Anti-CD19 Antibody Formulations

Field of the Invention

The present disclosure is related to stable lyophilized pharmaceutical formulations of an anti-CD19 antibody and provides methods of making and methods of using such formulations.

Background

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

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

The human CD19 molecule is a structurally distinct cell surface receptor expressed on the surface of human B cells, including, but not limited to, pre-B cells, B cells in early development {i.e., immature B cells), mature B cells through terminal differentiation into plasma cells, and malignant B cells. CD 19 is expressed by most pre-B acute lymphoblastic leukemias (ALL), non-Hodgkin's lymphomas, B cell chronic lymphocytic leukemias (CLL), pro-lymphocytic leukemias, hairy cell leukemias, common acute lymphocytic leukemias, and some Null-acute lymphoblastic leukemias (Nadler et al, J. Immunol., 131 :244-250 (1983), Loken et al, Blood, 70:1316-1324 (1987), Uckun et al, Blood, 71 :13- 29 (1988), Anderson et al, 1984. Blood, 63:1424-1433 (1984), Scheuermann, Leuk. Lymphoma, 18:385-397(1995)). The expression of CD19 on plasma cells further suggests it may be expressed on differentiated B cell tumors such as multiple myeloma, plasmacytomas, Waldenstrom's tumors (Grossbard et al., Br. J. Haematol, 102:509- 15(1998); Treon et al, Semin. Oncol, 30:248-52(2003)).

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

MOR208 (previously named XmAb5574) is an Fc engineered humanized monoclonal antibody that binds CD19. The increase in binding of MOR208 Fc to FcγR, due to XmAb engineered mutations, significantly enhances in-vitro antibody dependent cell-mediated cytotoxicity (ADCC), antibody dependent cell-mediated phagocytosis (ADCP), and direct cytotoxic effects (apoptosis) on tumor relative to the unmodified antibody. MOR208 has not been shown to mediate complement dependent cytotoxicity.

MOR208 has or is currently being studied in clinical trials in CLL, ALL and NHL. Specifically, a Phase I trial titled Safety and Tolerability of XmAb®5574 in Chronic Lymphocytic Leukemia, and a Phase IIa trial titled Study of Fc-Optimized Anti-CD19 Antibody (MOR208) to treat B-cell Acute Lymphoblastic Leukemia (B-ALL) are completed. A Phase IIa trial titled Study of Fc-Optimized Anti-CD19 Antibody (MOR208) to Treat Non-Hodgkin´s Lymphoma (NHL) has completed recruitment. And the following trials are ongoing: a Phase II/III trial titled A Trial to Evaluate the Efficacy and Safety of MOR208 With Bendamustine (BEN) Versus Rituximab (RTX) With BEN in Adult Patients With Relapsed or Refractory Diffuse Large B-cel Lymphoma (DLBCL) (B-MIND), a Phase II trial titled Study to Evaluate Efficacy and Safety of MOR208 With Idelalisib in R/R CLL/SLL Patients Pretreated With BTKi, a Phase II trial titled A Study to Evaluate the Safety and Efficacy of Lenalidomide With MOR208 in Patients With R-R DLBCL, and a Phase II trial titled Phase II MOR208 in Combination With Lenalidomide for Patients With Relapsed or Refractory CLL, SLL or PLL or Older Patients With Untreated CLL, SLL or PLL.

Therapeutic antibodies and antibody fragments are large and more complex molecules than traditional organic and inorganic drugs small molecules as antibodies possess multiple functional groups in addition to complex three-dimensional structures and, therefore, the formulation of such proteins poses special challenges. For a protein to remain biologically active, a formulation must preserve the conformational integrity of at least a core sequence of the protein's amino acids while at the same time protecting the protein's multiple functional groups from degradation. Formulations of antibodies may have short shelf lives and the formulated antibodies may lose biological activity resulting from chemical and physical instabilities during storage. The three most common pathways for protein degradation are protein aggregation,

deamidation and oxidation (Cleland et al., Critical Reviews in Therapeutic Drug Carrier Systems 10(4): 307-377 (1993)). In particular, aggregation can potentially lead to increased immune response in patients, leading to safety concerns and must be minimised or prevented.

Summary of the Invention

It is an object of the invention to provide formulations of anti-CD19 antibodies, and in particular formulations of anti-CD19 antibodies having a suitable shelf life.

Suitable formulations for therapeutic antibodies can be an aqueous pharmaceutical composition or a lyophilisate which can be reconstituted to provide a solution for administration to a patient.

Provided herein are lyophilized pharmaceutical formulations comprising an antibody. In an aspect, the formulation comprises an anti-CD19 antibody, a buffer, sucrose, and a surfactant, wherein the formulation has a pH of about 6.0 wherein said anti-CD19 antibody comprises a heavy chain constant domain of the sequence
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPDVFLFPPKPK
DTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQD
WLNGKEYKCKVSNKALPAPEEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK (SEQ ID NO: 8) and a light chain constant domain of the sequence
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC. (SEQ ID NO: 9).

In one aspect, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of about 20 mg/ml to about 125 mg/ml, a buffer, polysorbate in a concentration of about 0.005% (w/v) to about 0.06% (w/v), and pH of about 6.0, wherein the formulation further comprises a) trehalose in a concentration of about 180 mM to about 240 mM
or
b) Mannitol in a concentration of about 180 mM to about 240 mM and Sucrose in a concentration of about 10 mM to about 50mM.

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

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

In some embodiments, said anti-CD19 antibody comprises a variable heavy chain of the sequence
EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGTKYNEKF
QGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWGQGTLVTVSS
(SEQ ID NO: 10) and a variable light chain of the sequence
DIVMTQSPATLSLSPGERATLSCRSSKSLQNVNGNTYLYWFQQKPGQSPQLLIYRMSNLNSGVPD
RFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPITFGAGTKLEIK (SEQ ID NO: 11).

In some embodiments, said anti-CD19 antibody comprises a heavy chain constant domain of the sequence
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPDVFLFPPKPK
DTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQD
WLNGKEYKCKVSNKALPAPEEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK (SEQ ID NO: 8) and a light chain constant domain of the sequence
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC. (SEQ ID NO: 9).

In some embodiments, the anti-CD19 antibody in the formulation has a concentration of about 20 mg/ml to about 80 mg/ml. In some embodiments, the anti-CD19 antibody in the formulation has a

concentration of about 40 mg/ml.

In some embodiments, said buffer in the formulation is citrate buffer or phosphate buffer.

In some embodiments, said trehalose in the formulation is about 200 mM. In some embodiments, said trehalose in the formulation is 200 mM.

In some embodiments, said Mannitol in the formulation is about 219 mM and Sucrose in the formulation is about 29 mM. In some embodiments, said Mannitol in the formulation is 219 mM and Sucrose in the formulation is 29 mM.

In some embodiments, the formulation has a pH of about 6.0. In some embodiments, the formulation has a pH of 6.0.

In some embodiments, said polysorbate in the formulation is polysorbate 20. In some embodiments, said polysorbate (e.g., polysorbate 20) in the formulation is of about 0.005% (w/v) to about 0.06% (w/v). In some embodiments, said polysorbate (e.g., polysorbate 20) in the formulation is about 0.02% (w/v). In some embodiments, said polysorbate (e.g., polysorbate 20) in the formulation is 0.02% (w/v).

In a further aspect, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of about 40 mg/ml, citrate in a concentration of about 25 mM, trehalose in a concentration of about 200 mM, polysorbate in a concentration of about 0.02% (w/v), and a pH of about 6.0.

In a further aspect, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of 40 mg/ml, citrate in a concentration of 25 mM, trehalose in a concentration of 200 mM, polysorbate in a concentration of 0.02% (w/v), and a pH of 6.0.

In a further aspect, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of about 40 mg/ml, citrate in a concentration of about 25 mM, Mannitol in a concentration of about 219 mM, Sucrose in a concentration of about 29 mM, polysorbate in a concentration of about 0.02% (w/v), and a pH of about 6.0.

In a further aspect, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of 40 mg/ml, citrate in a concentration of 25 mM, Mannitol in a concentration of 219 mM, Sucrose in a concentration of 29 mM, polysorbate in a concentration of 0.02% (w/v), and a pH of 6.0.

In some embodiments, said anti-CD19 antibody in the formulation is not subject to prior lyophilization., e.g. is liquid In some embodiments, said anti-CD19 antibody in the formulation is a monoclonal antibody. In some embodiments, said anti-CD19 antibody in the formulation is a full length antibody. In some embodiments, said anti-CD19 antibody in the formulation is an IgG antibody. In some embodiments, said anti-CD19 antibody in the formulation is a humanized or a human antibody. In some embodiments, said anti-CD19 antibody in the formulation is an antibody fragment comprising an antigen-binding region. In some embodiments, the antibody fragment is a Fab or F(ab')2 fragment.

Description of Drawings

Figure 1: Provided are the amino acid sequences of MOR208.

Figure 2: Subvisible particle (SVP) count of MOR208 after 3 months at 40℃. Formulation 3 and Formulation 9 were compared and the mannitol/sucrose formulation generated more particles over time especially in the range between 2 µm and 1000 µm.

Detailed description of the invention

The term **"antibody"** means monoclonal antibodies, including any isotype, such as, IgG, IgM, IgA, IgD and IgE. An IgG antibody is comprised of two identical heavy chains and two identical light chains that are joined by disulfide bonds. Each heavy and light chain contains a constant region and a variable region. Each variable region contains three segments called "complementarity-determining regions" ("CDRs") or "hypervariable regions", which are primarily responsible for binding an epitope of an antigen. They are referred to as CDR1, CDR2, and CDR3, numbered sequentially from the N-terminus. The more highly conserved portions of the variable regions outside of the CDRs are called the "framework regions". An “antibody fragment” means an Fv, scFv, dsFv, Fab, Fab' F(ab')2

fragment, or other fragment, which contains at least one variable heavy or variable light chain, each containing CDRs and framework regions.

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

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

Human CD19 has the amino acid sequence of:

MPPPRLLFFLLFLTPMEVRPEEPLVVKVEEGDNAVLQCLKGTSDGPTQQLTWSRESPLKPFLKLSL
GLPGLGIHMRPLAIWLFIFNVSQQMGGFYLCQPGPPSEKAWQPGWTVNVEGSGELFRWNVSDLG
GLGCGLKNRSSEGPSSPSGKLMSPKLYVWAKDRPEIWEGEPPCLPPRDSLNQSLSQDLTMAPGS
TLWLSCGVPPDSVSRGPLSWTHVHPKGPKSLLSLELKDDRPARDMWVMETGLLLPRATAQDAGK
YYCHRGNLTMSFHLEITARPVLWHWLLRTGGWKVSAVTLAYLIFCLCSLVGILHLQRALVLRRKRK
RMTDPTRRFFKVTPPPGSGPQNQYGNVLSLPTPTSGLGRAQRWAAGLGGTAPSYGNPSSDVQA
DGALGSRSPPGVGPEEEEGEGYEEPDSEEDSEFYENDSNLGQDQLSQDGSGYENPEDEPLGPE
DEDSFSNAESYENEDEELTQPVARTMDFLSPHGSAWDPSREATSLGSQSYEDMRGILYAAPQLR
SIRGQPGPNHEEDADSYENMDNPDGPDPAWGGGGRMGTWSTR. (SEQ ID NO: 7)

"MOR208" is an anti-CD19 antibody. The amino acid sequence of the variable domains is provided in Figure 1. The amino acid sequence of the heavy and light chain Fc regions of MOR208 are provided in Figure 1. "MOR208" and "XmAb 5574" are used as synonyms to describe the antibody shown in Figure 1. The MOR208 antibody is described in US patent application serial number 12/377,251, which is incorporated by reference in its entirety.

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

In addition further antibodies specific for CD19 are described in WO2005012493 (US7109304), WO2010053716 (US12/266,999) (Immunomedics); WO2007002223 (US

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

The term **"pharmaceutical formulation"** refers to a preparation for administration to subjects. Such subjects may be humans.

A **"stable"** formulation is one that can be administered to patients after storage. In aspects, the formulation essentially retains its physical and chemical properties , as well as its biological activity upon storage. Various analytical techniques for measuring protein stability are available in the art and are reviewed in Peptide and Protein Drug Delivery, 247-301, Vincent Lee Ed., Marcel Dekker, Inc., New York, N.Y., Pubs. (1991) and Jones, A. Adv. Drug Delivery Rev. 10: 29-90 (1993), for example.

Stability can be evaluated qualitatively and/or quantitatively in a variety of different ways, including evaluation of aggregate formation (for example using size exclusion chromatography, by measuring turbidity, and/or by visual inspection); by assessing charge heterogeneity using ion exchange chromatography (IEC), size exclusion chromatography (HP-SEC), SDS-PAGE analysis to compare reduced and intact antibody; evaluating biological activity or antigen binding function of the antibody; etc. Instability may involve any one or more of: aggregation, deamidation (e.g. Asn deamidation), oxidation (e.g. Met oxidation), isomerization (e.g. Asp isomerization), clipping/hydrolysis/fragmentation (e.g. hinge region fragmentation), succinimide formation, unpaired cysteine(s), N-terminal extension, C-terminal processing, glycosylation differences, etc..

As used herein, **"biological activity"** of a monoclonal antibody refers to the ability of the antibody to bind to antigen. It can further include antibody binding to antigen and resulting in a measurable biological response which can be measured in vitro or in vivo.

As used herein, **"buffer"** refers to a buffered solution that resists changes in pH by the action of its acid-base conjugate components. The buffer of this invention preferably has a pH in the range

from about 4.5 to about 7.0, preferably from about 5.6 to about 7.0. In one embodiment the buffer has a pH of about 6.0 or a pH of 6.0. For example, a citrate buffer or a phosphate buffer are each an example of buffers that will control the pH in this range.

As used herein, a **"surfactant"** refers to a surface-active agent. Preferably the surfactant is a nonionic surfactant. Examples of surfactants herein include polysorbate (for example, polysorbate 20 and, polysorbate 80); poloxamer (e.g. poloxamer 188); Triton; sodium dodecyl sulfate (SDS); sodium laurel sulfate; sodium octyl glycoside; lauryl-, myristyl-, linoleyl-, or stearyl-sulfobetaine; lauryl-, myristyl-, linoleyl- or stearyl-sarcosine; linoleyl-, myristyl-, or cetyl-betaine; lauroamidopropyl-, cocamidopropyl-, linoleamidopropyl-, myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-betaine (e.g. lauroamidopropyl); myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-dimethylamine; sodium methyl cocoyl-, or disodium methyl oleyl-taurate; and the MONAQUAT™ series (Mona Industries, Inc., Paterson, N.J.); polyethyl glycol, polypropyl glycol, and copolymers of ethylene and propylene glycol (e.g. Pluronics, PF68 etc.); etc. In one embodiment, the surfactant herein is polysorbate 20.

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

"**Administered**" or "**administration**" includes but is not limited to delivery by an injectable form, such as, for example, an intravenous, intramuscular, intradermal or subcutaneous route or mucosal route, for example, as a nasal spray or aerosol for inhalation or as an ingestable solution, capsule or tablet.

A "**therapeutically effective amount**" of a compound or combination refers to an amount sufficient to at least partially arrest the clinical manifestations of a given disease or disorder and its complications. The amount that is effective for a particular therapeutic purpose will depend on the severity of the disease or injury as well as on the weight and general state of the subject. It will be understood that determination of an appropriate dosage may be achieved, using routine experimentation, by constructing a matrix of values and testing different points in the matrix, all of which is within the ordinary skills of a trained physician or clinical scientist.

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

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

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

"Binds the same epitope as" means the ability of an antibody or other binding agent to bind to CD19 and having the same epitope as the exemplified antibody. The epitopes of the exemplified antibody and other antibodies to CD19 can be determined using standard epitope mapping techniques. Epitope mapping techniques, well known in the art include Epitope Mapping Protocols in Methods in Molecular Biology, Vol. 66 (Glenn E.Morris, Ed., 1996) Humana Press, Totowa, New

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

Embodiments

In another embodiment, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of about 20 mg/ml to about 125 mg/ml, a buffer, polysorbate in a concentration of about 0.005% (w/v) to about 0.06% (w/v), and pH of about 6.0, wherein the formulation further comprises a) trehalose in a concentration of about 180 mM to about 240 mM

or

b) Mannitol in a concentration of about 180 mM to about 240 mM and Sucrose in a concentration of about 10 mM to about 50mM.

In another embodiment, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of about 20 mg/ml to about 125 mg/ml, a citrate buffer or phosphate buffer, polysorbate in a concentration of about 0.005% (w/v) to about 0,06% (w/v), and pH of about 6.0, wherein the formulation further comprises a) trehalose in a concentration of about 180 mM to about 240 mM

or

b) Mannitol in a concentration of about 180 mM to about 240 mM and Sucrose in a concentration of about 10 mM to about 50mM.

In some embodiments, said buffer in the formulation is in a concentration of 10 to 75 mM. In some embodiments, said buffer in the formulation is in a concentration of 20 to 50 mM. In some embodiments, said buffer in the formulation is in a concentration of 20 to 40 mM. In some embodiments, said buffer in the formulation is in a concentration of 20 to 30 mM. In some embodiments, said buffer in the formulation is in a concentration of about 25 mM. In some embodiments, said buffer in the formulation is in a concentration of 25 mM. In some embodiments, said buffer in the formulation is citrate buffer or phosphate buffer.

In some embodiments, said citrate buffer in the formulation is in a concentration of of 10 to 75 mM. In some embodiments, said citrate buffer in the formulation is in a concentration of 20 to 50 mM. In some embodiments, said citrate buffer in the formulation is in a concentration of 20 to 30 mM. In some embodiments, said citrate buffer in the formulation is in a concentration of about 25 mM. In some embodiments, said citrate buffer in the formulation is in a concentration of 25 mM.

In some embodiments, said phosphate buffer in the formulation is in a concentration of of 10 to 75 mM. In some embodiments, said phosphate buffer in the formulation is in a concentration of of 20 to 50 mM. In some embodiments, said phosphate buffer in the formulation is in a concentration of 20 to 30 mM. In some embodiments, said phosphate buffer in the formulation is in a concentration of about 25 mM. In some embodiments, said phosphate buffer in the formulation is in a concentration of 25 mM.

In another embodiment, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of about 20 mg/ml to about 125 mg/ml, a citrate buffer or phosphate buffer in a concentration of of 20 to 50 mM, polysorbate in a concentration of about 0.005% (w/v) to about 0,06% (w/v), and pH of about 6.0, wherein the formulation further comprises a) trehalose in a concentration of about 180 mM to about 240 mM
or
b) Mannitol in a concentration of about 180 mM to about 240 mM and Sucrose in a concentration of about 10 mM to about 50mM.

In another embodiment, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of about 20 mg/ml to about 125 mg/ml, a citrate buffer or phosphate buffer in a concentration of about 25 mM, polysorbate in a concentration of about 0.005% (w/v) to about 0,06% (w/v), and pH of about 6.0, wherein the formulation further comprises a) trehalose in a concentration of about 180 mM to about 240 mM

or

b) Mannitol in a concentration of about 180 mM to about 240 mM and Sucrose in a concentration of about 10 mM to about 50mM.

In another embodiment, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of about 20 mg/ml to about 125 mg/ml, a citrate buffer in a concentration of about 25 mM, polysorbate in a concentration of about 0.005% (w/v) to about 0,06% (w/v), and pH of about 6.0, wherein the formulation further comprises

a) trehalose in a concentration of about 180 mM to about 240 mM

or

b) Mannitol in a concentration of about 180 mM to about 240 mM and Sucrose in a concentration of about 10 mM to about 50mM.

In another embodiment, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of about 20 mg/ml to about 125 mg/ml, a phosphate buffer in a concentration of about 25 mM, polysorbate in a concentration of about 0.005% (w/v) to about 0,06% (w/v), and pH of about 6.0, wherein the formulation further comprises

a) trehalose in a concentration of about 180 mM to about 240 mM

or

b) Mannitol in a concentration of about 180 mM to about 240 mM and Sucrose in a concentration of about 10 mM to about 50mM.

In a further embodiment said anti-CD19 antibody is a full length antibody. In a further embodiment said anti-CD19 antibody is an IgGI, an IgG2, an IgG3 or an IgG4 antibody.

In another embodiment said anti-CD19 antibody is a humanized or a human antibody. In a further embodiment said anti-CD19 antibody is an antibody fragment comprising an antigen-binding region. In a further embodiment said antibody fragment is a Fab or F(ab')2 fragment.

In another embodiment said stable lyophilized pharmaceutical formulation is stable at 2-8℃ for at least 6 months, at least 12 months, at least 18 months, at least 24 months or at least 36 months.

In another embodiment said stable lyophilized pharmaceutical formulation is stable at about 40℃ for at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 or more weeks. In certain embodiments, said stable lyophilized pharmaceutical formulation is stable at about 40℃ for at least

about 1, 2, 3, 4, 5 or more months. In certain embodiments, said stable lyophilized pharmaceutical formulation is stable at about 25℃ for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or more months. In certain embodiments, said stable lyophilized pharmaceutical formulation is stable at about 5℃ for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, or more months. In certain embodiments, said stable lyophilized pharmaceutical formulation is stable at about 5+/-3 ℃ for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, or more months. In certain embodiments, said stable lyophilized pharmaceutical formulation is stable at about -20℃ for at least 1, 2, 3, 4, 5 , 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, or more months. In certain embodiments, said stable lyophilized pharmaceutical formulation is stable at 5℃ or -20℃ for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, or more months.

In a further embodiment the anti-CD19 antibody in the formulation retains at least 60%, at least 70%, at least 80%, at least 90%, at least 95% of its biological activity after storage. In some embodiment the biological activity is measured by antibody binding to CD19. In some embodiment the biological activity is measured by antibody binding to CD19 in a FACS CD19 binding assay. In some embodiment the biological activity is measured by ADCC activity of said anti-CD19 antibody. In some embodiment stable lyophilized pharmaceutical formulation is sterile.

In a further embodiment said stable lyophilized pharmaceutical formulation is suitable to be administered to a subject. In a further embodiment said said stable lyophilized pharmaceutical formulation is suitable for intravenous (IV) administration or subcutaneous administration.

In another aspect, provided herein is an article of manufacture comprising a container holding the stable lyophilized pharmaceutical formulation as disclosed herein. In an embodiment said container is a glass vial or a metal alloy container. In a further embodiment the metal alloy is 316L stainless steel or hastelloy.

In another aspect, provided herein is method of treating a disease or disorder in a subject comprising administering an effective amount of the formulation disclosed herein to the subject, wherein the disease or disorder is cancer. In another aspect, provided herein is the use of the stable

lyophilized pharmaceutical formulation as disclosed herein for the treatment of a disease or disorder in a subject comprising administering an effective amount of said formulation to the subject, wherein the disease or disorder is cancer. In a further aspect, provided herein is the use of the stable lyophilized pharmaceutical formulation as disclosed herein for the manufacture of a medicament for the treatment of a disease or disorder in a subject comprising administering an effective amount of said formulation to the subject, wherein the disease or disorder is cancer. In an embodiment the disease or disorder is non-Hodgkin's lymphoma (including each the subtypes described herein), chronic lymphocytic leukemia and/or acute lymphoblastic leukemia. In embodiments, the non-Hodgkin's lymphoma is selected from the group consisting of follicular lymphoma, small lymphocytic lymphoma, mucosa-associated lymphoid tissue, marginal zone, diffuse large B cell, Burkitt's, and mantle cell.

In some embodiments, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of about 20 mg/ml to about 125 mg/ml, a citrate buffer in a concentration of about 25 mM, polysorbate in a concentration of about 0.005% (w/v) to about 0,06% (w/v), and pH of about 6.0, wherein the formulation further comprises
a) trehalose in a concentration of about 180 mM to about 240 mM
or
b) Mannitol in a concentration of about 180 mM to about 240 mM and Sucrose in a concentration of about 10 mM to about 50mM,
wherein said anti-CD19 antibody comprises an HCDR1 region of sequence SYVMH (SEQ ID NO: 1), an HCDR2 region of sequence NPYNDG (SEQ ID NO: 2), an HCDR3 region of sequence GTYYYGTRVFDY (SEQ ID NO: 3), an LCDR1 region of sequence RSSKSLQNVNGNTYLY (SEQ ID NO: 4), an LCDR2 region of sequence RMSNLNS (SEQ ID NO: 5), and an LCDR3 region of sequence MQHLEYPIT (SEQ ID NO: 6).

In some embodiments, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of about 20 mg/ml to about 125 mg/ml, a citrate buffer in a concentration of about 25 mM, polysorbate in a concentration of about 0.005% (w/v) to about 0,06% (w/v), and pH of about 6.0, wherein the formulation further comprises
a) trehalose in a concentration of about 180 mM to about 240 mM
or
b) Mannitol in a concentration of about 180 mM to about 240 mM and Sucrose in a concentration of about 10 mM to about 50mM, wherein said anti-CD19 antibody comprises a variable heavy chain of the sequence

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGTKYNEKF QGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWGQGTLVTVSS (SEQ ID NO: 10) and a variable light chain of the sequence DIVMTQSPATLSLSPGERATLSCRSSKSLQNVNGNTYLYWFQQKPGQSPQLLIYRMSNLNSGVPD RFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPITFGAGTKLEIK (SEQ ID NO: 11).

In some embodiments, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of about 20 mg/ml to about 125 mg/ml, a citrate buffer in a concentration of about 25 mM, polysorbate in a concentration of about 0.005% (w/v) to about 0,06% (w/v), and pH of about 6.0, wherein the formulation further comprises

a) trehalose in a concentration of about 180 mM to about 240 mM

or

b) Mannitol in a concentration of about 180 mM to about 240 mM and Sucrose in a concentration of about 10 mM to about 50mM, wherein said anti-CD19 antibody comprises a heavy chain constant domain of the sequence ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPDVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQD WLNGKEYKCKVSNKALPAPEEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK (SEQ ID NO: 8) and a light chain constant domain of the sequence RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC. (SEQ ID NO: 9).

In some embodiments, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of about 40 mg/ml, a citrate buffer in a concentration of about 25 mM, polysorbate in a concentration of about 0.02% (w/v), and pH of about 6.0, wherein the formulation further comprises

a) trehalose in a concentration of about 200 mM

or

b) Mannitol in a concentration of about 219 mM and Sucrose in a concentration of about 29mM, wherein said anti-CD19 antibody comprises an HCDR1 region of sequence SYVMH (SEQ ID NO: 1), an HCDR2 region of sequence NPYNDG (SEQ ID NO: 2), an HCDR3 region of sequence GTYYYGTRVFDY (SEQ ID NO: 3), an LCDR1 region of sequence RSSKSLQNVNGNTYLY (SEQ ID NO: 4), an LCDR2 region of sequence RMSNLNS (SEQ

ID NO: 5), and an LCDR3 region of sequence MQHLEYPIT (SEQ ID NO: 6).

In some embodiments, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of about 40 mg/ml, a citrate buffer in a concentration of about 25 mM, polysorbate in a concentration of about 0.02% (w/v), and pH of about 6.0, wherein the formulation further comprises

a) trehalose in a concentration of about 200 mM

or

b) Mannitol in a concentration of about 219 mM and Sucrose in a concentration of about 29mM, wherein said anti-CD19 antibody comprises a variable heavy chain of the sequence EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGTKYNEKF QGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWGQGTLVTVSS (SEQ ID NO: 10) and a variable light chain of the sequence DIVMTQSPATLSLSPGERATLSCRSSKSLQNVNGNTYLYWFQQKPGQSPQLLIYRMSNLNSGVPD RFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPITFGAGTKLEIK (SEQ ID NO: 11).

In some embodiments, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of about 40 mg/ml, a citrate buffer in a concentration of about 25 mM, polysorbate in a concentration of about 0.02% (w/v), and pH of about 6.0, wherein the formulation further comprises

a) trehalose in a concentration of about 200 mM

or

b) Mannitol in a concentration of about 219 mM and Sucrose in a concentration of about 29mM, wherein said anti-CD19 antibody comprises a heavy chain constant domain of the sequence ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPDVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQD WLNGKEYKCKVSNKALPAPEEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK (SEQ ID NO: 8) and a light chain constant domain of the sequence RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC. (SEQ ID NO: 9).

In some embodiments, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of about 40 mg/ml, a citrate buffer

in a concentration of about 25 mM, polysorbate in a concentration of about 0.02% (w/v), and pH of about 6.0, wherein the formulation further comprises

a) trehalose in a concentration of about 200 mM

or

b) Mannitol in a concentration of about 219 mM and Sucrose in a concentration of about 29mM,

wherein said anti-CD19 antibody comprises a variable heavy chain having at least 85%, 86%, 87%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the sequence EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGTKYNEKF QGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWGQGTLVTVSS (SEQ ID NO: 10) and a variable light chain having at least 85%, 86%, 87%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the sequence DIVMTQSPATLSLSPGERATLSCRSSKSLQNVNGNTYLYWFQQKPGQSPQLLIYRMSNLNSGVPD RFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPITFGAGTKLEIK (SEQ ID NO: 11).

In some embodiments, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of about 40 mg/ml, a citrate buffer in a concentration of about 25 mM, polysorbate in a concentration of about 0.02% (w/v), and pH of about 6.0, wherein the formulation further comprises

a) trehalose in a concentration of about 200 mM

or

b) Mannitol in a concentration of about 219 mM and Sucrose in a concentration of about 29mM,

wherein said anti-CD19 antibody comprises a heavy chain constant domain having at least 85%, 86%, 87%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the sequence ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPDVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQD WLNGKEYKCKVSNKALPAPEEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK (SEQ ID NO: 8) and a light chain constant domain having at least 85%, 86%, 87%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the sequence RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC. (SEQ ID NO: 9).

In some embodiments, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of about 40 mg/ml, a citrate buffer

in a concentration of about 25 mM, polysorbate in a concentration of about 0.02% (w/v), and pH of about 6.0, wherein the formulation further comprises trehalose in a concentration of about 200 mM, wherein said anti-CD19 antibody comprises an HCDR1 region of sequence SYVMH (SEQ ID NO: 1), an HCDR2 region of sequence NPYNDG (SEQ ID NO: 2), an HCDR3 region of sequence GTYYYGTRVFDY (SEQ ID NO: 3), an LCDR1 region of sequence RSSKSLQNVNGNTYLY (SEQ ID NO: 4), an LCDR2 region of sequence RMSNLNS (SEQ ID NO: 5), and an LCDR3 region of sequence MQHLEYPIT (SEQ ID NO: 6).

In some embodiments, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of about 40 mg/ml, a citrate buffer in a concentration of about 25 mM, polysorbate in a concentration of about 0.02% (w/v), and pH of about 6.0, wherein the formulation further comprises Mannitol in a concentration of about 219 mM and Sucrose in a concentration of about 29mM, wherein said anti-CD19 antibody comprises an HCDR1 region of sequence SYVMH (SEQ ID NO: 1), an HCDR2 region of sequence NPYNDG (SEQ ID NO: 2), an HCDR3 region of sequence GTYYYGTRVFDY (SEQ ID NO: 3), an LCDR1 region of sequence RSSKSLQNVNGNTYLY (SEQ ID NO: 4), an LCDR2 region of sequence RMSNLNS (SEQ ID NO: 5), and an LCDR3 region of sequence MQHLEYPIT (SEQ ID NO: 6).

In some embodiments, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of about 40 mg/ml, a citrate buffer in a concentration of about 25 mM, polysorbate in a concentration of about 0.02% (w/v), and pH of about 6.0, wherein the formulation further comprises trehalose in a concentration of about 200 mM wherein said anti-CD19 antibody comprises a variable heavy chain of the sequence EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGTKYNEKF QGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWGQGTLVTVSS (SEQ ID NO: 10) and a variable light chain of the sequence DIVMTQSPATLSLSPGERATLSCRSSKSLQNVNGNTYLYWFQQKPGQSPQLLIYRMSNLNSGVPD RFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPITFGAGTKLEIK (SEQ ID NO: 11).

In some embodiments, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of about 40 mg/ml, a citrate buffer in a concentration of about 25 mM, polysorbate in a concentration of about 0.02% (w/v), and pH of about 6.0, wherein the formulation further comprises Mannitol in a concentration of about 219 mM and Sucrose in a concentration of about 29mM, wherein said anti-CD19 antibody comprises a variable heavy chain of the sequence

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGTKYNEKF
QGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWGQGTLVTVSS
(SEQ ID NO: 10) and a variable light chain of the sequence
DIVMTQSPATLSLSPGERATLSCRSSKSLQNVNGNTYLYWFQQKPGQSPQLLIYRMSNLNSGVPD
RFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPITFGAGTKLEIK (SEQ ID NO: 11).

In some embodiments, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of about 40 mg/ml, a citrate buffer in a concentration of about 25 mM, polysorbate in a concentration of about 0.02% (w/v), and pH of about 6.0, wherein the formulation further comprises trehalose in a concentration of about 200 mM, wherein said anti-CD19 antibody comprises a heavy chain constant domain of the sequence
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPDVFLFPPKPK
DTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQD
WLNGKEYKCKVSNKALPAPEEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK (SEQ ID NO: 8) and a light chain constant domain of the sequence
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC. (SEQ ID NO: 9).

In some embodiments, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of about 40 mg/ml, a citrate buffer in a concentration of about 25 mM, polysorbate in a concentration of about 0.02% (w/v), and pH of about 6.0, wherein the formulation further comprises Mannitol in a concentration of about 219 mM and Sucrose in a concentration of about 29mM, wherein said anti-CD19 antibody comprises a heavy chain constant domain of the sequence
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPDVFLFPPKPK
DTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQD
WLNGKEYKCKVSNKALPAPEEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK (SEQ ID NO: 8) and a light chain constant domain of the sequence
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC. (SEQ ID NO: 9).

In some embodiments, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of about 40 mg/ml, a phosphate buffer in a concentration of 20 to 40 mM, polysorbate in a concentration of about 0.02% (w/v), and pH of about 6.0, wherein the formulation further comprises trehalose in a concentration of about 200 mM, wherein said anti-CD19 antibody comprises a heavy chain constant domain of the sequence
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPDVFLFPPKPK
DTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQD
WLNGKEYKCKVSNKALPAPEEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK (SEQ ID NO: 8) and a light chain constant domain of the sequence
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC. (SEQ ID NO: 9).

In some embodiments, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of about 40 mg/ml, a phosphate buffer in a concentration of 20 to 40 mM, polysorbate in a concentration of about 0.02% (w/v), and pH of about 6.0, wherein the formulation further comprises Mannitol in a concentration of about 219 mM and Sucrose in a concentration of about 29mM, wherein said anti-CD19 antibody comprises a heavy chain constant domain of the sequence
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPDVFLFPPKPK
DTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQD
WLNGKEYKCKVSNKALPAPEEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK (SEQ ID NO: 8) and a light chain constant domain of the sequence
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC. (SEQ ID NO: 9).

In some embodiments, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of about 40 mg/ml, a phosphate buffer in a concentration of about 25 mM, polysorbate in a concentration of about 0.02% (w/v), and pH of about 6.0, wherein the formulation further comprises trehalose in a concentration

of about 200 mM, wherein said anti-CD19 antibody comprises a heavy chain constant domain of the sequence
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPDVFLFPPKPK
DTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQD
WLNGKEYKCKVSNKALPAPEEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK (SEQ ID NO: 8) and a light chain constant domain of the sequence
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC. (SEQ ID NO: 9).

In some embodiments, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of about 40 mg/ml, a phosphate buffer in a concentration of about 25 mM, polysorbate in a concentration of about 0.02% (w/v), and pH of about 6.0, wherein the formulation further comprises Mannitol in a concentration of about 219 mM and Sucrose in a concentration of about 29mM, wherein said anti-CD19 antibody comprises a heavy chain constant domain of the sequence
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPDVFLFPPKPK
DTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQD
WLNGKEYKCKVSNKALPAPEEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK (SEQ ID NO: 8) and a light chain constant domain of the sequence
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC. (SEQ ID NO: 9).

In some embodiments, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of 40 mg/ml, a citrate buffer in a concentration of 25 mM, polysorbate in a concentration of 0.02% (w/v), and pH of 6.0, wherein the formulation further comprises trehalose in a concentration of 200 mM, wherein said anti-CD19 antibody comprises an HCDR1 region of sequence SYVMH (SEQ ID NO: 1), an HCDR2 region of sequence NPYNDG (SEQ ID NO: 2), an HCDR3 region of sequence GTYYYGTRVFDY (SEQ ID NO: 3), an LCDR1 region of sequence RSSKSLQNVNGNTYLY (SEQ ID NO: 4), an LCDR2 region of sequence RMSNLNS (SEQ ID NO: 5), and an LCDR3 region of sequence MQHLEYPIT (SEQ ID NO: 6).

In some embodiments, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of 40 mg/ml, a citrate buffer in a concentration of 25 mM, polysorbate in a concentration of 0.02% (w/v), and pH of 6.0, wherein the formulation further comprises Mannitol in a concentration of 219 mM and Sucrose in a concentration of 29mM, wherein said anti-CD19 antibody comprises an HCDR1 region of sequence SYVMH (SEQ ID NO: 1), an HCDR2 region of sequence NPYNDG (SEQ ID NO: 2), an HCDR3 region of sequence GTYYYGTRVFDY (SEQ ID NO: 3), an LCDR1 region of sequence RSSKSLQNVNGNTYLY (SEQ ID NO: 4), an LCDR2 region of sequence RMSNLNS (SEQ ID NO: 5), and an LCDR3 region of sequence MQHLEYPIT (SEQ ID NO: 6).

In some embodiments, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of 40 mg/ml, a citrate buffer in a concentration of 25 mM, polysorbate in a concentration of 0.02% (w/v), and pH of 6.0, wherein the formulation further comprises trehalose in a concentration of 200 mM wherein said anti-CD19 antibody comprises a variable heavy chain of the sequence
EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGTKYNEKF
QGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWGQGTLVTVSS
(SEQ ID NO: 10) and a variable light chain of the sequence
DIVMTQSPATLSLSPGERATLSCRSSKSLQNVNGNTYLYWFQQKPGQSPQLLIYRMSNLNSGVPD
RFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPITFGAGTKLEIK (SEQ ID NO: 11).

In some embodiments, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of 40 mg/ml, a citrate buffer in a concentration of 25 mM, polysorbate in a concentration of 0.02% (w/v), and pH of 6.0, wherein the formulation further comprises Mannitol in a concentration of 219 mM and Sucrose in a concentration of 29mM, wherein said anti-CD19 antibody comprises a variable heavy chain of the sequence
EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGTKYNEKF
QGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWGQGTLVTVSS
(SEQ ID NO: 10) and a variable light chain of the sequence
DIVMTQSPATLSLSPGERATLSCRSSKSLQNVNGNTYLYWFQQKPGQSPQLLIYRMSNLNSGVPD
RFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPITFGAGTKLEIK (SEQ ID NO: 11).

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In some embodiments, provided herein is a stable lyophilized pharmaceutical formulation, the formulation comprising an anti-CD19 antibody in a concentration of 40 mg/ml, a citrate buffer in a concentration of 25 mM, polysorbate in a concentration of 0.02% (w/v), and pH of 6.0, wherein the formulation further comprises Mannitol in a concentration of 219 mM and Sucrose in a concentration of 29mM, wherein said anti-CD19 antibody comprises a heavy chain constant domain of the sequence

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPDVFLFPPKPK
DTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQD
WLNGKEYKCKVSNKALPAPEEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK (SEQ ID NO: 8) and a light chain constant domain of the sequence
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC. (SEQ ID NO: 9).

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ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPDVFLFPPKPK
DTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQD
WLNGKEYKCKVSNKALPAPEEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK (SEQ ID NO: 8) and a light chain constant domain of the sequence
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC. (SEQ ID NO: 9).

Working Examples

Working Example 1: Biophysical Characterization of MOR208

The biophysical properties of MOR208 were analyzed for the purpose of characterization, preformulation-screening and high concentration feasibility.

To assess the structural stability of MOR208, thermal melting experiments using DSF (Differential Scanning Fluorimetry) and CD spectroscopy were acquired. MOR208 samples were diluted to a concentration of 1.1 mg/mL. The hydrophobic dye sypro-orange was added to detect unfolding of the protein and respective melting curves were generated. The samples showed a relatively low melting temperature of 47 °C indicated by a step increase i n fluorescence.

MOR208 is a humanized monoclonal antibody binding to CD19 on B-cells and their progenitors. The Fc region has been engineered (S239D/ I332E) to enhance the effector functions supporting B-cell depletion. According to the current working hypothesis this Fc region format leads to CH2 domain flexibility, which results in enhanced ADCC potency but may be accompanied with adecreased melting temperature of the Fc domain.

Furthermore a pH screening study was performed using DSC, RALS and ITF to identify the most stable pH range for the protein. A pH range from 3.5 to 8.0 was covered in the study. The measurements were executed in a mixed buffer system covering the desired pH range. The most stable pH range for MOR208 was identified between pH 6.0 and 7.0 based on the results obtained.

Therefore two buffer systems were identified which provide sufficient buffer capacity in the pH range between pH 6.0 and 7.0 and are phamaceutical acceptable for parenteral use:

- Citrate (pH 5.5; 6.0)
- Histidine (pH 6.0; 6.5; 7.0)

Further DSC, RALS and ITF measurements were executed to test the thermal stability of MOR208 in 25 mM Citrate and 25 mM Histidine buffer. Only Citrate at a pH of 5.5 showed significantly lower transition temperatures where all other tested samples showed a comparable, thermal stability. This conclusion was also confirmed by RALS and ITF testing.

Furthermore the impact of ionic strength, sugars, polyols and polysorbate 20 on the thermal stability of MOR208 was tested. 25 mM Citrate buffer at pH 6.0 was selected as a basic buffer system for the

preparation of the samples containing NaCl or sugar or polysorbate 20. The measurements showed that the addition of NaCl increases the thermal stability of MOR208 based on the first transition temperature at 52°C. This observation was confirmed by ITF measurements. The results of the RALS testing did not identify any impact of NaCl on the thermal stability of MOR208.

The impact of sugars (trehalose and sucrose) and mannitol on the thermal stability of MOR208 was tested using concentrations of 200 mM for trehalose, 210 mM for sucrose and 180 mM mannitol/ 45 mM sucrose mixture. These concentrations were selected to shift the formulation into the isotonic range. The results of DSC, RALS and ITF testing indicated that trehalose and sucrose had a comparable impact on the thermal stability of MOR208 whereas the mixture of mannitol/sucrose slightly decreased the first transition temperature by approx. 0.8°C.

The impact of polysorbate 20 on the thermal stability of MOR208 was tested using concentrations of 0.02%, 0.04%, 0.08% and 0.12%. The results of the DSC and RALS measurements showed no significant impact of polysorbate 20 at the tested concentrations on the thermal stability of MOR208.

The impact of $MgCl_2$ on the thermal stability of MOR208 was tested using concentrations of 1, 5 and 10 mM. The results of the DSC and RALS measurements showed no significant impact of $MgCl_2$ at the tested concentrations on the thermal stability of MOR208.

Working Example 2: Stability Study of liquid formulations for MOR208

Based on the biophysical characterization of MOR208 the following 6 different liquid formulations were tested in a stability study:

The following formulations were selected for the stability study:

- Formulation F1: 10 mM Citrate, pH 6.0 + 0.01 % PS 20 + 150 mM NaCl
- Formulation F2: 25 mM Citrate, pH 6.0 + 0.02 % PS 20 + 125 mM NaCl
- Formulation F3: 25 mM Citrate, pH 6.0 + 0.02 % PS 20 + 200 mM Trehalose
- Formulation F4: 25 mM Citrate, pH 6.0 + 0.02 % PS 20 + 210 mM Sucrose
- Formulation F5: 25 mM Histidine, pH 6.0 + 0.02 % PS 20 + 140 mM NaCl
- Formulation F6: 25 mM Histidine, pH 6.0 + 0.02 % PS 20 + 230 mM Trehalose

- Formulation F7: 25 mM Succinat, pH 6.0 + 0.02 % PS 20 + 215 mM Trehalose
- Formulation F8: 25 mM Histidine, pH 6.5 + 0.02 % PS 20 + 240 mM Trehalose

The protein concentration was 20 mg/mL for all formulations. From each formulation 10 mL were filled in a 10 mL vial, closed with a coated rubber stopper and stored upside down at a controlled temperature of 25℃ for 12 weeks. Analytical testin g was performed prior storage (t0) after 4, 8 and 12 weeks.

Formulations 1, 3, 5 and 7 generated less subvisible particles over 12 weeks compared to the other formulations. The particles were measured by MFI. Formulation 5 showed the lowest number of SVP in the range >10µm and >25µm.

The aggregate and monomer level of the formulations was tested by HP SEC. Formulations 2, 3, 7 and 8 showed a higher increase in aggregates over 12 weeks compared to the other formulations.

The osmolality of formulation 4 increased after 4 weeks of storage which most probably indicates the degradation of sucrose contained in the formulation.

The shift in pH also indicates the degradation of sucrose in formulation 4.

Further HIC testing indicated that formulations 6, 7 and 8 showed a stronger decrease in peak 3 area% compared to the other formulations. Especially formulation 8 showed a strong reduction from 66.1% (initial value) to 52.2% after 12 weeks.

The physicochemical characterization of MOR208 identified the most stable pH range between pH 6.0 and 7.0.

Citrate, Histidine and Succinate buffer were selected as pharmaceutical acceptable buffer systems in the mentioned pH range. All buffer systems were used at a concentration of 25 mM providing sufficient buffer capacity for a protein concentration of 20 mg/mL. The addition of NaCl at a minimum concentration of 125 mM had a weak positive effect on the thermal stability of MOR208 (based on DSC data). The addition of sucrose, trehalose, mannitol/sucrose and polysorbate 20 did not alter the thermal stability of MOR208.

Based on the analytical results the most stable formulations were identified to be F1, F3 and F5.

- Formulation F1: 10 mM Citrate, pH 6.0 + 0.01 % PS 20 + 150 mM NaCl
- Formulation F3: 25 mM Citrate, pH 6.0 + 0.02 % PS 20 + 200 mM Trehalose
- Formulation F5: 25 mM Histidine, pH 6.0 + 0.02 % PS 20 + 140 mM NaCl

Samples of these 3 formulations were further kept at 25℃ for up to 8 months and again tested. Formulations 1 and 5 provided the highest stability but all liquid formulations generated subvisible particles after 8 months above the pharmacopeia specifications (NMT 6000 particles ≥ 10 μm per vial and NMT 600 particles ≥ 25 μm per vial). Therfore the desired shelf life of at least 24 months at 2-8℃ was not be reached by one of the tested liqui d formulations with 20 mg/mL protein.

This fact drove the decision to start the development of a lyophilized dosage form.

Working Example 3: Lyophilization feasibility study

Formulation 3 and a new Formulation 9 were involved in the lyophilization feasibility study with a concentration of MOR208 at 40mg/ml:

Formulation 3: 25 mM Citrate
200 mM Trehalose dihydrate
0.02% Polysorbate 20
pH 6.0

Formulation 9: 25 mM Citrate
219 mM Mannitol
29 mM Sucrose
0.02% Polysorbate 20
pH 6.0

Appearance of lyophilized Drug Product

The appearance of the lyo cake for both formulations was acceptable. The trehalose containing formulation was completely amorphous and shows a higher degree of shrinkage which is just a cosmetic observation and is typically not linked to product quality or stability. The mannitol containing formulation was partially cristalline and provides a cake of high pharmaceutical elegance without shrinkage.

The quality of the lyophilized MOR208 was tested and compared to the product quality prior lyophilization. The following **Table 1** summarizes the results of Formulations 3 and 9.

Table 1: Product quality comparison prior an after lyophilization

	Formulation 3		**Formulation 9**	
	Before Lyophilitzation	After Lyophilitzation	Before Lyophilitzation	After Lyophilitzation
Prior reconstitution				
Cake appearance	N/A	Acceptable cake appearance	N/A	Acceptable cake appearance
Moisture level	N/A	0.81-0.84%	N/A	1.19-1.36%
Post reconstitution				
Reconstitution time / n=2	N/A	48 sec	N/A	55 sec
Reconstitution behavior	N/A	foam formation	N/A	foam formation
Visual inspection	opalescent,colourless, no visible particles	opalescent,colourless, no visible particles	opalescent, colourless, no visible particles	opalescent, colourless, no visible particles
Turbidity	22 FNU	21 FNU	23 FNU	22 FNU
Osmolality / $mOsm \cdot kg^{-1}$	318	283	356	325
pH	6.1	6.1	6.1	6.1
UV scan	41.0 mg/mL	36.9 mg/mL	40.8 mg/mL	38.6 mg/mL
HPSEC (aggregate %)	0.8	0.8	0.7	0.8
HPSEC (monomer %)	99.2	99.2	99.3	99.2
HPSEC (fragments %)	0.0	0.0	0.0	0.0
Subvisible particles	n. d.	76 ≥ 10µm / mL 9 ≥ 25µm / mL	n. d.	71 ≥ 10µm / mL 12 ≥ 25µm / mL

IEC neutral peaks %	82.1	82.4	82.1	82.3
IEC post peaks %	6.6	6.3	6.5	6.3
IEC pre peaks %	11.4	11.3	11.4	11.4
CD16 binding	103	99	105	103
HIC pre peaks %	0.38	0.39	0.39	0.39
HIC peak 1%	0.98	1.19	0.97	1.22
HIC peak 2%	4.25	3.73	4.06	3.95
HIC peak 3%	86.59	86.66	86.75	86.82
HIC post peaks %	7.79	8.02	7.83	7.62

An additional lyophilization study was performed which only focused on subvisible particle (SVP) testing prior and post lyophilization. Results of the study are listed in **Table 2** clearly indicating that the lyophilization process did not increase the SVP count.

Table 2: Subvisible particle count of MOR208-F3 prior an post lyophilization

Sample	Sub-visible particles per mL (MFI)		
	2 - 1000 µm	≥ 10 µm	≥ 25 µm
MOR208-F3 before lyophilisation	847	46	5
lyo MOR208-F3 after reconstitution	892	15	2

Based on the test results no negative impact of the lyophilization process on product quality was observed. Both formulations provide an acceptable cake appearance. The reconstitution time is below 60 seconds for the formulation containing 40 mg protein per mL. Reconstitution is performed by adding 5 mL of water for injection. The moisture level of the mannitol/sucrose formulation is higher compared to the trehalose containing formulation which is due to the higher density of the lyo cake. The osmolality and the protein content (UV scan) decreased after lyophilization because the product was diluted due to the reconstitution procedure. The aggregation level did not increase during lyophilization and a low number of subvisible particles was counted after reconstitution. As a result both formulations are suitable for lyophilization of MOR208 and the lyophilization feasibility study was successfully finished. Following this study an accelerated stability study with both formulations was performed over a period of 3 months at 40℃.

Working Example 4: Accelerated Stability study

After the lyophilization feasibility study was successfully finalized a first stability study at 40℃ (75% rH) over 3 months was executed to compare both formulations. The stability testing included testing for color and visible particles. Furthermore the products were tested for HP-SEC (Aggregation), HIC, IEC, binding assay (CD16 Biacore), MFI (sub-visible particles) and SDS-Page.

HP-SEC testing showed a higher increase in aggregates for F9 after 3 months of storage **(Table 3)**. The aggregate level for F3 increased from 0.8% to 1.7% where the aggregate level of F9 increased from 0.8% to 2.6%.

Table 3: Aggregate testing by HP-SEC

	Formulation 3		Formulation 9	
	aggregates/%	monomer content/%	aggregates/%	monomer content/%
Initial value	0.8	99.3	0.8	99.2
1 month	1.4	98.6	2.2	97.8
2 months	1.8	98.2	2.1	97.9
3 months	1.7	98.3	2.6	97.4

The subvisible particles were tested for t0 and after 4 weeks, 8 weeks and 12 weeks with the MFI method. Comparing both formulations it was obvious that the mannitol/sucrose formulation generated more particles over time especially in the range between 2 µm and 1000 µm **(Figure 2)**.

Based on the analytical results, formulation 3 containing trehalose showed a higher stability at 40℃ compared to formulation 9. Differences in stability were seen in SVP (MFI) and aggregate level (HPSEC).

Conclusion:

The lyophilization feasibility study showed that MOR208 can be freeze dried without an impact on product quality using both formulations and a protein concentration of 40 mg/mL. The stability

conditions were 40℃ (75% rH) over a period of 3 mo nths. During this stability study the trehalose containing formulation was identified with a higher stability compared to the mannitol/sucrose formulation.

Working Example 5: Shelf life assignment

For a long term stability study MOR208 in Formulation 3 was put on real time storage at 5℃ ± 3℃ and accelerated storage at 25℃ ± 2℃ testing.

The performed stability studies comprise stability indicating and state of the art methods to monitor Drug Products regarding concentration, activity, purity, pharmaceutical and microbiological parameters during storage.

The following parameter methods were used and are considered to be the main stability indicating tests:

- Purity by HP-SEC:

Stability indicating properties of HP-SEC were shown by analysis of a relevant stress sample. Moreover, the capability of aggregate detection was verified by analytical ultracentrifugation.

- Homogeneity and purity by IEC and reduced/ non-reduced CGE:

For detection of fragments non-reducing CGE is applied; chemical modifications which lead to charge variants like deamidation were detected by IEC.

- Activity Assays:

The product specific activity assays CD19 binding assay (FACS), CD16 binding assay (SPR) and ADCC potency assay showed sensitivity to a relevant stress sample.

Results of the Real Time Storage at 5 ℃ ± 3 ℃ and the Accelerated Storage at 25 ℃ ± 3 ℃ are summarized in **Table 4** and **Table 5** respectively.

Table 4: MOR208 – Real Time Storage at 5 °C ± 3 °C

Parameter	Specification	0 months	6 months	12 months	18 months	24 months	36 months
Visible particles	Solution essentially free of foreign particles, may contain few white to whitish product-typical particles	Essentially free of foreign particles; no translucent, white to whitish particles contained					
IEC [%]	Report result Acidic Peak Group	24.1	24.7	24.5	24.4	24.3	24.3
	Report result Main Peak Group	66.3	65.5	65.5	66.0	65.8	65.6
	Report result Basic Peak Group	9.7	9.8	10.0	9.6	9.9	10.1
HP-SEC[%]	Monomer ≥ 92	98	97	98	98	98	98
	Aggregates ≤ 5	2	3	2	2	2	2
CGE reduced [%]	Σ heavy and light chains ≥ 90	97	97	96	97	97	96
CGE non reduced [%]	Main peak ≥ 85	92	93	93	93	93	93
	Fragments: Report result	6	6	6	6	6	6
Potency assay (ADCC) [%]	50 - 150 of standard material	78	118	99	98	99	97
CD19 binding assay (FACS) [%]	50 - 150 of standard material	97	92	96	106	109	83
CD16 binding assay (SPR) [%]	50 - 150 of standard material	96	97	101	100	100	93
Sub-visible Particles [particles/vial]	≥ 10 µm: ≤ 6000 particles/vial	10	7	347	93	167	113
	≥ 25 µm: ≤ 600 particles/vial	0	0	3	3	0	10

Sub-visible particles (MFI) [particles/mL]	Report result particles ≥ 2 to < 10 µm	3499	6027	21417	9265	5639	9632

Table 5: MOR208 – Accelerated Storage at 25 °C ± 3 °C

Parameter	Specification	0 months	1 months	3 months	6 months
Visible particles	Solution essentially free of foreign particles, may contain few white to whitish product-typical particles	Essentially free of foreign particles; no translucent, white to whitish particles contained			
IEC [%]	Report result Acidic Peak Group	24.1	23.9	24.6	24.8
	Report result Main Peak Group	66.3	66.0	64.7	64.1
	Report result Basic Peak Group	9.7	10.1	10.7	11.2
HP-SEC[%]	Monomer ≥ 92	98	98	98	97
	Aggregates ≤ 5	2	2	2	3
CGE reduced [%]	Σ heavy and light chains ≥ 90	97	97	97	96
CGE non reduced [%]	Main peak ≥ 85	92	93	93	93
	Fragments: Report result	6	6	6	6
Potency assay (ADCC) [%]	50 - 150 of standard material	78	99	102	97
CD19 binding assay (FACS) [%]	50 - 150 of standard material	97	97	93	101
CD16 binding assay (SPR) [%]	50 - 150 of standard material	96	99	104	91
Sub-visible Particles [particles/vial]	≥ 10 µm: ≤ 6000 particles/vial	10	117	433	50
	≥ 25 µm: ≤ 600 particles/vial	0	3	57	0
Sub-visible particles (MFI) [particles/mL]	Report result particles ≥ 2 to < 10 µm	3499	9861	9744	3777

Discussion of results:

Content and Activity

The functional activity of MOR208 was monitored with three different activity assays: CD19 binding assay (FACS), CD16 binding assay (SPR) and an ADCC based potency assay. With the combination of these three assays the antigen binding, the relevant effector binding as well as the major mode of action (ADCC) are covered.

Both binding assays show no clear or relevant tendencies over time. The ADCC based potency assay shows some increase over time (both under real term and accelerated conditions) but as the assay also shows a higher variability the tendency is not yet considered to be significant. In summary, all content and activity assays are well within the specification and do not indicate any critical changes in product quality over 36 months.

Purity

During 36 months of storage at the intended storage temperature 5 ± 3 °C none of the purity assays (i.e. HP-SEC, IEC, reducing and non-reducing CGE) indicate critical changes in product purity. Under accelerated conditions at 25 °C ± 2 °C the HP-SEC s hows only one change in the values at the latest testing point (decreased monomer/increased fragment values for one increment) but this is not reflected by long term data and is therefore considered negligible. IEC shows no tendencies under long term conditions but a clear tendency to a decreasing main peak group/increasing basic peak group under accelerated conditions.

In summary, all purity assays are well within the specification and do not indicate critical changes in product quality over 36 months.

Pharmaceutical Tests

During 36 months of storage at the intended storage temperature 5 ± 3 °C none of the pharmaceutical tests indicated a critical change over time. In summary, all pharmaceutical tests are well within the specification and do not indicate critical changes in product quality over 36 months.