, 6,316,444, 6,329,380, 6,344,459, 6,420,382, 6,479,512, 6,498,165, 6,544,988, 6,562,818, 6,586,423, 6,586,424, 6,740,665, 6,794,393, 6,875,767, 6,927,293, and 6,958,340, all of which are incorporated by reference herein in their entirety. In certain embodiments, the tyrosine kinase inhibitor is a small molecule kinase inhibitor that has been orally administered and that has been the subject of at least one Phase I clinical trial, more preferably at least one Phase II clinical, even more preferably at least one Phase III clinical trial, and most preferably approved by the FDA for at least one hematological or oncological indication. Examples of such inhibitors include, but are not limited to Erlotinib, Gefitinib, Lapatinib, Canertinib, BMS-599626 (AC-480), Neratinib, KRN-633, CEP-11981, Imatinib, Nilotinib, Dasatinib, AZM-475271, CP-724714, TAK-165, Sunitinib, Vatalanib, CP-547632, Vandetanib, Bosutinib, Lestaurtinib, Tandutinib, Midostaurin, Enzastaurin, AEE-788, Pazopanib, Axitinib, Motasenib, OSI-930, Cediranib, KRN-951, Dovitinib, Seliciclib, SNS-032, PD-0332991, MKC-I (Ro-317453; R-440), Sorafenib, ABT-869, Brivanib (BMS-582664), SU-14813, Telatinib, SU-6668, (TSU-68), L-21649, MLN-8054, AEW-541, and PD-0325901.

EGFR tyrosine kinase inhibitors as used herein include, but are not limited to compounds selected from the group consisting of but not limited to Erlotinib, lapatinib, Rociletinib (CO-1686), gefitinib, Dacomitinib (PF-00299804), Afatanib, Brigatinib (AP26113), WJTOG3405, NEJ002, AZD9291, HM61713, EGF816, ASP 8273, AC 0010. Examples of antibody EGFR inhibitors include Cetuximab, panitumumab, matuzumab, zalutumumab, nimotuzumab, necitumumab, Imgatuzumab (GA201, RO5083945), and ABT-806.

In some embodiments, the compound of the present invention is administered with a chemotherapeutic agent. The term "chemotherapeutic agent" refers to chemical compounds that are effective in inhibiting tumor growth. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphaorarnide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a carnptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CBI-TMI);

eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estrarnustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as the enediyne antibiotics (e.g. calicheamicin, especially calicheamicin (11 and calicheamicin 211, see, e.g., Agnew Chem Intl. Ed. Engl. 33:183-186 (1994); dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromomophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, canninomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idanrbicin, marcellomycin, mitomycins, mycophenolic acid, nogalarnycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptomgrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophospharnide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK®; razoxane; rhizoxin; sizofiran; spirogennanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylarnine; trichothecenes (especially T-2 toxin, verracurin A, roridinA and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobromtol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g. paclitaxel (TAXOL®, Bristol-Myers Squibb Oncology, Princeton, N.].) and doxetaxel (TAXOTERE®, Rhone-Poulenc Rorer, Antony, France); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine;

vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-1 1 ; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoic acid; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this definition are antihormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (Fareston); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

In some embodiments, the compound of the present invention is administered with a targeted cancer therapy. Targeted cancer therapies are drugs or other substances that block the growth and spread of cancer by interfering with specific molecules ("molecular targets") that are involved in the growth, progression, and spread of cancer. Targeted cancer therapies are sometimes called "molecularly targeted drugs", "molecularly targeted therapies", "precision medicines", or similar names. In some embodiments, the targeted therapy consists of administering the subject with a tyrosine kinase inhibitor as defined above.

In some embodiments, compound of the present invention is administered with an immunotherapeutic agent. The term "immunotherapeutic agent," as used herein, refers to a compound, composition or treatment that indirectly or directly enhances, stimulates or increases the body's immune response against cancer cells and/or that decreases the side effects of other anticancer therapies. Immunotherapy is thus a therapy that directly or indirectly stimulates or enhances the immune system's responses to cancer cells and/or lessens the side effects that may have been caused by other anti-cancer agents. Immunotherapy is also referred to in the art as immunologic therapy, biological therapy biological response modifier therapy and biotherapy. Examples of common immunotherapeutic agents known in the art include, but are not limited to, cytokines, cancer vaccines, monoclonal antibodies and non-cytokine adjuvants. Alternatively the immunotherapeutic treatment may consist of administering the subject with an amount of immune cells (T cells, NK, cells, dendritic cells, B cells…). Immunotherapeutic agents can be non-specific, i.e. boost the immune system generally so that the human body becomes more effective in fighting the growth and/or spread of cancer cells, or they can be specific, i.e. targeted to the cancer cells themselves immunotherapy regimens may combine the use of non-specific and specific immunotherapeutic agents. Non-specific immunotherapeutic agents are substances that stimulate or indirectly improve the immune system. Non-specific immunotherapeutic agents have been used alone as a main therapy for the treatment of cancer,

as well as in addition to a main therapy, in which case the non-specific immunotherapeutic agent functions as an adjuvant to enhance the effectiveness of other therapies (e.g. cancer vaccines). Non-specific immunotherapeutic agents can also function in this latter context to reduce the side effects of other therapies, for example, bone marrow suppression induced by certain chemotherapeutic agents. Non-specific immunotherapeutic agents can act on key immune system cells and cause secondary responses, such as increased production of cytokines and immunoglobulins. Alternatively, the agents can themselves comprise cytokines. Non-specific immunotherapeutic agents are generally classified as cytokines or non-cytokine adjuvants. A number of cytokines have found application in the treatment of cancer either as general non-specific immunotherapies designed to boost the immune system, or as adjuvants provided with other therapies. Suitable cytokines include, but are not limited to, interferons, interleukins and colony-stimulating factors. Interferons (IFNs) contemplated by the present invention include the common types of IFNs, IFN-alpha (IFN-α), IFN-beta (IFN-β) and IFN-gamma (IFN-γ). IFNs can act directly on cancer cells, for example, by slowing their growth, promoting their development into cells with more normal behaviour and/or increasing their production of antigens thus making the cancer cells easier for the immune system to recognise and destroy. IFNs can also act indirectly on cancer cells, for example, by slowing down angiogenesis, boosting the immune system and/or stimulating natural killer (NK) cells, T cells and macrophages. Recombinant IFN-alpha is available commercially as Roferon (Roche Pharmaceuticals) and Intron A (Schering Corporation). Interleukins contemplated by the present invention include IL-2, IL-4, IL-11 and IL-12. Examples of commercially available recombinant interleukins include Proleukin® (IL-2; Chiron Corporation) and Neumega® (IL-12; Wyeth Pharmaceuticals). Zymogenetics, Inc. (Seattle, Wash.) is currently testing a recombinant form of IL-21, which is also contemplated for use in the combinations of the present invention. Colony-stimulating factors (CSFs) contemplated by the present invention include granulocyte colony stimulating factor (G-CSF or filgrastim), granulocyte-macrophage colony stimulating factor (GM-CSF or sargramostim) and erythropoietin (epoetin alfa, darbepoietin). Treatment with one or more growth factors can help to stimulate the generation of new blood cells in subjects undergoing traditional chemotherapy. Accordingly, treatment with CSFs can be helpful in decreasing the side effects associated with chemotherapy and can allow for higher doses of chemotherapeutic agents to be used. Various-recombinant colony stimulating factors are available commercially, for example, Neupogen® (G-CSF; Amgen), Neulasta (pelfilgrastim; Amgen), Leukine (GM-CSF; Berlex), Procrit (erythropoietin; Ortho Biotech), Epogen (erythropoietin; Amgen), Arnesp (erytropoietin). In addition to having

specific or non-specific targets, immunotherapeutic agents can be active, i.e. stimulate the body's own immune response, or they can be passive, i.e. comprise immune system components that were generated external to the body. Passive specific immunotherapy typically involves the use of one or more monoclonal antibodies that are specific for a particular antigen found on the surface of a cancer cell or that are specific for a particular cell growth factor. Monoclonal antibodies may be used in the treatment of cancer in a number of ways, for example, to enhance a subject's immune response to a specific type of cancer, to interfere with the growth of cancer cells by targeting specific cell growth factors, such as those involved in angiogenesis, or by enhancing the delivery of other anticancer agents to cancer cells when linked or conjugated to agents such as chemotherapeutic agents, radioactive particles or toxins. Monoclonal antibodies currently used as cancer immunotherapeutic agents that are suitable for inclusion in the combinations of the present invention include, but are not limited to, rituximab (Rituxan®), trastuzumab (Herceptin®), ibritumomab tiuxetan (Zevalin®), tositumomab (Bexxar®), cetuximab (C-225, Erbitux®), bevacizumab (Avastin®), gemtuzumab ozogamicin (Mylotarg®), alemtuzumab (Campath®), and BL22. Other examples include anti-CTLA4 antibodies (e.g. Ipilimumab), anti-PD1 antibodies, anti-PDL1 antibodies, anti-TIMP3 antibodies, anti-LAG3 antibodies, anti-B7H3 antibodies, anti-B7H4 antibodies or anti-B7H6 antibodies. In some embodiments, antibodies include B cell depleting antibodies. Typical B cell depleting antibodies include but are not limited to anti-CD20 monoclonal antibodies [e.g. Rituximab (Roche), Ibritumomab tiuxetan (Bayer Schering), Tositumomab (GlaxoSmithKline), AME-133v (Applied Molecular Evolution), Ocrelizumab (Roche), Ofatumumab (HuMax-CD20, Gemnab), TRU-015 (Trubion) and IMMU-106 (Immunomedics)], an anti-CD22 antibody [e.g. Epratuzumab, Leonard et al., Clinical Cancer Research (Z004) 10: 53Z7-5334], anti-CD79a antibodies, anti-CD27 antibodies, or anti-CD19 antibodies (e.g. U.S. Pat. No. 7,109,304), anti-BAFF-R antibodies (e.g. Belimumab, GlaxoSmithKline), anti-APRIL antibodies (e.g. anti-human APRIL antibody, ProSci inc.), and anti-IL-6 antibodies [e.g. previously described by De Benedetti et al., J Immunol (2001) 166: 4334-4340 and by Suzuki et al., Europ J of Immunol (1992) 22 (8) 1989-1993, fully incorporated herein by reference]. The immunotherapeutic treatment may consist of allografting, in particular, allograft with hematopoietic stem cell HSC. The immunotherapeutic treatment may also consist in an adoptive immunotherapy as described by Nicholas P. Restifo, Mark E. Dudley and Steven A. Rosenberg “Adoptive immunotherapy for cancer: harnessing the T cell response, Nature Reviews Immunology, Volume 12, April 2012). In adoptive immunotherapy, the subject’s circulating lymphocytes, NK cells, are isolated amplified in vitro

and readministered to the subject. The activated lymphocytes or NK cells are most preferably be the subject's own cells that were earlier isolated from a blood or tumor sample and activated (or "expanded") in vitro.

In some embodiments, the compound of the present invention is administered with a radiotherapeutic agent. The term "radiotherapeutic agent" as used herein, is intended to refer to any radiotherapeutic agent known to one of skill in the art to be effective to treat or ameliorate cancer, without limitation. For instance, the radiotherapeutic agent can be an agent such as those administered in brachytherapy or radionuclide therapy. Such methods can optionally further comprise the administration of one or more additional cancer therapies, such as, but not limited to, chemotherapies, and/or another radiotherapy.

In one embodiment, said additional active compounds may be contained in the same composition or administrated separately.

In another embodiment, the pharmaceutical composition of the invention relates to combined preparation for simultaneous, separate or sequential use in the treatment of cancer in a subject in need thereof.

The invention also provides kits comprising the compound of the invention. Kits containing the compound of the invention find use in therapeutic methods.

A further aspect, the invention relates to a method of treating cancer in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a compound selected from the group consisting of BTN3A2 agonist or BTN3A2 expression activator.

In a further aspect, the present invention relates to a compound selected from the group consisting of BTN3A2 antagonist or BTN3A2 expression inhibitor for use in Vγ9Vδ2 T-cells inhibition or Vγ9Vδ2 T-cells–mediated lysis inhibition.

Accordingly, the subject according to the invention refers to any subject (preferably human) afflicted or at risk to be afflicted with autoimmune disease or inflammatory condition.

In some embodiments, the present invention relates to a compound selected from the group consisting of BTN3A2 antagonist or BTN3A2 expression inhibitor for use in the treatment of an auto-immune disease.

As used herein, an "autoimmune disease" is a disease or disorder arising from and directed at an individual's own tissues. Examples of autoimmune diseases include, but are not limited to Addison's Disease, Allergy, Alopecia Areata, Alzheimer's disease, Antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis, Ankylosing Spondylitis, Antiphospholipid Syndrome (Hughes Syndrome), arthritis, Asthma, Atherosclerosis,

Atherosclerotic plaque, autoimmune disease (e.g., lupus, RA, MS, Graves' disease, etc.), Autoimmune Hemolytic Anemia, Autoimmune Hepatitis, Autoimmune inner ear disease, Autoimmune Lymphoproliferative syndrome, Autoimmune Myocarditis, Autoimmune Oophoritis, Autoimmune Orchitis, Azoospermia, Behcet's Disease, Berger's Disease, Bullous Pemphigoid, Cardiomyopathy, Cardiovascular disease, Celiac Sprue/Coeliac disease, Chronic Fatigue Immune Dysfunction Syndrome (CFIDS), Chronic idiopathic polyneuritis, Chronic Inflammatory Demyelinating, Polyradicalneuropathy (CIPD), Chronic relapsing polyneuropathy (Guillain-Barré syndrome), Churg-Strauss Syndrome (CSS), Cicatricial Pemphigoid, Cold Agglutinin Disease (CAD), chronic obstructive pulmonary disease (COPD), CREST syndrome, Crohn's disease, Dermatitis, Herpetiformus, Dermatomyositis, diabetes, Discoid Lupus, Eczema, Epidermolysis bullosa acquisita, Essential Mixed Cryoglobulinemia, Evan's Syndrome, Exopthalmos, Fibromyalgia, Goodpasture's Syndrome, Hashimoto's Thyroiditis, Idiopathic Pulmonary Fibrosis, Idiopathic Thrombocytopenia Purpura (ITP), IgA Nephropathy, immunoproliferative disease or disorder (e.g., psoriasis), Inflammatory bowel disease (IBD), including Crohn’s disease and ulcerative colitis, Insulin Dependent Diabetes Mellitus (IDDM), Interstitial lung disease, juvenile diabetes, Juvenile Arthritis, juvenile idiopathic arthritis (JIA), Kawasaki's Disease, Lambert-Eaton Myasthenic Syndrome, Lichen Planus, lupus, Lupus Nephritis, Lymphoscytic Lypophisitis, Ménière's Disease, Miller Fish Syndrome/acute disseminated encephalomyeloradiculopathy, Mixed Connective Tissue Disease, Multiple Sclerosis (MS), muscular rheumatism, Myalgic encephalomyelitis (ME), Myasthenia Gravis, Ocular Inflammation, Pemphigus Foliaceus, Pemphigus Vulgaris, Pernicious Anaemia, Polyarteritis Nodosa, Polychondritis, Polyglandular Syndromes (Whitaker's syndrome), Polymyalgia Rheumatica, Polymyositis, Primary Agammaglobulinemia, Primary Biliary Cirrhosis/Autoimmune cholangiopathy, Psoriasis, Psoriatic arthritis, Raynaud's Phenomenon, Reiter's Syndrome/Reactive arthritis, Restenosis, Rheumatic Fever, rheumatic disease, Rheumatoid Arthritis, Sarcoidosis, Schmidt's syndrome, Scleroderma, Sjörgen's Syndrome, Stiff-Man Syndrome, Systemic Lupus Erythematosus (SLE), systemic scleroderma, Takayasu Arteritis, Temporal Arteritis/Giant Cell Arteritis, Thyroiditis, Type 1 diabetes, Type 2 diabetes, Ulcerative colitis, Uveitis, Vasculitis, Vitiligo, and Wegener's Granulomatosis.

In some embodiments, the present invention relates to a compound selected from the group consisting of BTN3A2 antagonist or BTN3A2 expression inhibitor for use in the treatment of an inflammatory condition.

The term "inflammatory condition" as used herein refers to acute or chronic localized or systemic responses to harmful stimuli, such as pathogens, damaged cells, physical injury or irritants, that are mediated in part by the activity of cytokines, chemokines, or inflammatory cells (e.g., neutrophils, monocytes, lymphocytes, macrophages) and is characterized in most instances by pain, redness, swelling, and impairment of tissue function. In some embodiments, the autoimmune disease or inflammatory condition is selected from the group consisting of arthritis, rheumatoid arthritis, acute arthritis, chronic rheumatoid arthritis, gouty arthritis, acute gouty arthritis, chronic inflammatory arthritis, degenerative arthritis, infectious arthritis, Lyme arthritis, proliferative arthritis, psoriatic arthritis, vertebral arthritis, and juvenile-onset rheumatoid arthritis, osteoarthritis, arthritis chronica progrediente, arthritis deformans, polyarthritis chronica primaria, reactive arthritis, and ankylosing spondylitis), inflammatory hyperproliferative skin diseases, psoriasis such as plaque psoriasis, gutatte psoriasis, pustular psoriasis, and psoriasis of the nails, dermatitis including contact dermatitis, chronic contact dermatitis, allergic dermatitis, allergic contact dermatitis, dermatitis herpetiformis, and atopic dermatitis, x-linked hyper IgM syndrome, urticaria such as chronic allergic urticaria and chronic idiopathic urticaria, including chronic autoimmune urticaria, polymyositis/dermatomyositis, juvenile dermatomyositis, toxic epidermal necrolysis, scleroderma, systemic scleroderma, sclerosis, systemic sclerosis, multiple sclerosis (MS), spino-optical MS, primary progressive MS (PPMS), relapsing remitting MS (RRMS), progressive systemic sclerosis, atherosclerosis, arteriosclerosis, sclerosis disseminata, and ataxic sclerosis, inflammatory bowel disease (IBD), Crohn's disease, colitis, ulcerative colitis, colitis ulcerosa, microscopic colitis, collagenous colitis, colitis polyposa, necrotizing enterocolitis, transmural colitis, autoimmune inflammatory bowel disease, pyoderma gangrenosum, erythema nodosum, primary sclerosing cholangitis, episcleritis, respiratory distress syndrome, adult or acute respiratory distress syndrome (ARDS), meningitis, inflammation of all or part of the uvea, iritis, choroiditis, an autoimmune hematological disorder, rheumatoid spondylitis, sudden hearing loss, IgE-mediated diseases such as anaphylaxis and allergic and atopic rhinitis, encephalitis, Rasmussen's encephalitis, limbic and/or brainstem encephalitis, uveitis, anterior uveitis, acute anterior uveitis, granulomatous uveitis, nongranulomatous uveitis, phacoantigenic uveitis, posterior uveitis, autoimmune uveitis, glomerulonephritis (GN), idiopathic membranous GN or idiopathic membranous nephropathy, membrano- or membranous proliferative GN (MPGN), rapidly progressive GN, allergic conditions, autoimmune myocarditis, leukocyte adhesion deficiency, systemic lupus erythematosus (SLE) or systemic lupus erythematodes such as cutaneous SLE, subacute cutaneous lupus erythematosus, neonatal lupus syndrome (NLE), lupus erythematosus

disseminatus, lupus (including nephritis, cerebritis, pediatric, non-renal, extra-renal, discoid, alopecia), juvenile onset (Type I) diabetes mellitus, including pediatric insulin-dependent diabetes mellitus (IDDM), adult onset diabetes mellitus (Type II diabetes), autoimmune diabetes, idiopathic diabetes insipidus, immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, tuberculosis, sarcoidosis, granulomatosis, lymphomatoid granulomatosis, Wegener's granulomatosis, agranulocytosis, vasculitides, including vasculitis, large vessel vasculitis, polymyalgia rheumatica, giant cell (Takayasu's) arteritis, medium vessel vasculitis, Kawasaki's disease, polyarteritis nodosa, microscopic polyarteritis, CNS vasculitis, necrotizing, cutaneous, hypersensitivity vasculitis, systemic necrotizing vasculitis, and ANCA-associated vasculitis, such as Churg-Strauss vasculitis or syndrome (CSS), temporal arteritis, aplastic anemia, autoimmune aplastic anemia, Coombs positive anemia, Diamond Blackfan anemia, hemolytic anemia or immune hemolytic anemia including autoimmune hemolytic anemia (AIHA), pernicious anemia (anemia perniciosa), Addison's disease, pure red cell anemia or aplasia (PRCA), Factor VIII deficiency, hemophilia A, autoimmune neutropenia, pancytopenia, leukopenia, diseases involving leukocyte diapedesis, CNS inflammatory disorders, multiple organ injury syndrome such as those secondary to septicemia, trauma or hemorrhage, antigen-antibody complex-mediated diseases, anti-glomerular basement membrane disease, anti-phospholipid antibody syndrome, allergic neuritis, Bechet's or Behcet's disease, Castleman's syndrome, Goodpasture's syndrome, Reynaud's syndrome, Sjogren's syndrome, Stevens-Johnson syndrome, pemphigoid such as pemphigoid bullous and skin pemphigoid, pemphigus, optionally pemphigus vulgaris, pemphigus foliaceus, pemphigus mucus-membrane pemphigoid, pemphigus erythematosus, autoimmune polyendocrinopathies, Reiter's disease or syndrome, immune complex nephritis, antibody-mediated nephritis, neuromyelitis optica, polyneuropathies, chronic neuropathy, IgM polyneuropathies, IgM-mediated neuropathy, thrombocytopenia, thrombotic thrombocytopenic purpura (TTP), idiopathic thrombocytopenic purpura (ITP), autoimmune orchitis and oophoritis, primary hypothyroidism, hypoparathyroidism, autoimmune thyroiditis, Hashimoto's disease, chronic thyroiditis (Hashimoto's thyroiditis); subacute thyroiditis, autoimmune thyroid disease, idiopathic hypothyroidism, Grave's disease, polyglandular syndromes such as autoimmune polyglandular syndromes (or polyglandular endocrinopathy syndromes), paraneoplastic syndromes, including neurologic paraneoplastic syndromes such as Lambert-Eaton myasthenic syndrome or Eaton-Lambert syndrome, stiff-man or stiff-person syndrome, encephalomyelitis, allergic encephalomyelitis, experimental allergic encephalomyelitis (EAE), myasthenia gravis, thymoma-associated myasthenia gravis,

cerebellar degeneration, neuromyotonia, opsoclonus or opsoclonus myoclonus syndrome (OMS), and sensory neuropathy, multifocal motor neuropathy, Sheehan's syndrome, autoimmune hepatitis, chronic hepatitis, lupoid hepatitis, giant cell hepatitis, chronic active hepatitis or autoimmune chronic active hepatitis, lymphoid interstitial pneumonitis, bronchiolitis obliterans (non-transplant) vs NSIP, Guillain-Barre syndrome, Berger's disease (IgA nephropathy), idiopathic IgA nephropathy, linear IgA dermatosis, primary biliary cirrhosis, pneumonocirrhosis, autoimmune enteropathy syndrome, Celiac disease, Coeliac disease, celiac sprue (gluten enteropathy), refractory sprue, idiopathic sprue, cryoglobulinemia, amylotrophic lateral sclerosis (ALS; Lou Gehrig's disease), coronary artery disease, autoimmune ear disease such as autoimmune inner ear disease (AGED), autoimmune hearing loss, opsoclonus myoclonus syndrome (OMS), polychondritis such as refractory or relapsed polychondritis, pulmonary alveolar proteinosis, amyloidosis, scleritis, a non-cancerous lymphocytosis, a primary lymphocytosis, which includes monoclonal B cell lymphocytosis, optionally benign monoclonal gammopathy or monoclonal garnmopathy of undetermined significance, MGUS, peripheral neuropathy, paraneoplastic syndrome, channelopathies such as epilepsy, migraine, arrhythmia, muscular disorders, deafness, blindness, periodic paralysis, and channelopathies of the CNS, autism, inflammatory myopathy, focal segmental glomerulosclerosis (FSGS), endocrine opthalmopathy, uveoretinitis, chorioretinitis, autoimmune hepatological disorder, fibromyalgia, multiple endocrine failure, Schmidt's syndrome, adrenalitis, gastric atrophy, presenile dementia, demyelinating diseases such as autoimmune demyelinating diseases, diabetic nephropathy, Dressler's syndrome, alopecia greata, CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyl), and telangiectasia), male and female autoimmune infertility, mixed connective tissue disease, Chagas' disease, rheumatic fever, recurrent abortion, farmer's lung, erythema multiforme, post-cardiotomy syndrome, Cushing's syndrome, bird-fancier's lung, allergic granulomatous angiitis, benign lymphocytic angiitis, Alport's syndrome, alveolitis such as allergic alveolitis and fibrosing alveolitis, interstitial lung disease, transfusion reaction, leprosy, malaria, leishmaniasis, kypanosomiasis, schistosomiasis, ascariasis, aspergillosis, Sampter's syndrome, Caplan's syndrome, dengue, endocarditis, endomyocardial fibrosis, diffuse interstitial pulmonary fibrosis, interstitial lung fibrosis, idiopathic pulmonary fibrosis, cystic fibrosis, endophthalmitis, erythema elevatum et diutinum, erythroblastosis fetalis, eosinophilic faciitis, Shulman's syndrome, Felty's syndrome, flariasis, cyclitis such as chronic cyclitis, heterochronic cyclitis, iridocyclitis, or Fuch's cyclitis, Henoch-Schonlein purpura, human immunodeficiency virus (HIV) infection, echovirus infection, cardiomyopathy, Alzheimer's

disease, parvovirus infection, rubella virus infection, post-vaccination syndromes, congenital rubella infection, Epstein-Barr virus infection, mumps, Evan's syndrome, autoimmune gonadal failure, Sydenham's chorea, post-streptococcal nephritis, thromboangitis ubiterans, thyrotoxicosis, tabes dorsalis, chorioiditis, giant cell polymyalgia, endocrine ophthamopathy, chronic hypersensitivity pneumonitis, keratoconjunctivitis sicca, epidemic keratoconjunctivitis, idiopathic nephritic syndrome, minimal change nephropathy, benign familial and ischemia-reperfusion injury, retinal autoimmunity, joint inflammation, bronchitis, chronic obstructive airway disease, silicosis, aphthae, aphthous stomatitis, arteriosclerotic disorders, aspermiogenese, autoimmune hemolysis, Boeck's disease, cryoglobulinemia, Dupuytren's contracture, endophthalmia phacoanaphylactica, enteritis allergica, erythema nodosum leprosum, idiopathic facial paralysis, chronic fatigue syndrome, febris rheumatica, Hamman-Rich's disease, sensoneural hearing loss, haemoglobinuria paroxysmatica, hypogonadism, ileitis regionalis, leucopenia, mononucleosis infectiosa, traverse myelitis, primary idiopathic myxedema, nephrosis, ophthalmia symphatica, orchitis granulomatosa, pancreatitis (e.g. chronic pancreatitis), polyradiculitis acuta, pyoderma gangrenosum, Quervain's thyreoiditis, acquired splenic atrophy, infertility due to antispermatozoan antobodies, non-malignant thymoma, vitiligo, SCID and Epstein-Barr virus-associated diseases, acquired immune deficiency syndrome (AIDS), parasitic diseases such as Lesihmania, toxic-shock syndrome, food poisoning, conditions involving infiltration of T cells, leukocyte-adhesion deficiency, immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, diseases involving leukocyte diapedesis, multiple organ injury syndrome, antigen-antibody complex-mediated diseases, antiglomerular basement membrane disease, allergic neuritis, autoimmune polyendocrinopathies, oophoritis, primary myxedema, autoimmune atrophic gastritis, sympathetic ophthalmia, rheumatic diseases, mixed connective tissue disease, nephrotic syndrome, insulitis, polyendocrine failure, peripheral neuropathy, autoimmune polyglandular syndrome type I, adult-onset idiopathic hypoparathyroidism (AOIH), alopecia totalis, dilated cardiomyopathy, epidermolisis bullosa acquisita (EBA), hemochromatosis, myocarditis, nephrotic syndrome, primary sclerosing cholangitis, purulent or nonpurulent sinusitis, acute or chronic sinusitis, ethmoid, frontal, maxillary, or sphenoid sinusitis, an eosinophil-related disorder such as eosinophilia, pulmonary infiltration eosinophilia, eosinophilia-myalgia syndrome, Loffler's syndrome, chronic eosinophilic pneumonia, tropical pulmonary eosinophilia, bronchopneumonic aspergillosis, aspergilloma, or granulomas containing eosinophils, anaphylaxis, seronegative spondyloarthritides, polyendocrine autoimmune disease, sclerosing cholangitis, sclera, episclera, chronic

mucocutaneous candidiasis, Bruton's syndrome, transient hypogammaglobulinemia of infancy, Wiskott-Aldrich syndrome, ataxia telangiectasia, autoimmune disorders associated with collagen disease, rheumatism, neurological disease, ischemic re-perfusion disorder, reduction in blood pressure response, vascular dysfunction, antgiectasis, tissue injury, cardiovascular ischemia, hyperalgesia, cerebral ischemia, and disease accompanying vascularization, allergic hypersensitivity disorders, glomerulonephritides, reperfusion injury, reperfusion injury of myocardial or other tissues, dermatoses with acute inflammatory components, acute purulent meningitis or other central nervous system inflammatory disorders, ocular and orbital inflammatory disorders, granulocyte transfusion-associated syndromes, cytokine-induced toxicity, acute serious inflammation, chronic intractable inflammation, pyelitis, pneumonocirrhosis, diabetic retinopathy, diabetic large-artery disorder, endarterial hyperplasia, peptic ulcer, valvulitis, and endometriosis.

A further aspect, the invention relates to a method of treating auto-immune disease in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a compound selected from the group consisting of BTN3A2 agonist or BTN3A2 expression activator.

A further aspect, the invention relates to a method of treating inflammatory condition in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a compound selected from the group consisting of BTN3A2 agonist or BTN3A2 expression activator.

The term “BTN3A2 antagonist” has its general meaning in the art and refers to a compound that selectively inactivates the BTN3A2. The term “BTN3A2 antagonist” refers to any compound that can directly or indirectly inhibits the signal transduction cascade related to the BTN3A2. As used herein, the term “selectively inactivates” refers to a compound that preferentially inactivates BTN3A2 with a greater affinity and potency, respectively, than its interaction with the other sub-types or isoforms of the BTN3A family (BTN3A1 and BTN3A3). BTN3A2 antagonist also refers to a compound that decrease the BTN3A2 activity level. Compounds that prefer BTN3A2, but that may also inactivate other BTN3A sub-types, as partial or full antagonists are contemplated. Typically, a BTN3A2 antagonist is a small organic molecule, a polypeptide, an aptamer or an antibody.

Tests and assays for determining whether a compound is a BTN3A2 antagonist are well known by the skilled person in the art such as described in Williams and Barclay, 1988; Ruddy et al., 1997; Rhodes et al., 2001.

In another embodiment, the compound of the invention is an aptamer. Aptamers are a class of molecule that represents an alternative to antibodies in term of molecular recognition. Aptamers are oligonucleotide sequences with the capacity to recognize virtually any class of target molecules with high affinity and specificity. Such ligands may be isolated through Systematic Evolution of Ligands by EXponential enrichment (SELEX) of a random sequence library, as described in Tuerk C. and Gold L., 1990. The random sequence library is obtainable by combinatorial chemical synthesis of DNA. In this library, each member is a linear oligomer, eventually chemically modified, of a unique sequence. Possible modifications, uses and advantages of this class of molecules have been reviewed in Jayasena S.D., 1999. Peptide aptamers consists of a conformationally constrained antibody variable region displayed by a platform protein, such as E. coli Thioredoxin A that are selected from combinatorial libraries by two hybrid methods (Colas et al., 1996). Then after raising aptamers directed against the target of the invention as above described, the skilled man in the art can easily select those blocking or inactivating the target.

In another embodiment, the compound of the invention is an antibody (the term including "antibody portion") directed against the target such as described above and which is a BTN3A2 antagonist.

In one embodiment, the compound of the invention is a BTN3A2 expression inhibitor.

An "inhibitor of expression" refers to a natural or synthetic compound that has a biological effect to inhibit the expression of a gene.

The target expression inhibitors for use in the present invention may be based on antisense oligonucleotide constructs. Anti-sense oligonucleotides, including anti-sense RNA molecules and anti-sense DNA molecules, would act to directly block the translation of the target mRNA by binding thereto and thus preventing protein translation or increasing mRNA degradation, thus decreasing the level of the target proteins, and thus activity, in a cell. For example, antisense oligonucleotides of at least about 15 bases and complementary to unique regions of the mRNA transcript sequence encoding the target can be synthesized, e.g., by conventional phosphodiester techniques and administered by e.g., intravenous injection or infusion. Methods for using antisense techniques for specifically alleviating gene expression of genes whose sequence is known are well known in the art (e.g. see U.S. Pat. Nos. 6,566,135; 6,566,131; 6,365,354; 6,410,323; 6,107,091; 6,046,321; and 5,981,732).

Small inhibitory RNAs (siRNAs) can also function as a target expression inhibitors for use in the present invention. The target gene expression can be reduced by contacting the subject or cell with a small double stranded RNA (dsRNA), or a vector or construct causing the

production of a small double stranded RNA, such that the target expression is specifically inhibited (i.e. RNA interference or RNAi). Methods for selecting an appropriate dsRNA or dsRNA-encoding vector are well known in the art for genes whose sequence is known (e.g. see Tuschl, T. et al. (1999); Elbashir, S. M. et al. (2001); Hannon, GJ. (2002); McManus, MT. et al. (2002); Brummelkamp, TR. et al. (2002); U.S. Pat. Nos. 6,573,099 and 6,506,559; and International Patent Publication Nos. WO 01/36646, WO 99/32619, and WO 01/68836).

Ribozymes can also function as a target expression inhibitors for use in the present invention. Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Engineered hairpin or hammerhead motif ribozyme molecules that specifically and efficiently catalyze endonucleolytic cleavage of the target mRNA sequences are thereby useful within the scope of the present invention. Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, which typically include the following sequences, GUA, GUU, and GUC. Once identified, short RNA sequences of between about 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site can be evaluated for predicted structural features, such as secondary structure, that can render the oligonucleotide sequence unsuitable. The suitability of candidate targets can also be evaluated by testing their accessibility to hybridization with complementary oligonucleotides, using, e.g., ribonuclease protection assays.

Both antisense oligonucleotides and ribozymes useful a target inhibitors can be prepared by known methods. These include techniques for chemical synthesis such as, e.g., by solid phase phosphoramadite chemical synthesis. Alternatively, anti-sense RNA molecules can be generated by in vitro or in vivo transcription of DNA sequences encoding the RNA molecule. Such DNA sequences can be incorporated into a wide variety of vectors that incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Various modifications to the oligonucleotides of the invention can be introduced as a means of increasing intracellular stability and half-life. Possible modifications include but are not limited to the addition of flanking sequences of ribonucleotides or deoxyribonucleotides to the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2'-O-methyl rather than phosphodiesterase linkages within the oligonucleotide backbone.

Antisense oligonucleotides siRNAs and ribozymes of the invention may be delivered in vivo alone or in association with a vector. In its broadest sense, a "vector" is any vehicle capable of facilitating the transfer of the antisense oligonucleotide siRNA or ribozyme nucleic acid to

the cells and preferably cells expressing the target. Preferably, the vector transports the nucleic acid to cells with reduced degradation relative to the extent of degradation that would result in the absence of the vector. In general, the vectors useful in the invention include, but are not limited to, plasmids, phagemids, viruses, other vehicles derived from viral or bacterial sources that have been manipulated by the insertion or incorporation of the antisense oligonucleotide siRNA or ribozyme nucleic acid sequences. Viral vectors are a preferred type of vector and include, but are not limited to nucleic acid sequences from the following viruses: retrovirus, such as moloney murine leukemia virus, harvey murine sarcoma virus, murine mammary tumor virus, and rouse sarcoma virus; adenovirus, adeno-associated virus; SV40-type viruses; polyoma viruses; Epstein-Barr viruses; papilloma viruses; herpes virus; vaccinia virus; polio virus; and RNA virus such as a retrovirus. One can readily employ other vectors not named but known to the art.

Preferred viral vectors are based on non-cytopathic eukaryotic viruses in which non-essential genes have been replaced with the gene of interest. Non-cytopathic viruses include retroviruses (e.g., lentivirus), the life cycle of which involves reverse transcription of genomic viral RNA into DNA with subsequent proviral integration into host cellular DNA. Retroviruses have been approved for human gene therapy trials. Most useful are those retroviruses that are replication-deficient (i.e., capable of directing synthesis of the desired proteins, but incapable of manufacturing an infectious particle). Such genetically altered retroviral expression vectors have general utility for the high-efficiency transduction of genes in vivo. Standard protocols for producing replication-deficient retroviruses (including the steps of incorporation of exogenous genetic material into a plasmid, transfection of a packaging cell lined with plasmid, production of recombinant retroviruses by the packaging cell line, collection of viral particles from tissue culture media, and infection of the target cells with viral particles) are provided in KRIEGLER (A Laboratory Manual," W.H. Freeman C.O., New York, 1990) and in MURRY ("Methods in Molecular Biology," vol.7, Humana Press, Inc., Cliffton, N.J., 1991).

Preferred viruses for certain applications are the adeno-viruses and adeno-associated viruses, which are double-stranded DNA viruses that have already been approved for human use in gene therapy. The adeno-associated virus can be engineered to be replication deficient and is capable of infecting a wide range of cell types and species. It further has advantages such as, heat and lipid solvent stability; high transduction frequencies in cells of diverse lineages, including hemopoietic cells; and lack of superinfection inhibition thus allowing multiple series of transductions. Reportedly, the adeno-associated virus can integrate into human cellular DNA in a site-specific manner, thereby minimizing the possibility of insertional mutagenesis and

variability of inserted gene expression characteristic of retroviral infection. In addition, wild-type adeno-associated virus infections have been followed in tissue culture for greater than 100 passages in the absence of selective pressure, implying that the adeno-associated virus genomic integration is a relatively stable event. The adeno-associated virus can also function in an extrachromosomal fashion.

Other vectors include plasmid vectors. Plasmid vectors have been extensively described in the art and are well known to those of skill in the art. See e.g., SANBROOK et al., "Molecular Cloning: A Laboratory Manual," Second Edition, Cold Spring Harbor Laboratory Press, 1989. In the last few years, plasmid vectors have been used as DNA vaccines for delivering antigen-encoding genes to cells in vivo. They are particularly advantageous for this because they do not have the same safety concerns as with many of the viral vectors. These plasmids, however, having a promoter compatible with the host cell, can express a peptide from a gene operatively encoded within the plasmid. Some commonly used plasmids include pBR322, pUC18, pUCl9, pRC/CMV, SV40, and pBlueScript. Other plasmids are well known to those of ordinary skill in the art. Additionally, plasmids may be custom designed using restriction enzymes and ligation reactions to remove and add specific fragments of DNA. Plasmids may be delivered by a variety of parenteral, mucosal and topical routes. For example, the DNA plasmid can be injected by intramuscular, intradermal, subcutaneous, or other routes. It may also be administered by intranasal sprays or drops, rectal suppository and orally. It may also be administered into the epidermis or a mucosal surface using a gene-gun. The plasmids may be given in an aqueous solution, dried onto gold particles or in association with another DNA delivery system including but not limited to liposomes, dendrimers, cochleate and microencapsulation.

Typically the compounds according to the invention as described above are administered to the patient in a therapeutically effective amount.

By a "therapeutically effective amount" of the compound of the present invention as above described is meant a sufficient amount of the compound at a reasonable benefit/risk ratio applicable to any medical treatment. It will be understood, however, that the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific compound employed; the specific composition employed, the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific

compound employed; the duration of the treatment; drugs used in combination or coincidential with the specific compound employed; and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of the compound at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. However, the daily dosage of the products may be varied over a wide range from 0.01 to 1,000 mg per adult per day. Typically, the compositions contain 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, 50.0, 100, 250 and 500 mg of the compound of the present invention for the symptomatic adjustment of the dosage to the patient to be treated. A medicament typically contains from about 0.01 mg to about 500 mg of the compound of the present invention, preferably from 1 mg to about 100 mg of the compound of the present invention. An effective amount of the drug is ordinarily supplied at a dosage level from 0.0002 mg/kg to about 20 mg/kg of body weight per day, especially from about 0.001 mg/kg to 7 mg/kg of body weight per day.

In a particular embodiment, the compound according to the invention may be used in a concentration between 0.01 μM and 20 μM, particularly, the compound of the invention may be used in a concentration of 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 20.0 μM.

According to the invention, the compound of the present invention is administered to the subject in the form of a pharmaceutical composition. Typically, the compound of the present invention may be combined with pharmaceutically acceptable excipients, and optionally sustained-release matrices, such as biodegradable polymers, to form therapeutic compositions. "Pharmaceutically" or "pharmaceutically acceptable" refer to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to a mammal, especially a human, as appropriate. A pharmaceutically acceptable carrier or excipient refers to a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type.

In the pharmaceutical compositions of the present invention for oral, sublingual, subcutaneous, intramuscular, intravenous, transdermal, local or rectal administration, the active principle, alone or in combination with another active principle, can be administered in a unit administration form, as a mixture with conventional pharmaceutical supports, to animals and human beings. Suitable unit administration forms comprise oral-route forms such as tablets, gel capsules, powders, granules and oral suspensions or solutions, sublingual and buccal administration forms, aerosols, implants, subcutaneous, transdermal, topical, intraperitoneal, intramuscular, intravenous, subdermal, transdermal, intrathecal and intranasal administration forms and rectal administration forms.

Typically, the pharmaceutical compositions contain vehicles which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized water or physiological saline, permit the constitution of injectable solutions. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. Solutions comprising compounds of the invention as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. The compound of the present invention can be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. The carrier can also be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetables oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin. Sterile injectable solutions are prepared by incorporating the active

compounds in the required amount in the appropriate solvent with several of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized agent of the present inventions into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the typical methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the compound of the present invention plus any additional desired ingredient from a previously sterile-filtered solution thereof. The preparation of more, or highly concentrated solutions for direct injection is also contemplated, where the use of DMSO as solvent is envisioned to result in extremely rapid penetration, delivering high concentrations of the active agents to a small tumor area. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above, but drug release capsules and the like can also be employed. For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

In a further aspect, the present invention relates to a method of screening a candidate compound for use as a drug for treating cancer in a subject in need thereof, wherein the method comprises the steps of:

- providing a BTN3A2, providing a cell, tissue sample or organism expressing a BTN3A2,
- providing a candidate compound such as a small organic molecule, a polypeptide, or an antibody,
- measuring the BTN3A2 activity,
- and selecting positively candidate compounds that induce or inhibit BTN3A2 activity.

Methods for measuring BTN3A2 activity are well known in the art (Williams and Barclay, 1988; Ruddy et al., 1997; Rhodes et al., 2001). For example, measuring the BTN3A2

activity involves determining a Ki on the BTN3A2 cloned and transfected in a stable manner into a CHO cell line, measuring cancer cell migration and invasion abilities, measuring cancer cell growth, measuring cancer cell proliferation, measuring BTN3A2 pathway signalling, and measuring Vγ9Vδ2 T-cells activity in the present or absence of the candidate compound.

Tests and assays for screening and determining whether a candidate compound is a BTN3A2 agonist or antagonist are well known in the art (Williams and Barclay, 1988; Ruddy et al., 1997; Rhodes et al., 2001). In vitro and in vivo assays may be used to assess the potency and selectivity of the candidate compounds to induce or inhibit BTN3A2 activity.

Activities of the candidate compounds, their ability to bind BTN3A2 and their ability to induce or inhibit BTN3A2 activity may be tested using isolated cancer cell, cancer cell lines or CHO cell line cloned and transfected in a stable manner by the human BTN3A2.

The invention will be further illustrated by the following figures and examples. However, these examples and figures should not be interpreted in any way as limiting the scope of the present invention.

FIGURES:

Figure 1: BTN3A1 and BTN3A2/A3 expression is also necessary for BrHPP-mediated Vγ9Vδ2-T cells activation. Co-culture experiments of Vγ9Vδ2-T cells with HEK293T and HL-60 BTN3A KO clones, upon BrHPP treatment. Data plotted represents the mean+SEM of 3 independent experiments performed with 2 different Vγ9Vδ2-T cells donors (>90% purity) each time in co-culture experiments performed with CRISPR KO clones (Fig. 1-A (left) and 1-B (left)). 2 independent experiments with 2 different Vγ9Vδ2-T cells donors each time were run in co-culture assays performed with BTN3A triple KO cells re-expressing different BTN3A combinations (Fig. 1-A (right) and 1-B (right)). Statistical significance was established using Two-way ANOVA Test with Bonferroni correction (* $p < 0.05$). **A)** Analysis of (I) degranulation (CD107+) of Vγ9Vδ2-T cells when challenged with the indicated HEK293T BTN3A KO clones, as well as Mock cells used as positive control. II) Vγ9Vδ2-T cells were challenged with HEK293T triple KO cells, transiently transfected with pCNA3.1, either EV or encoding the indicated BTN3A paralogs, in single transfection or co-transfection. In both experiments target cells were pre-treated with escalating concentrations of Bromohydrin-pyrophosphate (10nM, 100 nM and 1 μM). **B)** Analysis of degranulation (CD107+) of Vγ9Vδ2-T cells when challenged with the indicated HL-60 BTN3A KO clones, as well as Mock cells used as positive control (I). In II), Vγ9Vδ2-T cells were challenged with

HL-60 triple KO cells, transiently transfected with pCNA3.1, either EV or encoding the indicated BTN3A paralogs, in single transfection or co-transfection. In both experiments target cells were pre-treated with escalating concentrations of Bromohydrin-pyrophosphate (10nM, 100 nM and 1 μM). Triple KO cells were transfected 48 hours before the co-culture's setting up. Mock Triple KO cells were used as negative control.

EXAMPLE:

Example 1:

Role of BTN3A2 protein in the recognition of AML blasts by Vγ9Vδ2 T-cells.

Vγ9Vδ2 T-cells are the main peripheral blood γδT-cell subpopulation. This T-cell subset stands out as having the capacity to "sense" infected and malignant cells. Most primate Vγ9Vδ2 T cells are specifically activated by small organic non-peptidic pyrophosphate molecules, also called phosphoantigens (PAg). Among them, isopentenyl pyrophosphate (IPP) tends to accumulate in p53-null cancers. BTN3A/CD277 molecules expression on target cells has been shown to trigger the PAg-induced activation of human Vγ9Vδ2 T cells. BTN3A molecules are members of the human butyrophilin (BTN), a type I transmembrane glycoproteins superfamily sharing strong homologies with the costimulatory molecules of the B7 family. The BTN3A cluster is composed of 3 isoforms (BTN3A1, BTN3A2, and BTN3A3) exhibiting 95% identity and mainly distinguished by their intracellular part (e.g., presence or absence of a B30.2/SPRY domain). PAg binding on B30.2 domain of BTN3A1 isoform has been shown to specifically trigger Vγ9Vδ2 T-cells activation. If BTN3A2 isoform, lacking the B30.2 intracellular domain, cannot trigger PAg-mediated Vγ9Vδ2 T-cells activation, it appears to be necessary to fully activate Vγ9Vδ2 T-cells against their targets. Interestingly, BTN3A2 is the most expressed BTN3A paralog in several AML cell lines as shown by transcriptome analysis. For the first time, we could demonstrate BTN3A2 protein expression using a unique novel BTN3A2 monoclonal antibody. Thus, we postulated that BTN3A2 overexpression might sensitize AML blasts to Vγ9Vδ2 T-cells–mediated lysis.

Thereby, the main goal of our work was to elucidate the role of BTN3A2 in the recognition mechanism of AML blasts by Vγ9Vδ2 T-cells. First, we confirmed using co-immunoprecipitation, FRET and PLA experiments that BTN3A2 interacted with BTN3A1 and to a lesser extent with BTN3A3. Next, we generated BTN3A2 knock-out HL-60 cells, as well as a BTN3A1/A3 double KO using CRISPR/Cas9-gene editing. Vγ9Vδ2 T-cells were challenged against WT and KO HL-60 cells, in presence of natural BTN3 ligand (Aminobisphosphonates) or using our previously described BTN3 specific agonist mAb.

Surprisingly in leukemias lacking BTN3A1 expression, BTN3A2 was still, although to a lesser extent, able to induce AML killing using agonist BTN3A mAb. This finding could unmask new therapeutic opportunities to treat AML patients with Vγ9Vδ2 T-cells immunotherapy.

Example 2:

Materials and Methods

CRISPR plasmid construction

pSpCas9(BB)-2A-GFP (PX458) was a gift from Feng Zhang (Addgene plasmid # 48138) (Ran et al., 2013). PX458 plasmid was linearized using BbsI (37°C, one hour digestion). sgRNA targeting the sequence 5'-GAGTGAGCAGCTGGACCAAGAGG-3' within the signal peptide of BTN3A2 (Third exon) was devised manually. The guide oligos targeting the different BTN3A paralogs were purchased from Invitrogen The guide oligos for the top and bottom strand contain overhangs for ligation into the pair of BbsI sites in PX458. The sgRNA primers were annealed in a thermocycler by using the following parameters: 95 °C for 5 min; ramp down to 85 °C at 2 °C/s, then ramp down to 25°C at 0.1°C/s. sgRNA primers were cloned into PX458 plasmid by incubating them with PX458 and T4 ligase (New England Biolabs) during one hour at 37°C. Then, 20 uL of E. Coli DH5-α strain (from Invitrogen) were transformed with 2uL ligation product and immediately seeded on LB-agar ampicillin plates (50 ug/ml). Primary screening of clones with the correct insertion was performed with a BbsI/AgeI double digestion of plasmids' minipreps. Confirmation of positive clones carrying the correct insertion was assessed by Sanger sequencing (GATC Biotech).

CRISPR clones transfection and clonal isolation

BxPC-3 and HAP-1 CRISPR clones were seeded the day before transfection at 70% confluency approximately. HL-60 clones were cultured at a concentration of 0.5 million cells/ml the day before transfection. BxPC-3 and HAP-1 cells were then transfected with FuGENE®HD (Promega®) at a 4:1 ratio (Reagent/DNA ratio), HEK293T with lipofectamine LTX (Invitrogen®) and HL-60 cells were transfected by nucleofection (Lonza, V kit, program T-019). 48 hours after transfection, the BxPC-3, HAP-1, HEK293T and HL-60 clones expressing the highest GFP levels were sorted and isolated by FACS (BD FACSAria™ II). For clonal isolation, 250 GFP+ BxPC-3 cells were cultured in ClonaCell™-TCS Medium (StemCell™ Technologies) (Hydroximethylcellulose-based semisolid medium) with1% Penicillin/Streptomycin, 20% RPMI 1640 with 10% FBS in order to allow the formation of monoclonal colonies, while HL-60, HEK293T and HAP-1 individual clones were seeded in 96-well plates (one cell/well) in RPMI-1640, DMEM and IMDM medium, respectively, with 50%

FBS. Thirty days later, BxPC-3 colonies were manually picked from the semisolid medium plate and seeded into 24-well plates. At confluence, BxPC-3 clones were subsequently seeded into 12-well plates, 6-well plates and T-25 flasks. Once T-25 flasks were confluent, the half of cells was frozen (50% RPMI 1640, 40% FBS and 10% DMSO) and the other half was derived for protein extraction. Similarly, HL-60, HEK293T and HAP-1 clones were expanded until the obtaining of 10 million cells or confluence in T-25 flasks. In the same way, the half of cells was frozen and the other half was derived for protein extraction (for HL-60 cells) or DNA extraction (for HAP-1 cells).

BTN3A vectors used during experiments

pCDNA3.1 BTN3Ax-3xFlag plasmids were generated by cloning 3xFlag BTN3Ax-ORF from donor vector SIGMA_p3XFLAG-myc-CMV25 expression vector (e6276) by gateway recombination (Katzen, 2007). STOP codons were generated at the end of BTN3Ax sequences in order to avoid expression of c-myc tag, c-terminal to BTN3A protein sequences. 3xFlag tag is located N-terminal to BTN3Ax coding sequence. pcDNA3.1 BTN3Ax-eGFP plasmids were purchased from GenScript ®.BTN3Ax-eGFP ORFs from these plasmids were cloned into pDONR221 gateway adaptor plasmid (cloning site: HindIII-EcoRI), then transferred to pMSCV-PURO-GVA retroviral vector plasmids by gateway recombination (attB). BTN3A1-Link-emGFP transfection plasmid was a kind gift from E. Scotet (Nantes University). During the experiment focusing on the expression of BTN3A1 to the cell membrane compartment when co-expressed with A2 or A3, HEK293T cells BTN3A KO (CRISPR-Cas9) were co-transfected with the aforementioned 3xFlag constructs and with pCDNA3.1 BTN3Ax-Link-CFP/YFP plasmids. After 24hrs, cells were labelled with anti-Flag mAb (Invitrogen™) followed by anti-mouse-PE (BD Biosciences) and analysed by FACS. For1 CFP or YFP constructs used in FRET experiments, cDNA was synthetized (GeneArt) and cloned into pCDN3.1-Zeo+ backbone (Invitrogen). BTN3A1 sequence used as reference was the NM_007048, for BTN3A2, the reference coding sequence was the NM_001197246 and NM_006994 for BTN3A3. CFP, YFP and CFP-YFP fusion protein sequences were kindly provided by Pascal Schneider and Cristian Smulski (Lausanne).

Retroviral transduction of HL-60 single KO cell clones

Retroviral particles were obtained by transfection of Phoenix A cells with pMSCV-PURO-BTN3Ax retroviral vector plasmids described above. Cells were transfected with lipofectamine LTX at 50% confluency at supernatants were collected two times per day during two days. Filtered supernatants were added to HL-60 single KO cells culture medium at 1:1

ratio. After 48 hours, 1 μg/ml puromycin was added to infected cells during 10 days. After this period, cells were analysed by western blot.

Protein extraction and Western Blot of CRISPR clones

10 cm-culture dishes were placed on ice, washed in PBS, cells were dissociated and lysed in 250 μl of ice-cold HNTG buffer (50 mM HEPES pH 7, 50 mM NaF, 1 mM EGTA, 150 mM NaCl, 1% Triton X-100, 10% glycerol, and 1.5 mM MgCl2) in the presence of protease inhibitors (Roche Applied Science) and 100 μM Na3VO4. Protein quantification in all cell lysates was performed by Bradford (Biorad quantification kit) and by direct measurement (Protein A280) performed with Nanodrop® ND-1000 (ThermoFisher™ Scientific). Proteins were resolved by SDS-PAGE 12%, followed by western blotting. Primary antibodies used were: anti-BTN3A 20.1, 103.2 and 146.1 mAb, anti-GAPDH (Flarebio® Biotech LLC). Primary antibodies binding was detected with peroxidase conjugated anti-mouse IgG and anti-rabbit IgG antibody (Jackson Laboratory). Immunoreactive bands were detected using enhanced chemiluminescent reagents West Dura and West Pico (Pierce).

Genomic PCR and sequence analysis of CRISPR clones

Primers flanking the CRISPR plasmid putative cut sites on BTN3A1, BTN3A2 and BTN3A3 were manually devised and purchased from Invitrogen®. The PCR reaction (using specific primers) was performed with Phusion Hot Start II DNA Polymerase (ThermoFisher™ Scientific). The PCR reaction was performed in a thermocycler by using the following parameters: 1. - Initial denaturation at 98.0°C during 30s, 2.-Denaturation at 98.0°C during 20s, 3. – Primer annealing at 67°C during 20s, 4. – Extension at 72°C during 30°C and 5. – Final extension at 72°C during 5 minutes. 35 amplification cycles were performed on each PCR reaction.

Once obtained, PCR products were purified with Nucleospin® Gel and PCR Clean-up (MACHEREY-NAGEL GmbH & Co). DNA concentration of purified PCR products was measured with Nanodrop® ND-1000 (ThermoFisher™ Scientific). Sanger sequencing of purified PCR products was subsequently performed (GATC Biotech). Quality of sequencing results was visually assessed with FinchTV (Geospiza™). DNA sequences were aligned one to each other with the multiple sequence alignment tool MUSCLE (EMBL-EBI). If two or more sequences were observed from the CRISPR plasmid cut site for one purified PCR product, each allelic sequence was then analysed cloning the PCR pool into pCR®-Blunt plasmid (ThermoFisher™ Scientific).

Purified PCR products cloning into pCR®-Blunt plasmid and allelic sequencing

pCR®-Blunt plasmid was linearized performing a digestion with StuI during two hours at 37°C. Then, the digestion product was run on a 2% agarose gel. The 3.5 Kb band was extracted from the gel using Nucleospin® Gel and PCR Clean-up (MACHEREY-NAGEL GmbH & Co). Purified PCR products were ligated into linearized pCR®-Blunt plasmid incubating them in the presence of T4 ligase at room temperature during two hours. Afterwards, 20 uL of E. Coli DH5-α strain (from Invitrogen) were transformed with 2uL ligation product, overgrowth during one hour in S.O.C. medium (Invitrogen®) and seeded on LB-agar Kanamycin plates (50 ug/ml). The day after, colonies were picked and cultured in 2 ml LB kanamycin overnight. Then, plasmid DNA was isolated from minipreps with Nucleospin® Plasmid (MACHEREY-NAGEL GmbH & Co). Plasmids concentration was measured with Nanodrop® ND-1000 (ThermoFisher™ Scientific). Sanger sequencing of purified PCR products was subsequently performed (GATC Biotech). Quality of sequencing results was visually assessed with FinchTV (Geospiza™). DNA sequences were aligned one to each other with the multiple sequence alignment tool MUSCLE (EMBL-EBI).

Transcriptomic analysis of BTN3A KO CRISPR clones and IFNγ-treated HL-60 cells by RT q-PCR

Primers for RT q-PCR experiments were devised with AmplifX 1.5.4 software and purchased from invitrogen™. Total RNA was isolated using RNEasy Mini Kit (Qiagen®). To quantify BTN3A expression levels, equal amounts of cDNA were synthesized using random primers (Invitrogen™, Carlsbad, CA). Power SYBR Green PCR master mix was used as fluorescent dye during experiments (Applied Biosystems™, Carlsbad, CA). GAPDH served as internal control. All RT q-PCRs performed using SYBR Green were conducted at 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 minute. The specificity of the reaction was verified by melt curve analysis. Cq data are normalized relative to GAPDH expression. WT or not treated cells were considered the sample calibrator of during the experiments.

Degranulation/cytokines induction assay

For CD107a+b, IFN-γ and TNF-α expression assays, target cells were pretreated for 2 hours at 37°C with unspecific IgG1 mAb (negative control, 1 μg/ml), BTNA-specific activating mAb (20.1, single dose 1 μg/ml) or escalating doses of BrHPP (10 nM, 100 nM and 1 μ M). For N-BPs (zoledronate) treatment, target cells were pre-incubated overnight with escalating doses of this drug (5, 10 and 20 μ M). Treated cells were next extensively washed and cultured together with V γ 9V ∂ 2-T cells at 37° C in complete RPMI 1640 medium in the presence of

Golgistop® and FITC-labelled CD107a and CD107b-specific mAbs (Miltenyi™ Biotech). After 4 hr, cells were harvested and stained with PE-labelled Pan gd TCR-specific mAb (Miltenyi™ Biotech), AF-700 anti-CD3 antibody (BD Biosciences) and LIVE/DEAD™ Fixable near-IR Dead Cell Stain reagent (Invitrogen™). After 20 minutes incubation, cells were permeabilized, subsequently stained with APC-labelled IFNγ and eFluor450-labelled TNFα-specific mAbs (BD Biosciences), and analysed by flow cytometry. Data were collected on FACS LSRII and analysed with FlowJo X software (Tree Star®). WT cells were were used as positive control during functional assays and triple KO cells, as negative control. Statistical significance was established using Two-way ANOVA Test with Bonferroni correction (* $p < 0.05$).

Co-immunoprecipitation of BTN3A-bound proteins

HEK293T triple KO cells were transiently co-transfected 48 hours before cell lysis with the indicated plasmids. HL-60 BT3A1 KO cells stably expressing BTN3A1-eGFP were obtained one week before the experiment setting-up. Two days after, transfected 10 cm-culture dishes were placed on ice, washed in PBS, and cells were dissociated and lysed in 1 ml of ice-cold HNTG buffer (50 mM HEPES pH 7, 50 mM NaF, 1 mM EGTA, 150 mM NaCl, 1% Triton X-100, 10% glycerol, and 1.5 mM MgCl2) in the presence of protease inhibitors (Roche Applied Science) and 100 μM Na3VO4 during 15 minutes at 4°C, then, lysates were centrifuged at 13.000 rpm during 15 min and supernatants were collected. Protein quantification in all cell lysates was performed by Bradford (Biorad quantification kit) and by direct measurement (Protein A280) performed with Nanodrop® ND-1000 (ThermoFisher™ Scientific). Cell lysates were incubated with CL-4B sepharose beads (Sigma-Aldrich®) to remove proteins that bind the sepharose beads in a non-specific manner (4°C, 45 min). Thereafter, pre-cleared cell lysates were incubated overnight at 4°C with 1 μg of anti-GFP antibody (Roche). The day after, protein G sepharose beads are added to the cell lysates to recover primary antibody and associated proteins (45 min, 4°C). Finally, sepharose beads are pulled down by centrifugation. Co-immunoprecipitated proteins were eluted in 2x laemmli buffer, heated at 95°C during 5 min and loaded into 10% SDS-PAGE polyacrylamide gel to allow the visualization of co-immunoprecipitated proteins by western blot. In order to visualize the 3x-Flag proteins bounded to BTN3Ax-eGFP, PVDF membranes were incubated with rabbit anti-Flag antibody (Sigma-Aldrich), followed by HRP-conjugated goat anti-rabbit immunostaining. To visualize endogen BTN3A proteins from HL-60 cells, membranes were incubated with 20.1 or anti-GFP antibody (Roche), then with HRP-conjugated goat anti-mouse IgG. Immunoreactive bands were detected using enhanced chemiluminescent reagents West Dura and West Pico (Pierce).

BTN3A expression assay of HL-60 cells treated with IFNγ and Ruxolitinib

60.000 HL-60 cells were cultured in IMDM medium with 10% FBS in 96-well plates and treated with IFNγ (100 U/ml), IFNγ+ Ruxolitinib (0.5 μM) or vehicle (DMSO). Afterwards, treated HL-60 cells were labelled with aqua live/dead staining (Thermofisher Scientific ®) and with AF647-20.1 antibody or AF647-IgGI control isotype in order to analyse only the effects of those drugs in the expression of BTN3A on live cells. Data were collected on LSRII and analysed with FlowJo X software (Tree Star®). Cells treated with 1% DMSO were used as negative control during the assay.

FRET experiments

Experiments were performed using a MacsQuant VYB Analyser (Miltenyi). YFP signal was recorded using the 488 nm laser with a 525/50 filter, CFP signal was recorded using the 405 nm laser with a 450/50 filter and FRET signal was recorded using the 405 nm laser with a 525/50 filter. HEK 293T cells were transiently transfected with CFP and YFP fusion receptors and analysed 20–24 h post-transfection. Positive FRET cells were gated using an CFP–YFP fusion protein as positive control and a co-transfection of CFP and YFP as negative control according to Banning et al. and Schneider et al (Banning et al., 2010).

Results

IFNγ treatment induces BTN3A expression on AML cells

In 2004, E. Compte and co-workers evidenced BTN3A up-regulation on HUVEC cells (human vein endothelial cells) when stimulated with IFNγ. IFNγ is considered one the main cytokines involved in Th1 immune responses. Vγ9Vδ2-T cells respond to BTN3A stimulus secreting, among others, IFNγ. IFNγ induces the expression of a vast array of membrane proteins involved in immune recognition as HLA molecules, as well as co-inhibitory (PD-L1) and co-stimulatory molecules (CD80 and CD86). We were wondering if IFNγ could induce BTN3A overexpression on cancer cells, thus reinforcing BTN3A signaling towards Vγ9Vδ2-T cells.

First, HL-60 cells were incubated during 12 hours with 100 UI/ml IFNγ, cells were harvested after 4, 6, 8 and 12 hours of treatment and cDNAs obtained from RNA extracts were subjected to RT q-PCR analysis (data not shown). Results show up-regulation of all BTN3A paralogs, mainly BTN3A3 (4-fold increase after 12hours), at mRNA level, upon IFNγ stimulation. BTN3A2 and BTN3A1 mRNA levels doubled their expression levels compared to not-treated cells after 12 hours incubation.

Then, BTN3A expression level at cell surface was assessed by flow cytometry. FACS analysis confirmed BTN3A overexpression at membrane level after 48 hours incubation with IFNγ (data not shown). IFNγ-treated HL-60 cells increased PD-L1 surface expression as well, while the control CD33 expression remained stable among different conditions (data not shown). IFNγ+Ruxolitinib (Jak1/2 inhibitor) treatment was added latter to the assay with the aim to elucidate if IFNγ-mediated BTN3A overexpression depends on STAT1 activation. BTN3A expression was measured on HL-60 and HEK293T WT and triple KO cells, as well as on BxPC-3 cells. IFNγ treatment significantly induced an increase of BTN3A surface expression on HL-6O cells (B). IFNγ induction of BTN3A expression is mostly balanced when Ruxolitinib is added to the treatment. HL-60 triple KO cells BTN3A levels remained stable along the different treatments (data not shown). Results look similar for HEK293T cells. BxPC-3 and HAP-1 WT cells also showed overexpression of BTN3A at membrane level upon IFNγ treatment (data not shown). Thus, independently of the cell line tested, IFNγ induced BTN3A overexpression at cell membrane level (p-value ≤ 0.001).

Both BTN3A1 and BTN3A2/A3 expression are required for IPP-induced Vγ9Vδ2-T cells stimulation on cancerous cells

In order to study the role of BTN3A proteins in different cell lines, BTN3A KO clones were obtained by CRISPR (data not shown) on HEK293T (Kidney embryonic, suitable for transfection) and HL-60 cells (AML). CRISPR KO clones used during co-culture assays were validated by Sanger sequencing (data not shown), western blot (data not shown), RT q-PCR (data not shown) and flow cytometry (data not shown). We chose a leukemic cell line to check the role of BTN3A in a cancerous context. Functional assays measuring degranulation (CD107+), IFNγ and TNFα production by Vγ9Vδ2-T cells provided to us information about how Vγ9Vδ2-T cells react against different BTN3A KO clones. We also wanted to find out the BTN3A requirements each activation method has in order to efficiently activate Vγ9Vδ2-T cells. Thus, target cells were pre-treated with three different activators of Vγ9Vδ2-T cells (Zoledronic acid, BrHPP and 20.1) during co-culture experiments.

Although BTN3A surface expression levels of all the cell lines tested are quite different (data not shown), the two cell lines effectively induced degranulation and cytokines production upon zoledronic acid treatment (data not shown). As expected, the cell line that stimulated the most Vγ9Vδ2-T cells was HL-60, whose BTN3A surface expression levels were the highest on FACS analysis (data not shown). We obtained HEK293T triple KO for BTN3A1, BTN3A2 and BTN3A3 and transfected them with each BTN3A plasmid. The expression levels were

investigated and depicted using a pan BTN3A mAb (20.1), or mAbs specific for BTN3A2, or recognizing BTN3A2 and BTN3A3.

The assays performed with KO clones shows that cells lacking the expression of BTN3A1 on one hand or BTN3A2+BTN3A3 on the other weren't able to activate Vγ9Vδ2-T cells, neither triple KO cells (data not shown). It was true for the two other cell lines derived from CML (HAP-1) and pancreatic cancer (BxPC-3) tested, upon ZA stimulation (data not shown). The ability of BTN3A3 and especially BTN3A2 KO clones in activating Vγ9Vδ2-T cells was impaired, mainly at suboptimal concentrations of zoledronic acid, although differences were not significant in some cases. The effects of BTN3A2 and BTN3A3 KO might be due to altered BTN3A surface expression. Thus, BTN3A2 KO clones used in this experiment express BTN3A at levels that are 40-60% below those encountered on Mock cells. By its part, BTN3A3 KO clones (only in HL-60) express 5-30% less BTN3A at cell membrane than Mock cells.

Co-culture assays on triple BTN3A KO cells transiently transfected with all the possible combinations of BTN3A proteins were performed on HEK293T cells (data not shown), as well as in HL-60 (data not shown), in order to compare the effects of BTN3A re-expression in both cells. Re-expression of either BTN3A1+BTN3A2, BTN3A1+BTN3A3 or BTN3A1+BTN3A2+ BTN3A3 provided the maximum reconstitution of Vγ9Vδ2-T cells degranulation under ZA stimulation, with only some significant differences found between the re-expression of BTN3A1+BTN3A2 and BTN3A1+BTN3A3 at the higher dose of ZA tested (20 μM) ($p<0.01$). These differences might be explained, at least in the case of HL-60 cells, by the higher transfection efficiency BTN3A1+BTN3A2 combination showed previous to co-culture assays (data not shown).

BTN3A1 reconstitution provided only a weak ability to reconstitute Vγ9Vδ2-T cells functions and only at the higher dose of ZA tested (20μM). This activation reached for BTN3A1+BTN3A2 or BTN3A1+BTN3A3 was not further increased when all the three BTN3A proteins are rescued at the same time, meaning that there is no additive effect in terms of Vγ9Vδ2-T cells activation when BTN3A2 and BTN3A3 are co-expressed. Furthermore, in both cells, BTN3A1+A2+A3 co-transfection induced Vγ9Vδ2-T cells degranulation at lower levels than BTN3A1+BTN3A2 reconstitution, although not significantly ($p>0.05$). Interestingly, transfection efficiency was comparable or even higher (for HL-60 cells) in BTN3A1+A2+A3 condition.

IFNγ and TNFα production by Vγ9Vδ2-T cells when challenged by different BNT3A clones in different cell lines was also measured in parallel to degranulation and trends were largely superimposable to those obtained when measuring CD107 (data not shown).

Similar BTN3A requirements for ZA and BrHPP-mediated Vγ9Vδ2-T cells activation

In parallel to co-culture experiments performed with ZA stimulation, BrHPP-induced BTN3A activation was also investigated. Bromohydrin pyrophosphate (BrHPP) is a synthetic compound, active at nanomolar concentrations similarly to natural phosphoantigens from MEP pathway (non-mevalonate pathway) of isoprenoid biosynthesis (24), as (E)-4-Hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP).

Generally, Vγ9Vδ2-T cells response to BTN3A KO clones (Figure 1 A and B-I, left side) under BrHPP stimulation follows the same criteria than IPP-boosted Vγ9Vδ2-T cells activation. Thus, A1, A2/A3 and triple KO clones didn't activate Vγ9Vδ2-T cells under BrHPP treatment. BTN3A2 KO clones also poorly activated Vγ9Vδ2-T cells at suboptimal concentrations. BTN3A2 and A3 abrogation inhibited Vγ9Vδ2-T cells degranulation compared to Mock cells.

Functional assays performed with triple KO cells transiently transfected with different BTN3A combinations (Figure 1 A-II and B-II, right side) yielded similar results to those obtained under ZA-stimulation. As it was seen before, BTN3A1+A2, BTN3A1+A3 and BTN3A1+A2+A3 combinations of BTN3A proteins, and in a lesser extent BTN3A1 alone, successfully activated Vγ9Vδ2-T cells when pre-incubated with BrHPP (Figure 1-II) in both HEK293T (3A-II) and HL-60 (3A-II). No overt benefit in terms of Vγ9Vδ2-T cells activation was observed when all the BTN3A proteins are re-introduced in cells compared to A1+A2, as well as A1+A3, double transfection. Moreover, in this case, double co-transfections (BTN3A1+BTN3A2 and BTN3A1+BTN3A3) significantly induced more degranulation than triple co-transfection, in HEK293T cells ($p<0.01$). In HL-60 cells, significant differences are found between BTN3A1+BTN3A2 and BTN3A1+BTN3A3 co-transfection at the higher dose of ZA tested (20 μM) ($p<0.01$).

Once again, and in the same line than experiments performed with ZA stimulation, BTN3A3 shows redundancy of function with BTN3A2, as it helps triggering Vγ9Vδ2-T cells activation similarly to BTN3A2 when co-expressed with BTN3A1, while absence of both, BTN3A2 and BTN3A3 proteins in target cells totally abrogates Vγ9Vδ2-T cells activation under BrHPP stimulation.

Its worth to note that BTN3A1 by itself can activate Vγ9Vδ2-T cells when ectopically expressed although weakly, since A2/A3 KO cells cannot trigger Vγ9Vδ2-T cells activation, as they don't express BTN3A at detectable levels in cell surface (data not shown).

20.1-mediated Vγ9Vδ2-T cells activation mainly depends upon BTN3A1 expression on target cells

During co-culture assays, we also challenged the ability of 20.1 BTN3A activating antibody to activate Vγ9Vδ2-T cells when one or more BTN3A proteins are lacking. Triple KO and A2/A3 KO cells were not able to activate Vγ9Vδ2-T cells when pre-incubated with 20.1 antibody, independently on the cell line tested. Interestingly, using HEK293T cells, 20.1 antibody stimulated although to a lesser extent, compared to HL-60 cells ($p=0.0003$).

In addition, BTN3A1 deletion reduced the most Vγ9Vδ2-T cells mediated IFNγ, TNFα production and cytolysis of HL-60 cells (data not shown). Regarding the relative relevance of each BTN3A paralog on 20.1-mediated Vγ9Vδ2-T cells activation, BTN3A2 and BTN3A3 expression display only limited implications on 20.1-mediated Vγ9Vδ2-T cells activation compared to BTN3A1, as differences in terms of Vγ9Vδ2-T cells activation were not significant compared to Mock in all the cell lines tested (data not shown).

Last confirmation of BTN3A1 predominant role on 20.1-mediated activation came from functional assays performed on HL-60 single KO clones reconstituted to re-express its missing isoform. There, a significant increase on Vγ9Vδ2-T cells degranulation (data not shown), which was not observed upon BTN3A2 and BTN3A3 reconstitution, became evident when BTN3A1 KO cells expressed again its missing protein.

Functional assays performed on HL-60 and HEK293T triple KO cells transiently transfected with all the possible combinations of BTN3A paralogs pointed out again the relevance of BTN3A2/A3 co-expression along with BTN3A1 in order to properly express the latter at cell surface (data not shown), as higher levels of BTN3A were found at membrane level when BTN3A1 was co-expressed along with other BTN3A proteins. Significant differences were found between Triple KO and A2+A3-transfected HL-60 cells, when targets were pre-incubated with 20.1, compared to 20.1-treated HL-60 triple KO cells (data not shown). Although critical for 20.1-mediated activation, BTN3A1 transfected cells activated Vγ9Vδ2-T cells to a lesser extent than the combination of BTN3A1+A2/A3. In the same way, percentage of BTN3A+ clones achieved when cell are transfected with BTN3A1 is the lowest among all the conditions, and is lower than those obtained when BTN3A1 is co-expressed with BTN3A2/A3. Then, we decided to compare transfection efficiency in targets with degranulation levels of Vγ9Vδ2-T cells when challenged with these targets (data not shown). Dot plots

evidence that combinations of BTN3A1 with BTN3A2, BTN3A3 or both reach the highest Vγ9Vδ2-T cells degranulation levels due to higher percentages of successfully transfected target cells. BTN3A1 reconstitution efficiently stimulates Vγ9Vδ2-T cells degranulation, given the low levels of BTN3A+ cells obtained during transfection. BTN3A3 reconstitution, among all single transfections, was the most effective to rescue Vγ9Vδ2-T cells degranulation upon 20.1 treatment on our studied cell models, since its expression levels are also the highest as measured by flow cytometry (data not shown) among all single transfections. BTN3A2 single transfection was the less efficient in terms of promoting Vγ9Vδ2-T cells degranulation, among all single transfection. Finally, co-culture assays performed with 20.1 BTN3A-activating mAb on BTN3A triple KO cells transiently re-expressing one or more BTN3A paralogs evidence that there is no additive effect between BTN3A2 and BTN3A3 to trigger Vγ9Vδ2-T cells activation (data not shown), as triple KO cells re-expressing BTN3A2+BTN3A3 (black dots) show no advantage in triggering Vγ9Vδ2-T cells degranulation compared to triple KO cells transiently expressing BTN3A3 alone. All These findings unveil the relevance of BTN3A1 as the most important BTN3A protein for 20.1-mediated Vγ9Vδ2-T cells stimulation.

BTN3A1 helps BTN3A2 and BTN3A3 to localize at cell membrane compartment

An additional experiment was performed in order to find out how BTN3A proteins are expressed at membrane level when concomitantly expressed with other BTN3A proteins. We first confirmed the data obtained by Vantourout et al. who previously reported BTN3A1 increased membrane localization when co-expressed with BTN3A2 (Vantourout et al., 2018) (data not shown). In addition, BTN3A1 surface expression was significantly enhanced when cells were transfected with the three BTN3A proteins although either BTN3A2 or BTN3A3 were sufficient to elicit maximum BTN3A1 expression (data not shown).

Interstingly, BTN3A1 expression increased the expression levels on the cell surface of BTN3A2 (data not shown) and BTN3A3 (data not shown). This enhancement in BTN3A2 and BTN3A3 membrane localization when co-expressed with BTN3A1 is lost when all the three proteins are co-expressed at the same time. All these experiments demonstrate that BTN3A2 and BTN3A3 expression at cell surface is enhanced by BTN3A1. They might also compete somehow for binding to BTN3A1 since BTN3A2/A3 increase in surface expression, when co-transfected along with BTN3A1, come back to normal (expression levels encountered in single transfection of BTN3A2/BTN3A3) when all the three proteins are co-expressed at the same time.

All BTN3A proteins interact between each other in Co-Immunoprecipitation assays

With the aim to better understand the complex interactions governing BTN3A biology, and taking in mind that others have reported BTN3A1 forming homo- and heterodimers, all the possible combinations of BTN3A proteins were transiently transfected in triple KO HEK93T cells.

Western blot analysis of co-immunoprecipitated proteins showed that all BTN3A proteins interact between each other (data not shown). Homotypic and heterotypic interactions were both found. Thus, BTN3A1-eGFP was pulled down along with BTN3A1-3xFlag, BTN3A2-3xFlag and BTN3A3-3xFlag, BTN3A2-eGFP did the same with BTN3A2-3xFlag and BTN3A3-3xFlag, finally BTN3A3- eGFP co-immunoprecipitated BTN3A3-3xFlag.

Then, endogenous BTN3A proteins were also challenged in order to see if we could obtain similar results when pulling-down endogenous BTN3A proteins that are usually expressed at much lower levels on physiological context than in cells transfected to ectopically express proteins. For this purpose, HL-60 BTN3A1 KO cells stably transduced with pMSCV-BTN3A1eGFP-PURO or EV retroviral particles to reconstitute BTN3A1 expression were subjected to protein extraction. Endogenous BTN3A2 and BTN3A3 co-immunoprecipitated with BTN3A1-eGFP.

Analysis of BTN3A homotypic and heterotypic interactions by Fluorescence Resonance Energy transfer (FRET) and Proximity Ligation Assay (PLA).

Next, the ability BTN3A proteins have to form homo and heterodimeric complexes between them was challenged by FRET. As previously seen in Co-IP experiments, all three BTN3A proteins interacted one to each other with different efficiencies (data not shown). Thus, BTN3A1 robustly co-localize with itself, as 84% of analysed cells showed BTN3A1 co-localization. BTN3A1 strongly interacts with BTN3A2 as well (72% positive cells). However only 32% of cells were positive when measuring BTN3A1+A3 co-localization. BTN3A2 strongly co-localize with itself (66% FRET-positive cells) and seems to be the strongest BTN3A3 interactor (36% of FRET-positive cells). Finally, BTN3A3 proteins poorly co-localize with the others and BTN3A3 homotypic interaction was weakest among all BTN3A3 interactions measured.

FRET analysis of BTN3A interactions upon N-BPs treatment was also performed (data not shown), but only modest non-significant increases in BTN3A1-A1 and BTN3A1-A3 co-localization were observed. The same experiment was also performed by PLA using N-BP treatments and, in this case, a significant increase was observed in A1-A1 as well as in A1-A3 co-localization. Thus, BTN3A interactions that are increased upon phosphoantigens burst,

compared to not-treated cells, would be those involving BTN3A1 and another BTN3A protein expressing a B30.2 domain.

We can conclude from co-localization studies that interactions between BTN3A members are by far more complex than expected initially, since all BTN3A proteins are known to interact and co-localize in cells.

REFERENCES:

Throughout this application, various references describe the state of the art to which this invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.

CLAIMS:

1. A compound selected from the group consisting of BTN3A2 agonist or BTN3A2 expression activator for use in the treatment of cancer in a subject in need thereof.

2. The compound for use according to claim 1, wherein the BTN3A2 agonist is selected from the group consisting of small organic molecule, polypeptide, or antibody.

3. The compound for use according to any of claims 1 or 2, wherein the cancer is acute myeloid leukemia.

4. The compound for use according to any of claims 1 or 2, wherein the cancer is pancreatic cancer.

5. A compound selected from the group consisting of BTN3A2 antagonist or BTN3A2 expression inhibitor for use in the treatment of an auto-immune disease.

6. A compound selected from the group consisting of BTN3A2 antagonist or BTN3A2 expression inhibitor for use in the treatment of an inflammatory condition.

7. The compound for use according to any of claims 1 or 2, wherein the BTN3A2 antagonist is selected from the group consisting of small organic molecule, a polypeptide, an aptamer or an antibody.

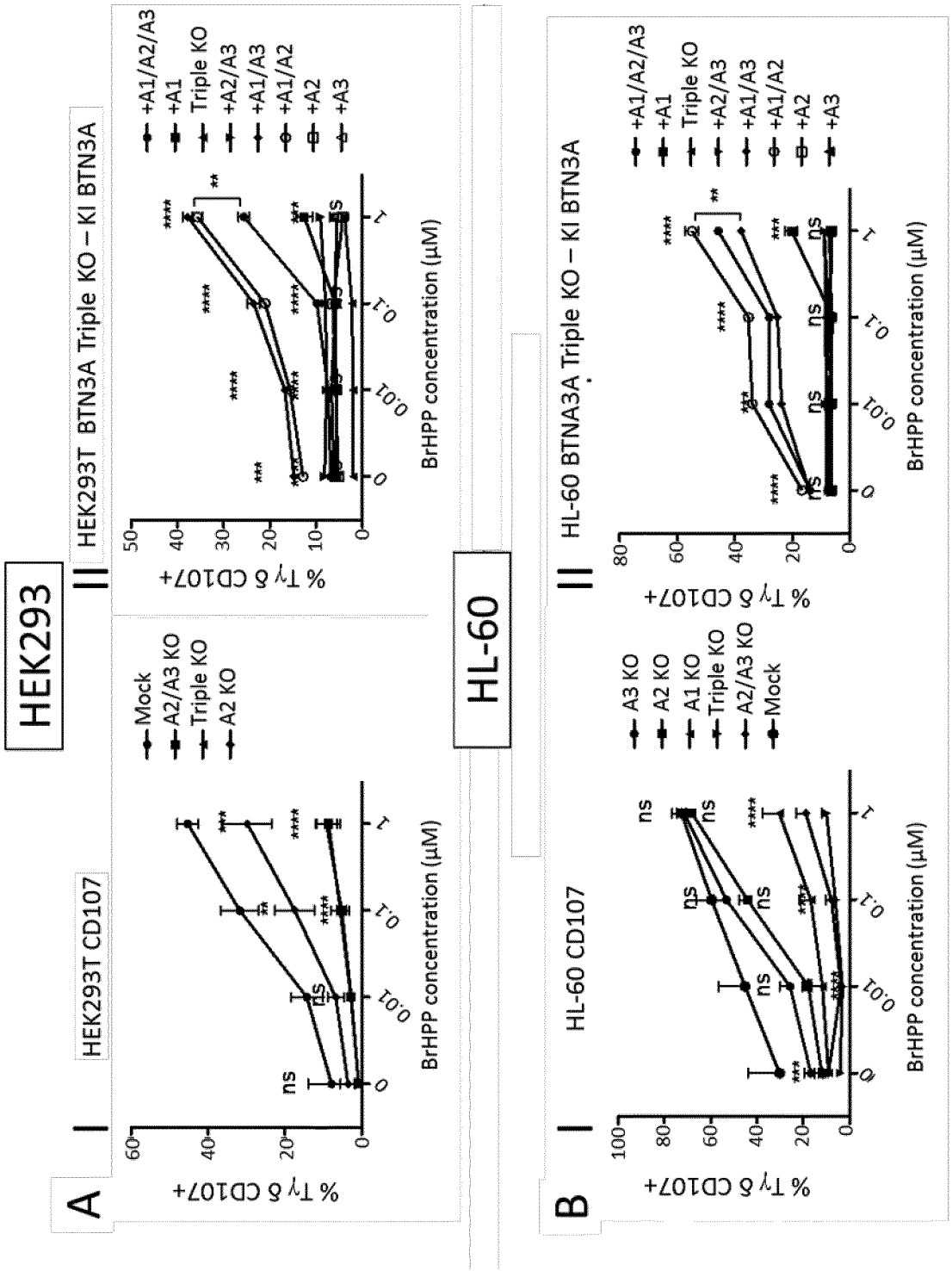
A
HEK293
HEK293T CD107
% Tγδ CD107+
BrHPP concentration (µM)
Mock
A2/A3 KO
Triple KO
A2 KO
HEK293T BTN3A Triple KO – KI BTN3A
+A1/A2/A3
+A1
Triple KO
+A2/A3
+A1/A3
+A1/A2
+A2
+A3
B
HL-60
HL-60 CD107
A3 KO
A2 KO
A1 KO
Triple KO
A2/A3 KO
Mock
HL-60 BTNA3A Triple KO – KI BTN3A

Figure 1A & 1B

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/061227

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/395 A61P35/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2015/353643 A1 (OLIVE DANIEL [FR] ET AL) 10 December 2015 (2015-12-10) page 1, paragraph 12 - page 2, paragraph 27 page 12, paragraph 148 - paragraph 149 claims 1-11 paragraph [0160] - paragraph [0164] -----	1-4
X	WO 2012/080351 A1 (INST NAT SANTE RECH MED [FR]; UNIV AIX MARSEILLE II [FR]; INST PAOLI C) 21 June 2012 (2012-06-21) page 3, line 25 - page 4, line 30 page 22, line 5 - line 14 claims 1-14 page 23, line 23 - page 24, line 21 page 51, line 23 - page 52, line 4 ----- -/--	1,2,4

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

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 August 2019	10/10/2019

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

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

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AUDREY BENYAMINE ET AL: "BTN3A molecules considerably improve V[gamma]9V[delta]2T cells-based immunotherapy in acute myeloid leukemia", ONCOIMMUNOLOGY, vol. 5, no. 10, 2 October 2016 (2016-10-02), pages e1146843-1, XP055433234, DOI: 10.1080/2162402X.2016.1146843 page 2, left-hand column, paragraph 2 - paragraph 3 page 8, left-hand column, paragraph 2 abstract -----	1-3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2019/061227

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-4

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-4

A compound selected from the group consisting of BTN3A2 agonist or BTN3A2 expression activator for use in the treatment of cancer in a subject in need thereof.

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2. claims: 5-7

A compound selected from the group consisting of BTN3A2 antagonist or BTN3A2 expression inhibitor for use in the treatment of an auto-immune disease or for use in the treatment of an inflammatory condition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2019/061227

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2015353643 A1	10-12-2015	US 2015353643 A1	10-12-2015
		US 2019201513 A1	04-07-2019
WO 2012080351 A1	21-06-2012	EP 2651441 A1	23-10-2013
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		US 2014322235 A1	30-10-2014
		WO 2012080351 A1	21-06-2012
		WO 2012080769 A1	21-06-2012