Title: LIQUID COMPOSITIONS FOR ANTI-CD 19 ANTIBODY-DRUG CONJUGATES

Abstract: The present invention concerns stable pharmaceutical compositions for anti-CD 19 antibody-drug conjugates, for example for anti-CD 19 antibody conjugated, for instance, to a maytansinoid. The invention further relates to medicaments and treatments using the pharmaceutical compositions of the invention. Additionally, the invention relates to a kit comprising at least one of the pharmaceutical compositions of the invention.
LIQUID COMPOSITIONS FOR ANTI-CD19 ANTIBODY-DRUG CONJUGATES

This application claims priority to European Patent Application No. 15305725.2 filed on May 13, 2015, the entire contents of which are incorporated herein by this reference.

The present invention concerns stable pharmaceutical compositions for anti-CD19 antibody-drug conjugates, for example for anti-CD19 antibody conjugated, for instance, to a maytansinoid. The invention further relates to medicaments and treatments using the pharmaceutical compositions of the invention. Additionally, the invention relates to a kit comprising at least one of the pharmaceutical compositions of the invention.

CD19 is the earliest differentiation antigen of the B lymphocyte lineage, expressed on most B-cells, but not detected on plasma cells, stem cells, or on normal myeloid lineage. Therefore, CD19 is expressed on tumor cells from all B cell-derived neoplasms (B-cell non-Hodgkin's lymphoma, acute lymphoblastic leukemia, chronic lymphocytic leukemia), except myeloma.

HuB4-DM4 is an anti-CD19 antibody-drug conjugate composed of a humanized IgG1 monoclonal antibody, huB4, which specifically targets the CD19 antigen, conjugated through a disulfide link to the maytansinoid derivative DM4, a potent tubulin inhibitor. The structure and the uses of the HuB4-DM4 conjugate (SAR3419) have been previously described, for example, in the international patent application WO2012156455. HuB4-DM4 is currently in Phase-II clinical trials. After binding to the CD19 antigen, the HuB4-DM4 conjugate undergoes internalization and intracellular release of DM4.

The instabilities of antibodies are a major obstruction to commercial development of antibody drugs. For instance, certain prior liquid antibody preparations have short shelf lives and antibodies may lose biological activity resulting from chemical and physical instabilities during the storage.

Antibody-drug conjugates (ADCs) have a modified stability profile in comparison to the unconjugated, naked, monoclonal antibody. The modified stability profile results for example from the addition of the linker and drug moieties, as well as from the hydrophobicity of the drug itself, or is due to the hydrophobicity of grafted lateral chains. ADCs therefore have a higher tendency to aggregate in comparison to the naked monoclonal antibodies. Aggregates are known to induce undesired immune responses. Formulations developed for naked antibodies can therefore not be used for an antibody drug conjugate of the same antibody and formulations of ADCs should therefore be specifically developed for each antibody-drug conjugate.
For example, SAR3419 was formulated during phase I clinical studies in 10mM citrate, 135mM NaCl at pH 5.5, a formulation that was equally used for the naked antibody.

The inventors discovered that said formulation, when used for the ADC, leads to high amounts of visible and sub-visible particles and said ADC therefore only has limited stability upon storage.

The inventors developed new compositions comprising the anti-CD19 antibody-drug conjugate SAR3419 and showed that the compositions of the invention improve the stability of the anti-CD19 antibody-drug conjugate (Figures 1-3).

It has been further shown that the compositions of the invention comprising an anti-CD19 antibody-drug conjugate, an acetate or histidine buffer, sucrose, mannitol, and polysorbate, and having a pH of 5.0 to 6.5 minimize the formation of ADC aggregates such as dimers and sub-visible particles when the compositions are stored, for example, for 1 week at 40°C. In particular, size exclusion chromatography (SEC) analysis demonstrated that, after 1 week at 40°C, the tested compositions have a content of ADC monomer that is more than 95% of the total peak area (Figure 4), and dimer concentrations that are less than 4% of the total peak area (Figure 5).

The inventors further demonstrated in long term studies that the compositions comprising an anti-CD19 antibody-drug conjugate, an acetate or histidine buffer, sucrose, mannitol, and polysorbate, and having a pH of 5.0 to 6.5 have a decreased amount of dimers after storage at about 25°C for 6 months compared to a reference composition comprising the same anti-CD19 ADC in 10mM citrate, 135mM NaCl at pH 5.5 (Figure 3).

In another example, the compositions of the invention show a higher thermal stability with, for example, regards to particle formation in long term studies, for instance less amounts of small aggregates in the sub-visible particle size are observed (smaller amounts of particles that are bigger than 25µm or bigger than 10µm) in comparison to the composition comprising the same anti-CD19 ADC in 10mM citrate, 135mM NaCl at pH 5.5 (Figures 6 and 7).

The present invention thus defines suitable pharmaceutical compositions for anti-CD19 antibody-drug conjugates, such as anti-CD19 antibodies conjugated to maytansinoid, for example the anti-CD19 antibody-drug conjugate SAR3419, comprising an anti-CD19 antibody-drug conjugate, an acetate or histidine buffer, sucrose, mannitol, and polysorbate, having a pH of 5.0 to 6.5.
Summary of the Invention

The present invention thus relates to a pharmaceutical composition comprising:

a) an anti-CD19 antibody-drug conjugate,

b) at least one buffer agent selected from the group consisting of acetate and histidine,

c) sucrose,

d) mannitol, and

e) polysorbate,

wherein the pH of the composition is 5.0 to 6.5.

In one embodiment, the composition comprises less than 0.01% w/v of polysorbate. In a further embodiment the polysorbate is polysorbate 80.

In a further embodiment, the composition comprises 0.0025% w/v to 0.01% w/v of the surfactant polysorbate 80, for example 0.0025% w/v to 0.0075%, 0.004% w/v to 0.0075%, for instance 0.004% to 0.006%, for example 0.0045% to 0.0055% w/v, for instance 0.005% polysorbate 80.

The present invention is based on the surprising finding that pharmaceutical compositions having a pH value of 5.0 to 6.5, comprising an acetate or histidine buffer, sucrose, mannitol, polysorbate improve the stability of anti-CD19 antibody-drug conjugate.

In one embodiment, the compositions of the invention comprise less than 10mg/ml sodium chloride, for example, less than 7mg/ml sodium chloride. In one embodiment the composition comprises 2mg/ml sodium chloride or less. In another embodiment the composition does not comprise sodium chloride.

In one embodiment, the compositions of the invention comprise 1 to 100mM of at least one buffer agent, for example 5 to 50mM, 5 to 20mM, 5 to 15mM, 8 to 12mM, for instance 10mM.

In one embodiment, the at least one buffer agent is acetate. In a further embodiment, the pH of the composition comprising acetate as a buffer agent is 5.3 to 5.7, for instance 5.5.

In another embodiment, the at least one buffer agent is histidine. In a further embodiment, the pH of the composition comprising histidine as a buffer agent is 5.8 to 6.2, for instance 6.0.

In one embodiment, the composition is a liquid composition.

In one embodiment, the composition comprises 1 to 20mg/ml of an anti-CD19 antibody-drug conjugate, for example 1 to 18mg/ml, 1 to 16mg/ml, 1 to 14mg/ml, 1 to 12mg/ml, 1 to 10mg/ml, 2 to 9mg/ml, 3 to 8mg/ml, 4 to 7mg/ml, for instance 4.5 to 5.5mg/ml, such as 5mg/ml.
In one embodiment the composition comprises 10 to 150mM of the excipient sucrose, for example 30 to 150mM, 40 to 80mM, 50 to 80mM, 60 to 80mM, 65 to 80mM, 65 to 75mM, 70 to 75mM, for example 73mM. In a further example the composition comprises for example 120 to 150mM sucrose, 140mM to 150mM, for example 146mM sucrose.

In another embodiment, the composition comprises 100 to 300mM of the excipient mannitol, for example 120 to 280mM, 140 to 260mM, 160mM to 240mM, 180 to 220mM, 190 to 210mM, 200 to 210mM, 204 to 208mM, for example 206mM. In a further example the composition comprises for example 120 to 140mM mannitol, 125mM to 135mM, for example 132mM mannitol.

In one embodiment, the invention relates to a pharmaceutical composition comprising

a) 5 mg/ml anti-CD19 antibody-drug conjugate,
b) 10mM acetate buffer,
c) 73mM sucrose,
d) 206mM mannitol, and
d) 0.005%w/v polysorbate 80,
wherein the pH is 5.5.

In one embodiment, the anti-CD19 antibody is conjugated to a maytansinoid, for example DM4.

In a further embodiment, the anti-CD19 antibody is conjugated to a maytansinoid through a cleavable linker, for example a N-succinimidyl 4-(2-pyridyldithio)butanoate (SPDB) linker.

In a particular embodiment, the anti-CD19 antibody-drug conjugate comprises an antibody which binds specifically to the CD19 antigen. In a further embodiment, the anti-CD19 antibody of the anti-CD19 antibody-drug conjugate comprises a light chain variable domain comprising three sequential complementarity-determining regions (CRDs) having amino acid sequences SEQ ID NOS: 1, 2, and 3, and a heavy chain variable domain comprising sequential complementarity-determining regions having amino acid sequences SEQ ID NOS: 4, 5, and 6.

In a further embodiment, the antibody of anti-CD19 antibody-drug conjugate comprises a light chain consisting of the amino acid sequence SEQ ID NO: 7 and a heavy chain consisting of the amino acid sequence represented by SEQ ID NO: 8.

In one embodiment, the anti-CD19 antibody-drug conjugate is coltuximab ravtansine.
The inventors have developed a pharmaceutical composition of an anti-CD19 antibody-drug conjugate having a higher stability than the same anti-CD19 antibody-drug conjugate when present in a composition comprising 10mM citrate, 135mM NaCl at pH 5.5. Therefore, in one embodiment the pharmaceutical composition provides an anti-CD19 antibody-drug conjugate having a higher stability in comparison to a reference composition of the same anti-CD19 antibody-drug conjugate.

An improved stability refers for example to an increased physical stability when stored at storage condition or exposed to stress, in particular thermal stress.

**Detailed Description of the Invention**

**Definitions**

As used herein, a sequence "at least 85% identical to a reference sequence" is a sequence having, on its entire length, 85%, or more, in particular 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% sequence identity with the entire length of the reference sequence.

A percentage of "sequence identity" may be determined by comparing the two sequences, optimally aligned over a comparison window, wherein the portion of the polypeptide sequence in the comparison window may comprise additions or deletions (i.e. gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. Optimal alignment of sequences for comparison is conducted by global pairwise alignment, e.g. using the algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48: 443. The percentage of sequence identity can be readily determined for instance using the program Needle, with the BLOSUM62 matrix, and the following parameters gap-open=10, gap-extend=0.5.

In the context of the invention, a "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain group with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. Examples of groups of amino acids that have side chains with similar chemical properties include 1) aliphatic side chains: glycine, alanine, valine, leucine, and isoleucine; 2) aliphatic-hydroxyl side chains: serine and threonine; 3) amide-containing
side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains: lysine, arginine, and histidine; 6) acidic side chains: aspartic acid and glutamic acid; and 7) sulfur-containing side chains: cysteine and methionine. Conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine-tryptophane, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine.

As used herein, the term "subject" or "individual" are used interchangeably and may be, for example, a human or a non-human mammal. For example, the subject is a mouse, a rat, a bat; a ferret; a rabbit; a feline (cat); a canine (dog); a primate (monkey), an equine (horse); a human, including man, woman and child. In one embodiment a "subject" refers to a human.

**Anti-CD19 antibody-drug conjugate**

As used herein, "conjugate", "immunoconjugate", "antibody-drug conjugate" or "ADC" have the same meaning and are interchangeable.

The anti-CD19 antibody-drug conjugate in context of the invention comprises two primary components, an anti-CD19 antibody and a growth inhibitory agent.

Accordingly, in one embodiment, the antibody-drug conjugate in context of the invention comprises an anti-CD19 antibody linked or conjugated to at least one growth inhibitory agent, such as a cytotoxic agent or a radioactive isotope.

As used herein, the term "an anti-CD19 antibody" refers to an antibody that specifically recognizes and binds the CD19 antigen, in particular when the CD19 antigen is exposed on the cell surface.

As used herein, the term "CD19" refers to a type I transmembrane glycoprotein of the immunoglobulin Ig superfamily, with expression restricted to B cells. CD19 is involved in B-cell fate and differentiation through the modulation of B-cell receptor signaling at multiple stages of B-cell development. CD19 is ubiquitously expressed on B cells, as it is found expressed from the early pre-B stage throughout B-cell differentiation up to mature B cells, before it is down-modulated at the plasma cell stage. The pattern of CD19 expression is maintained in B-cell malignancies, covering all subtypes of B-cell lymphoma, from indolent to aggressive forms, as well as B-cell chronic lymphocytic leukemia and non-T acute lymphoblastic leukemia, and allows the targeting of tumor indications of early B cells, such as acute lymphoblastic leukemia (ALL), which cannot be targeted by rituximab.
A "growth inhibitory agent," or "anti-proliferative agent," which can be used indifferently, refers to a compound or composition which inhibits growth of a cell, especially tumour cell, either in vitro or in vivo.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term "cytotoxic agent" is intended to include chemotherapeutic agents, enzymes, antibiotics, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof, and the various antitumor or anticancer agents disclosed below. In some embodiments, the cytotoxic agent is a taxoid, vincas, taxanes, a maytansinoid or maytansinoid analog such as DM1 or DM4, a small drug, a tomaymycin or pyrrolobenzodiazepine derivative, a cryptophycin derivative, a leptomycin derivative, an auristatin or dolastatin analog, a prodrug, topoisomerase II inhibitors, a DNA alkylating agent, an anti-tubulin agent, a CC-1 065 or CC-1 065 analog.

As used herein "maytansinoids." denotes maytansinoids and maytansinoid analogs. Maytansinoids are drugs that inhibit microtubule formation and that are highly toxic to mammalian cells.

Examples of suitable maytansinoids include maytansinol and maytansinol analogs.

Examples of suitable maytansinol analogues include those having a modified aromatic ring and those having modifications at other positions. Such suitable maytansinoids are disclosed in U.S. Patent Nos. 4,424,219; 4,256,746; 4,294,757; 4,307,016; 4,313,946; 4,315,929; 4,331,598; 4,361,650; 4,362,663; 4,364,866; 4,450,254; 4,322,348; 4,371,533; 6,333,410; 5,475,092; 5,585,499; and 5,846,545.

In one embodiment, the cytotoxic agent is a maytansinoid such as DM1, formally termed A^-deacetyl-A^- (3-mercapto-1-oxopropyl)-maytansine, or DM4, formally termed A^-deacetyl-A^- (4-methyl-4-mercapto-1-oxopentyl)-maytansine.

The term "radioactive isotope" is intended to include radioactive isotopes suitable for treating cancer, such as At211, Bi212, Er169, I131, I125, Y90, In111, P32, Re186, Re188, Sm153, Sr89, and radioactive isotopes of Lu. Such radioisotopes generally emit mainly beta-radiation. In an embodiment the radioactive isotope is alpha-emitter isotope, more precisely Thorium 227 which emits alpha-radiation.

In some embodiments, the antibodies of the present invention are covalently attached, directly or via a cleavable or non-cleavable linker, to the at least one growth inhibitory agent.

"Linker", as used herein, means a chemical moiety comprising a covalent bond or a chain of atoms that covalently attaches a polypeptide to a drug moiety.
The conjugates may be prepared by *in vitro* methods. In order to link a drug or prodrug to the antibody, a linking group is used. Suitable linking groups are well known in the art and include disulfide groups, thioether groups, acid labile groups, photolabile groups, peptidase labile groups and esterase labile groups. Conjugation of an antibody in context of the invention with cytotoxic agents or growth inhibitory agents may be made using a variety of bifunctional protein coupling agents including but not limited to N-succinimidyl pyridyldithiobutyrate (SPDB), butanoic acid 4-[(5-nitro-2-pyridinyl)dithio]-2,5-dioxo-1-pyrrolidinyl ester (nitro-SPDB), 4-(Pyridin-2-ylisulfanyl)-2-sulfo-butryric acid (sulfo-SPDB), N-succinimidyl (2-pyridyldithio) propionate (SPDP), succinimidyl (N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl)-hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4- dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al (1987). Carbon labeled 1-isothiocyanatobenzyl methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody (WO 94/1 1026).

The linker may be a "cleavable linker" facilitating release of the cytotoxic agent or growth inhibitory agent in the cell. For example, an acid-labile linker, a peptidase-sensitive linker, an esterase labile linker, a photolabile linker or a disulfide-containing linker (See e.g. U.S. Patent No. 5,208,020) may be used. The linker may be also a "non-cleavable linker" (for example SMCC linker) that might lead to better tolerance in some cases.

According to an embodiment, in the conjugate in context of the invention, the growth inhibitory agent is a maytansinoid, in particular DM1 or DM4. In said conjugate, the antibody is conjugated to said at least one growth inhibitory agent by a linking group. In particular said linking group is a non-cleavable linker, such as N-succinimidyl 4-(2-pyridyldithio)butanoate (SPDB).

DM4 and a method of conjugating DM4 to the huB4 antibody through a SPDB linker are described in, for example, the US Patent Application No. 2004/0235840.

According to an embodiment, the drug-to-antibody ratio of the conjugate ranges from 1 to 10, for instance from 3 to 5, in particular from 3 to 4, for example 3.5.

It should be noted that the drug-to-antibody ratio can vary with the nature of the antibody and of the drug (i.e. the growth-inhibitory agent) used along with the experimental conditions used for the conjugation (like the ratio growth-inhibitory
agent/antibody, the reaction time, the nature of the solvent and of the cosolvent if any). Thus the contact between the antibody and the growth-inhibitory agent leads to a mixture comprising several conjugates differing from one another by different drug-to-antibody ratios; optionally the naked antibody; optionally aggregates. The drug-to-antibody that is determined is thus a mean value.

Methods to determine the drug-to-antibody ratio are known to the skilled in the art and consist in measuring spectrophotometrically the ratio of the absorbance of a solution of substantially purified conjugate at $A_0$ and 280 nm. 280 nm is a wavelength generally used for measuring protein concentration, such as antibody concentration. The wavelength $A_0$ is selected so as to allow discriminating the drug from the antibody, i.e. as readily known to the skilled person, $A_0$ is a wavelength at which the drug has a high absorbance and $A_0$ is sufficiently remote from 280 nm to avoid substantial overlap in the absorbance peaks of the drug and antibody. $A_0$ may be selected as being 252 nm in the case of maytansinoid molecules. The average drug-to-antibody ratio is then calculated from the ratio of the drug concentration to that of the antibody: Drug-to-antibody ratio = $c_D/ c_A$.

An "antibody" may be a natural or conventional antibody in which two heavy chains are linked to each other by disulfide bonds and each heavy chain is linked to a light chain by a disulfide bond. There are two types of light chain, lambda ($\lambda$) and kappa ($\kappa$). There are five main heavy chain classes (or isotypes) which determine the functional activity of an antibody molecule: IgM, IgD, IgG, IgA and IgE. Each chain contains distinct sequence domains. The light chain includes two domains or regions, a variable domain (VL) and a constant domain (CL). The heavy chain includes four domains, a variable domain (VH) and three constant domains (CH1, CH2 and CH3, collectively referred to as CH). The variable regions of both light (VL) and heavy (VH) chains determine binding recognition and specificity to the antigen. The constant region domains of the light (CL) and heavy (CH) chains confer important biological properties such as antibody chain association, secretion, trans-placental mobility, complement binding, and binding to Fc receptors (FcR). The Fv fragment is the N-terminal part of the Fab fragment of an immunoglobulin and consists of the variable portions of one light chain and one heavy chain. The specificity of the antibody resides in the structural complementarity between the antibody combining site and the antigenic determinant. Antibody combining sites are made up of residues that are primarily from the hypervariable or complementarity determining regions (CDRs). Occasionally, residues from nonhypervariable or framework regions (FR) influence the overall domain structure and hence the combining site.
"Complementarity Determining Regions" or "CDRs" refer to amino acid sequences which together define the binding affinity and specificity of the natural Fv region of a native immunoglobulin binding site. The light and heavy chains of an immunoglobulin each have three CDRs, designated CDR1-L, CDR2-L, CDR3-L and CDR1-H, CDR2-H, CDR3-H, respectively. A conventional antibody antigen-binding site, therefore, includes six CDRs, comprising the CDR set from each of a heavy and a light chain V region.

"Framework Regions" (FRs) refer to amino acid sequences interposed between CDRs, i.e. to those portions of immunoglobulin light and heavy chain variable regions that are relatively conserved among different immunoglobulins in a single species. The light and heavy chains of an immunoglobulin each have four FRs, designated FR1-L, FR2-L, FR3-L, FR4-L, and FR1-H, FR2-H, FR3-H, FR4-H, respectively.

As used herein, a "human framework region" is a framework region that is substantially identical (about 85%, or more, in particular 90%, 95%, 97%, 99% or 100%) to the framework region of a naturally occurring human antibody.

In the context of the invention, CDR/FR definition in an immunoglobulin light or heavy chain is to be determined based on Kabat et al. (1991) Sequences of Proteins of Immunological Interest (DHHS, Washington, DC), 5th Ed.).

As used herein, the term "antibody" denotes conventional antibodies and fragments thereof, as well as single domain antibodies and fragments thereof, in particular variable heavy chain of single domain antibodies, and chimeric, humanized, bispecific or multispecific antibodies.

As used herein, antibody or immunoglobulin also includes "single domain antibodies" which have been more recently described and which are antibodies whose complementary determining regions are part of a single domain polypeptide. Examples of single domain antibodies include heavy chain antibodies, antibodies naturally devoid of light chains, single domain antibodies derived from conventional four-chain antibodies, engineered single domain antibodies. Single domain antibodies may be derived from any species including, but not limited to mouse, human, camel, llama, goat, rabbit and bovine. Single domain antibodies may be naturally occurring single domain antibodies known as heavy chain antibody devoid of light chains. In particular, Camelidae species, for example camel, dromedary, llama, alpaca and guanaco, produce heavy chain antibodies naturally devoid of light chain. Camelid heavy chain antibodies also lack the CH1 domain.

The variable heavy chain of these single domain antibodies devoid of light chains are known in the art as "VHH" or "nanobody". Similar to conventional VH domains, VHHs contain four FRs and three CDRs. Nanobodies have advantages over conventional antibodies: they are about ten times smaller than IgG molecules, and as a consequence
properly folded functional nanobodies can be produced by *in vitro* expression while achieving high yield. Furthermore, nanobodies are very stable, and resistant to the action of proteases. The properties and production of nanobodies have been reviewed by Harmsen and De Haard (Harmsen and De Haard (2007) *Appl. Microbiol. Biotechnol.* 77:1 3-22).

The term "monoclonal antibody." or "mAb" as used herein refers to an antibody molecule of a single amino acid composition that is directed against a specific antigen, and is not to be construed as requiring production of the antibody by any particular method. A monoclonal antibody may be produced by a single clone of B cells or hybridoma, but may also be recombinant, *i.e.* produced by protein engineering.

"Fragments" of (conventional) antibodies comprise a portion of an intact antibody, in particular the antigen binding region or variable region of the intact antibody. Examples of antibody fragments include Fv, Fab, F(\(\text{ab}'\))\(_2\), Fab', dsFv, (dsFv)\(_2\), scFv, sc(Fv)\(_2\), diabodies, bispecific and multispecific antibodies formed from antibody fragments. A fragment of a conventional antibody may also be a single domain antibody, such as a heavy chain antibody or VHH.

The term "Fab" denotes an antibody fragment having a molecular weight of about 50,000 Da and antigen binding activity, in which about a half of the N-terminal side of H chain and the entire L chain, among fragments obtained by treating IgG with a protease, papain, are bound together through a disulfide bond.

The term "F(\(\text{ab}'\))\(_2\)" refers to an antibody fragment having a molecular weight of about 100,000 Da and antigen binding activity, which is slightly larger than the Fab bound via a disulfide bond of the hinge region, among fragments obtained by treating IgG with a protease, pepsin.

The term "Fab'" refers to an antibody fragment having a molecular weight of about 50,000 Da and antigen binding activity, which is obtained by cutting a disulfide bond of the hinge region of the F(\(\text{ab}'\))\(_2\) fragment.

A single chain Fv ("scFv") polypeptide is a covalently linked VH::VL heterodimer which is usually expressed from a gene fusion including VH and VL encoding genes linked by a peptide-encoding linker. The human scFv fragment includes CDRs that are held in appropriate conformation, in particular by using gene recombination techniques. Divalent and multivalent antibody fragments can form either spontaneously by association of monovalent scFvs, or can be generated by coupling monovalent scFvs by a peptide linker, such as divalent sc(Fv)\(_2\).

"dsFv" is a VH::VL heterodimer stabilised by a disulphide bond.

"(dsFv)?" denotes two dsFv coupled by a peptide linker.
The term "bispecific antibody," or "BsAb" denotes an antibody which combines the antigen-binding sites of two antibodies within a single molecule. Thus, BsAbs are able to bind two different antigens simultaneously. Genetic engineering has been used with increasing frequency to design, modify, and produce antibodies or antibody derivatives with a desired set of binding properties and effector functions as described for instance in EP 2 050 764 A1.

The term "multispecific antibody," denotes an antibody which combines the antigen-binding sites of two or more antibodies within a single molecule.

The term "diabodies," refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) in the same polypeptide chain (VH-VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites.

In a particular embodiment, the epitope-binding fragment is selected from the group consisting of Fv, Fab, F(ab')2, Fab', dsFv, (dsFv)2, scFv, sc(Fv)2, diabodies and VHH.

In one embodiment, the anti-CD19 antibody-drug conjugate comprises and anti-CD19 antibody which binds specifically to the CD19 antigen.

In a particular embodiment, the conjugate comprises an anti-CD19 antibody which comprises a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 1, CDR2-L of SEQ ID NO: 2 and CDR3-L of sequence SEQ ID NO: 3, and a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 4, CDR2-H of sequence SEQ ID NO: 5 and CDR3-H of sequence SEQ ID NO: 6.

In a further embodiment, the conjugate in context of the invention comprises an antibody which comprises:

- a light chain variable domain identified in the amino acid sequence represented by SEQ ID NO: 7 or a sequence at least 85%, more particularly at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical thereto, in particular provided that said sequence contains the sequences SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3, and

- a heavy chain variable domain identified in the amino acid sequence represented by SEQ ID NO: 8 or a sequence at least 85%, more particularly at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical thereto, in particular provided that said sequence contains the sequences SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6.
Methods to identify the variable domain amino acid sequence within a given light or heavy chain sequence are known to the skilled in the art.

In another embodiment, the conjugate in context of the invention comprises the humanized anti-CD19 antibody huB4 described in Roguska et al. (Proc. Natl. Acad. Sci. USA, 91:969-973, 1994).

In a further embodiment, the conjugate comprises an antibody which comprises:

- a light chain of sequence

EIVLTQSPAIMSASSGVTMCASSGNYMHWQQKPGTPRSSYDTSKLAGVPA RFSGSGSTDDSTISMEPDAATYYCHQGNYTGGGTKLEIKRTVAAPSVIFPPSD

EQLKSGTASVCLNNYFPREAKVQWKVDNALQSGQESVTEQDSKDSTYSLSSTLTLEK
SKA YEHKVYACEVTHQGLSSPVTKSFNRCGE (SEQ ID NO: 7), or a sequence at least 85%, more particularly at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical thereto, in particular provided that said sequence contains the sequences SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3, and

- a heavy chain of sequence

QVQLVQPGAEVKPGASVKLSDKTSGYTFTSNWNVHVPAPGQGLEWIGEIDPSDSYTVNYQNNFQGKDALTVDKSTSTAYMEVSSLRSDTTAVYYCARGSNPYKYAMDYWGQGTSVTVSSTKGPSVFPLAPSSGTSGGTALGCLVFYPFEPVTWNSGALTSGVHTFPAVLQGSLSSSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKEPKSCDKTHTCPPCPAPELLGGPSVFIPKDKTLISRTPEVTCCSVVVDHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTVRVSVTLHQLDNLNGKEYKCKVSNKAPPIEKTISAKAGQPREDQYPVTLPSSREDLTNKQVSLTLCKVFYPDSIAVEWESNGQPENNYKTTPVLDSDGSLFYSKLVTDKSRLWWQGNGFCSVCSVMHEALHNHYTQKSLSLSQGK (SEQ ID NO: 8), or a sequence at least 85%, more particularly at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical thereto, in particular provided that said sequence contains the sequences SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6.

In a further aspect of the invention, the anti-CD19 antibody comprised by the conjugate is a humanized antibody or a fragment of a humanized antibody obtained, for example by resurfacing.

The term "humanized antibody" refers to an antibody which is initially wholly or partially of non-human origin and which has been modified to replace certain amino acids, in particular in the framework regions of the heavy and light chains, in order to avoid or minimize an immune response in humans. The constant domains of a humanized antibody are most of the time human CH and CL domains. In an embodiment, a humanized antibody has constant domains of human origin.
In a further aspect of the invention, the anti-CD19 antibody comprised by the conjugate is a chimeric antibody or a fragment of a chimeric antibody.

The term "chimeric antibody" refers to an engineered antibody which in its broadest sense contains one or more region(s) from one antibody and one or more regions from one or more other antibody(ies). In particular a chimeric antibody comprises a VH domain and a VL domain of an antibody derived from a non-human animal, in association with a CH domain and a CL domain of another antibody, in particular a human antibody. As the non-human animal, any animal such as mouse, rat, hamster, rabbit or the like can be used. A chimeric antibody may also denote a multispecific antibody having specificity for at least two different antigens. In an embodiment, a chimeric antibody has variable domains of mouse origin and constant domains of human origin.

The goal of humanization is a reduction in the immunogenicity of a xenogenic antibody, such as a murine antibody, for introduction into a human, while maintaining the full antigen binding affinity and specificity of the antibody. Humanized antibodies, or antibodies adapted for non-rejection by other mammals, may be produced using several technologies such as resurfacing and CDR grafting. As used herein, the resurfacing technology uses a combination of molecular modeling, statistical analysis and mutagenesis to alter the non-CDR surfaces of antibody variable regions to resemble the surfaces of known antibodies of the target host.

Strategies and methods for the resurfacing of antibodies, and other methods for reducing immunogenicity of antibodies within a different host, are disclosed in U.S. Patent No. 5,639,641. Briefly, in a particular method, (1) position alignments of a pool of antibody heavy and light chain variable regions is generated to give a set of heavy and light chain variable region framework surface exposed positions wherein the alignment positions for all variable regions are at least about 98% identical; (2) a set of heavy and light chain variable region framework surface exposed amino acid residues is defined for a rodent antibody (or fragment thereof); (3) a set of heavy and light chain variable region framework surface exposed amino acid residues that is most closely identical to the set of rodent surface exposed amino acid residues is identified; (4) the set of heavy and light chain variable region framework surface exposed amino acid residues defined in step (2) is substituted with the set of heavy and light chain variable region framework surface exposed amino acid residues identified in step (3), except for those amino acid residues that are within 5 Å of any atom of any residue of the complementarity-determining regions of the rodent antibody; and (5) the humanized rodent antibody having binding specificity is produced.

In one embodiment, the anti-CD19 antibody-drug conjugate in context of the invention is the HuB4-DM4 conjugate (SAR3419) that has been previously described in the international patent application WO2012/156455, which is herein incorporated by reference.

In one embodiment, the anti-CD19 antibody-drug conjugate in context of the invention is coltuximab ravtansine.

"Coltuximab_ravtansine" is the International Nonproprietary Name for an immunoglobulin G1-kappa, anti-[Homo sapiens CD19 (B lymphocyte surface antigen B4, Leu-12)], chimeric monoclonal antibody conjugated to maytansinoid DM4 according to the «International Nonproprietary Names for Pharmaceutical Substances (INN) » as published by the WHO in WHO Drug Information, Vol. 27, No. 2, 2013.

**Pharmaceutical Compositions**

As herein used, the term "pharmaceutical compositions" refers to liquid preparations which are in such form as to permit the biological activity of the active ingredients to be unequivocally effective, and which contain no additional components which are significantly toxic to the subjects to which the composition would be administered. Such compositions are sterile. "Pharmaceutically acceptable" excipients (vehicles, additives) are those which are suitable for administration to a subject.

A "pharmaceutical formulation" or "formulation" refers to the process but also the product of a process in which an active drug is combined with chemical substances to produce a final medicinal product, the final formulation therefore refers to medicinal products such as capsules, pills, tablets, emulsions or compositions. Therefore, in one embodiment, a pharmaceutical formulation is a pharmaceutical composition.
In one embodiment the pharmaceutical composition of the invention is stable.

"Stability" refers to chemical stability and physical stability and can be evaluated qualitatively and/or quantitatively using various analytical techniques that are described in the art and are reviewed in for example 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). Those methods include the evaluation of aggregate formation (for example using size exclusion chromatography, by measuring turbidity, and/or by visual inspection); by assessing charge heterogeneity using cation exchange chromatography or capillary zone electrophoresis; amino -terminal or carboxy- terminal sequence analysis; mass spectrometric analysis; SDS-PAGE analysis to compare reduced and intact ADC; peptide map (for example tryptic or LYS-C) analysis; evaluating biological activity or antigen binding function of the ADC; etc. Instability may involve any one or more of: aggregation, deamidation (e.g. Asndeamidation), oxidation (e.g. Met oxidation), isomerization (e.g. Asp isomeriation), clipping/hydro lysis/fragmentation (e.g. hinge region fragmentation), succinimide formation, unpaired cysteine(s), N-terminal extension, C-terminal processing, glycosylation changes, etc. A "deamidated" ADC is one in which one or more asparagine residue thereof has been modified, e.g. to an aspartic acid or an iso-aspartic acid by a post-translational modification. In order to measure stability a sample of the composition of the invention may be tested in a stability study, wherein a sample is exposed for a selected time period to a stress condition followed by quantitative and qualitative analysis of the chemical and physical stability using an adequate analytical technique.

In context of the present invention stability refers to physical stability. Accordingly, in one embodiment a composition of the invention which is stable is physical stable.

"Physical stability" refers substantially, in context of the invention, to an antibody-drug conjugate having low amounts of visible particle formation, low amounts of soluble aggregate formation, in particular dimers, and low amounts of hydrolysis of free drug related products. Methods to access the physical stability are known to the skilled in the art and include for example HIAC/ROYCO particle counter to quantify the presence of visible particles, size exclusion chromatography (SEC) to quantify the presence of aggregates, such as soluble aggregates, for instance dimers, dynamic light scattering (DLS), and reverse- phase HPLC to quantify dimers and/or free drug related products and differential scanning calorimetry (DSC). For size exclusion chromatography (SEC) for example a difference of 0.5% of the content might be considered as significantly different in the context of the invention under the tested conditions depending on the column used, operating pressure, and velocity of the buffer.
"Free drug related products" herein refers to degradation products of the drug which is conjugated to the monoclonal antibody or to degradation products resulting from the drug and linker of the ADC.

In one embodiment, a maytansinoid such as DM4 is linked to the anti-CD19 antibody via SPDB. In the same embodiment, free drug related products are, for example, DM4-TBA, maysine and maysine degradation products. The released small molecules DM4-TBA, maysine and maysine degradation products are also called free maytansinoids.

"DM4-TBA" with the chemical structure (I)

![Chemical structure of DM4-TBA](attachment:image1)

results from the cleavage of linker attached to lysine residues or residual weakly bound linker.

"Residual weakly bound linkers" herein refer to linkers that spontaneously hydrolyze. The SPDB linker for example mainly reacts with lysine but may also react with other residues like tyrosine or cysteine leading to esters or thioesters which are Residual weakly bound linkers.

"Maysine" with the chemical structure (II)

![Chemical structure of Maysine](attachment:image2)

may be formed by beta elimination of the secondary ester of the side chain.
"Mavsine degradation products" are formed by fragmentation and oxidation of maysine.

In context of the present invention a "stable composition" is one in which the ADC is physical stable when stored at storage condition or exposed to stress, in particular thermal stress.

"Storage condition" herein refers to the storage of the composition for example at 5°C to 25°C, for example storage at 5°C or 25°C for a certain period of time, for example from 1 week to 6 months, such as 1 week, 1 month, 2 months, 3 months, 4 months, 5 months and 6 months.

The term "stress" or "stress condition" in context of the invention refers to thermal stress. Methods and conditions to simulate thermal stress are diverse and known to the skilled in the art. Thermal stress refers for example to the storage at decreased or increased temperatures for an amount of time, in one example samples may be stored at 5°C, 25°C and 40°C, wherein for instance 40°C refer to a stress condition. In one embodiment, a stress condition or thermal stress refers to storage at 40°C for 1 week.

Accordingly, stability can be measured at a selected temperature for a selected time period for instance by storing a sample at 40°C for 1 week or by storing a sample at 5°C and 25°C for 1 week up to 6 months and by using for instance SEC, HIAC/ROYCO particle counter, reverse-phase HPLC for qualitative and quantitative analysis.

In one embodiment, stable refers to at least one of the following characteristics

i) the composition has a monomer content in % of more than 90% in relation to the total area of all peaks when measured by SEC, and/or

ii) the composition has a dimer content in % of less than 4% in relation to the total area of all peaks when measured by SEC, and/or

iii) the composition has a sub-visible particle content of a particles having a size bigger than 10μM which is less than 1000 particles per vial when measured by HIAC/ROYCO particle counter, and/or

iv) the composition has a sub-visible particle content of a particles having a size bigger than 25μM which is less than 200 particles per vial when measured by HIAC/ROYCO particle counter.

In one embodiment, the vial size used in HIAC/ROYCO particle counter measurements is 25 ml.

In one embodiment the composition is stable for 1 week at 40°C.

In a further embodiment the composition is stable for up to 6 months at 5°C or 25°C.
In the same embodiments, stable refers to a composition having a monomer content in % of more than 90%, more than 91%, more than 92%, more than 93%, more than 94%, more than 95%, more than 96%, more than 97% or more than 98% in relation to the total area of all peaks when measured by SEC.

In the same embodiments, stable refers to a composition having a dimer content in % of less than 4%, less than 3.5%, less than 3%, in relation to the total area of all peaks when measured by SEC.

In the same embodiments, stable refers to a composition having a subvisible particle content of particles having a size bigger than 10μM which is less than 1000 particles, less than 900 particles, less than 800 particles, less than 700 particles, less than 600 particles per vial when measured when measured by HIAC/ROYCO particle counter.

In the same embodiments, stable refers to a composition having a subvisible particle content of particles having a size bigger than 25μM which is less than 200 particles, less than 180 particles, less than 160 particles, less than 140 particles, less than 120 particles per vial when measured when measured by HIAC/ROYCO particle counter.

In one embodiment the compositions of the invention have an increased physical stability.

In a further embodiment the compositions of the invention have higher stability.

In a further embodiment the compositions of the invention have a higher stability against stress, in particular thermal stress.

Terms such as "decreased", "higher", "less", "smaller", "increased", "lower" or "less" or such alike denote quantitative differences between two states and refer to at least statistically significant differences between the two states.

"Improved stability", "higher stability" and/or "increased stability" in context of the invention refer to physical stability that has been qualitatively and/or quantitatively evaluated as described above and which is increased in comparison to the physical stability of a reference composition of the same ADC.

In certain embodiments, the reference composition is a composition comprising the same anti-CD19 antibody-drug conjugate, citrate, NaCl at pH 5 to 6. In certain embodiments, the reference composition is a composition comprising the same anti-CD19 antibody-drug conjugate, 10mM citrate and 135mM NaCl at pH 5.5

In accordance with the above, in one embodiment the pharmaceutical composition of the present invention has at least one feature selected from the group consisting of:

(a) a decreased amount of dimers after storage at about 25°C for 6 months as measured by Size Exclusion Chromatography (SEC), and/or
(b) a decreased amount of sub-visible particles after storage at about 25°C for 6 months as measured by HIAC/ROYCO particle counter, wherein the particles have a size bigger than 1000 µM,
c) a decreased amount of sub-visible particles after storage at about 25°C for 6 months as measured by HIAC/ROYCO particle counter, wherein the particles have a size bigger than 250 µM, compared to a reference composition.

Furthermore, in accordance with the above, in one embodiment the pharmaceutical composition of the present invention has at least one feature selected from the group consisting of:
(a) the composition is stable to thermal stress of 1 week at 40°C,
(b) the composition is stable during storage at 5°C for 6 months, and/or
(c) the composition is stable during storage at 25°C for 6 months, wherein stability refers to at least one of the following characteristics:
i) the composition has a monomer content in % of more than 90%, more than 91%, more than 92%, more than 93%, more than 94%, more than 95% in relation to the total area of all peaks when measured by SEC,
ii) the composition has a dimer content in % of less than 4%, less than 3.5%, less than 3%, in relation to the total area of all peaks when measured by SEC, and/or
iii) the composition has a sub-visible particle content of particles having a size bigger than 1000 µM which is less than 1000 particles, less than 900 particles, less than 800 particles, less than 700 particles, less than 600 particles per vial when measured when measured by HIAC/ROYCO particle counter, and/or
iv) the composition has a sub-visible particle content of a particles having a size bigger than 250 µM which is less than 200 particles, less than 180 particles, less than 160 particles, less than 140 particles, less than 120 particles per vial when measured when measured by HIAC/ROYCO particle counter.

In one embodiment, the composition comprises less than 7 mg/ml sodium chloride, less than 6 mg/ml, less than 5 mg/ml, less than 2 mg/ml sodium chloride, for example no sodium chloride.

When the composition does not comprise sodium chloride, the composition is essentially free from sodium chlorate.

As used herein the term "essentially" denotes a composition wherein no sodium chlorate molecules are actively, i.e. are intended to be added. Trace amounts of sodium
chlorate may be present in a concentration below 5mg/ml, 3mg/ml, 2mg/ml, 1mg/ml, for example below 0.5mg/ml, in particular below 0.05mg/ml.

In one embodiment, the anti-CD19 antibody-drug conjugate of the composition is comprised in a therapeutically effective amount.

In a pharmacological sense in context of the present invention a "therapeutically effective amount" or "effective amount" of anti-CD19 antibody-drug conjugate refers to an amount effective in the prevention or treatment of a disorder for the treatment of which the antibody is effective. Accordingly, the composition may comprise 0.1 to 100mg/ml of the anti-CD19 antibody-drug conjugate, 0.1 to 50mg/ml, 0.1 to 20mg/ml, 1 to 20mg/ml anti-CD19 antibody-drug conjugate, for example 1 to 18mg/ml, 1 to 16mg/ml, 1 to 14mg/ml, 1 to 12mg/ml, 1 to 10mg/ml, 2 to 9mg/ml, 3 to 8mg/ml, 4 to 7mg/ml, for instance 4.5 to 5.5mg/ml, such as 5mg/ml. Ranges intermediate to the above recited concentrations are also intended to be part of this invention. For example, ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be included.

As used herein "buffer" refers to a buffered solution that resists changes in pH by the action of its acid-base conjugate components. The "pH" herein refers to the acidity or basicity of the composition at 25°C. Standard methods to measure the pH of a composition are known to the skilled in the art. In one example the pH is typically measured using a pH meter at 25°C. Typically, measuring pH consists of calibrating the instrument, placing the electrodes in a well-mixed sample, and then reading the pH directly from the pH meter.

The composition thus comprises 1 to 100mM of at least one buffer agent, 1 to 50mM of at least one buffer agent, 1 to 30mM of at least one buffer agent, 1 to 15mM of at least one buffer agent, for example 5 to 15mM such as 5mM, 6mM, 7mM, 8mM, 9mM, 10mM, 11mM, 12mM, 13mM, 14mM, 15mM of at least one buffer agent. In one embodiment, the composition comprises 10mM of at least one buffer agent.

The inventors showed that acetate and histidine buffers strongly reduce the formation of dimers and sub-visible particles and thus improve stability of the anti-CD19 antibody-drug conjugate in comparison to for example a citrate buffer.

Therefore, the at least one buffer agent in context of the invention is acetate or histidine.

In one embodiment, the at least one buffer agent may be 2, 3 or more buffer agents. Therefore, in one embodiment the at least one buffer agent is two buffer agents.
In one example the two buffer agents might be acetate and histidine, wherein the resulting buffer is an acetate-histidine buffer or histidine-acetate buffer.

In one embodiment, the at least one buffer is acetate, in the same embodiment the pH of the composition is 5 to 6.5, for example 5.0 to 6.0, 5.2 to 5.8, for example 5.3 to 5.7, for instance 5.4 to 5.6, such as 5.4, 5.5 or 5.6.

As known by the skilled in the art an acetate buffer consists of a mixture of acetate, for example sodium acetate as the base and acetic acid as the acid. To prepare an acetate buffer of a specific concentration and pH the skilled in the art must calculate the amount of for example sodium acetate or sodium acetate tri-hydrate that has to be mixed with acetic acid. For example for 1 ml of a 10mM acetate buffer with pH 5.5, 0.17mg of sodium acetate trihydrate is mixed with 0.08mg acetic acid, wherein the acetic acid is usually used for pH adjustment.

In one embodiment, the at least one buffer is histidine, in the same embodiment the pH of the composition is 5 to 6.5, for example 5.5 to 6.5, 5.7 to 6.3, for example 5.8 to 6.2, for instance 5.9 to 6.1, such as 5.9, 6.0 or 6.1.

The inventors further discovered a stabilizing effect of the excipient sucrose, wherein sucrose reduces the sub-visible particle formation.

The compositions of the invention thus comprise sucrose as an excipient.

In one embodiment, the composition comprises 10 to 150mM of sucrose, for example 30 to 150mM, 40 to 80mM, 50 to 80mM, 60 to 80mM, 65 to 75mM, 70 to 75mM, for example 73mM. In a further example the composition comprises for example 120 to 150mM sucrose, 140mM to 150mM, for example 146mM sucrose.

The composition further comprises 100 to 300mM of mannitol, for example 120 to 280mM, 140 to 260mM, 160mM to 240mM, 180 to 220mM, 190 to 210mM, 200 to 210mM, 204 to 208mM, for example 206mM. In a further example the composition comprises for example 120 to 140mM mannitol, 125mM to 135mM, for example 132mM mannitol.

The inventors discovered that adding small amounts of the surfactant polysorbate 80 reduces aggregation, in particular dimerization of the formulated anti-CD19 antibody-drug conjugate and/or minimizes the formation of sub-visible particles in the composition. They further discovered that the amount of polysorbate 80 should not exceed the amount of 0.01% of polysorbate 80.

The term "surfactant" and "detergent" may be used herein interchangeably.

A "polysorbate" is an emulsifier derived from PEG-ylated sorbitan (a derivative of sorbitol) esterified with fatty acids. This class of agents comprises, among others, polysorbates 20, 21, 40, 60, 61, 65, 80, 81, 85, and 120.
In one embodiment the composition comprises the surfactant polysorbate 20 (common commercial brand names include Alkest TW 20 and Tween 20) and/or polysorbate 80 (common commercial brand names include Alkest TW 80, Canarcel, Poegasorb 80, Tween 80).

In one embodiment, the composition comprises less than 0.01% w/v of polysorbate. In a further embodiment the polysorbate is polysorbate 80 (PS80).

In a further embodiment, the composition comprises 0.0025% w/v to 0.01% w/v of the surfactant polysorbate 80, for example 0.0025% w/v to 0.0075%, 0.004% w/v to 0.0075%, for instance 0.004% to 0.006%, for example 0.0045 to 0.0055% w/v, for instance 0.005% of polysorbate 80.

According to one example the composition of the invention comprises 5mg/ml anti-CD19 antibody-drug conjugate, 10mM acetate buffer at pH 5.5, 73mM sucrose, 206mM mannitol, and less than 0.01% w/v PS80.

According to a further example the composition of the invention comprises 5mg/ml anti-CD19 antibody-drug conjugate, 10mM acetate buffer at pH 5.5, 73mM sucrose, 206mM mannitol, and 0.005% w/v polysorbate 80.

According to another example the composition of the invention comprises 5mg/ml anti-CD19 antibody-drug conjugate 0.01% w/v PS80.

According to a further example the composition of the invention comprises 5mg/ml anti-CD19 antibody-drug conjugate, 10mM histidine buffer at pH 6.0, 73mM sucrose, 206mM mannitol, and 0.005% w/v polysorbate 80.

According to a further example the composition of the invention comprises 5mg/ml anti-CD19 antibody-drug conjugate, 10mM histidine buffer at pH 6.0, 146mM sucrose, 132mM mannitol, and 0.005% w/v polysorbate 80.

In one embodiment one or more other pharmaceutically acceptable carriers, excipients or stabilizers such as those described in Remington’s Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980) may be included in the composition provided that they do not significantly adversely affect the desired characteristics of the composition. Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed and include additional buffering agents; co-solvents; antioxidants including ascorbic acid and methionine; chelating agents such as EDTA; metal complexes (e.g. Zn-protein complexes); biodegradable polymers such as polyesters; and/or salt-forming counter-ions such as sodium.

The composition of the invention may also be combined with one or more other therapeutic agents as necessary for the particular indication being treated, in particular those with complementary activities that do not adversely affect the anti-CD19 antibody-
drug conjugate of the composition. Such therapeutic agents are suitably present in combination in amounts that are effective for the purpose intended.

Medicaments and Treatments using the Pharmaceutical Composition

In one embodiment, the invention provides a method of treating or preventing a disease or disorder comprising administering to a subject in need thereof a therapeutically effective amount of a pharmaceutical composition of the invention.

The invention also relates to a pharmaceutical composition of the invention for use as a medicament. The invention further refers to the use of a pharmaceutical composition of the invention for the preparation of a medicament for treating a disease or disorder in a subject. In one embodiment, the invention refers to the use of a pharmaceutical composition for treating a disease or disorder in a subject.

In the context of the invention, the term "treating" or "treatment", refers to a therapeutic use (i.e. on a subject having a given disease) and means reversing, alleviating, inhibiting the progress of one or more symptoms of such disorder or condition. Therefore treatment does not only refer to a treatment that leads to a complete cure of the disease, but also to treatments that slow down the progression of the disease and/or prolong the survival of the subject.

By "preventing," is meant a prophylactic use (i.e. on a subject susceptible of developing a given disease).

A "disease," or "disorder" is any condition that would benefit from treatment with the antibody anti-CD19-drug conjugate. This includes chronic and acute disorders or diseases including those pathological conditions which predisposes the subject to the disorder in question.

The term "in need of treatment" refers to a subject having already the disorder as well as those in which the disorder is to be prevented.

In one embodiment, the disorder refers to B-cell diseases such as such as lymphoma, leukemia, or autoimmune disease.

In a further embodiment, the B-cell disease is a CD19+ B-cell malignancy.

As used herein, the term "CD19+ B-cell malignancy" refers to any malignancy expressing the CD19 cell surface antigen. Said CD19+ B-cell malignancies can be for example leukemia, such as acute lymphoblastic leukemia (ALL) or lymphoma, such as a Non-Hodgkin's lymphoma (NHL). In one embodiment, the Non-Hodgkin's lymphoma symptom can be a Diffuse Large B-cell lymphoma (DLBCL), a folicular lymphoma (FL), a
Mantle cell lymphoma (MCL), a Marginal zone lymphoma (MZL), a Small lymphocytic
lymphoma (SLL) or a Waldenstrom macroglobulinemia (WM).

In one particular embodiment, said subject has already been treated for a CD19+
B-cell malignancy. In particular, said patient may have failed therapy, such as a
chemotherapy or an antibody therapy, such as rituximab therapy.

In a particular embodiment, said Non-Hodgkin’s lymphoma is a relapsed or
refractory B-cell non-Hodgkin’s lymphoma.

In another particular embodiment, said Non-Hodgkin’s lymphoma symptom is a B-
cell non-Hodgkin’s lymphoma expressing CD19.

In another particular embodiment, said subject has already been treated for the
Non-Hodgkin’s lymphoma symptom. In particular, said patient may have failed therapy,
such as a chemotherapy or a rituximab therapy.

In another particular embodiment, said Non-Hodgkin’s lymphoma symptom is a
rituximab resistant disease.

In another particular embodiment, said patient has received a autologous or
allogeneic stem cell transplant.

An “effective amount” refers to an amount effective, at dosages and for periods of
time necessary, to achieve the desired therapeutic or prophylactic result.

A “therapeutically effective amount” of the pharmaceutical composition of the
invention may vary according to factors such as the disease state, age, sex, and weight of
the individual, and the ability of the anti-CD19 antibody-drug conjugate in context of the
invention, to elicit a desired therapeutic result. A therapeutically effective amount
encompasses an amount in which any toxic or detrimental effects of the anti-CD19
antibody-drug conjugate are outweighed by the therapeutically beneficial effects. A
therapeutically effective amount also encompasses an amount sufficient to confer benefit,
e.g., clinical benefit.

A physician or veterinarian having ordinary skill in the art can readily determine
and prescribe the effective amount of the pharmaceutical composition of the present
invention required. For example, the physician or veterinarian could start doses of the
composition of the invention at levels lower than that required in order to achieve the
desired therapeutic effect and gradually increase the dosage until the desired effect is
achieved.

In adults, the dose of the composition may be for example 55 mg/m² given
administered weekly for example for 4 weeks then bi-weekly.

In one embodiment, the pharmaceutical composition is administered to a subject in
accordance with known methods, such as intravenous administration, e.g., as a bolus or
by continuous infusion over a period of time that is typically comprised between 10 minutes and 4 hours, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intratumoral, peritumoral, intralesional, perilesional or intrathecal administration, for instance by intramuscular or subcutaneous administration.

In another embodiment, the composition is administered by nebulisation.

For the prevention or treatment of disease, the appropriate dosage of the composition of the invention will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the anti-CD19 antibody-drug conjugate comprised in the composition is administered for preventive or therapeutic purposes, previous therapy, the subject's clinical history and response to the anti-CD19 antibody-drug conjugate of the composition, and the discretion of the attending physician. The composition is suitably administered to the subject at one time or over a series of treatments. Depending on the type and severity of the disease, about 5 to 72 mg/m² of the anti-CD19 antibody-drug conjugate of the composition an initial candidate dosage for administration to the subject, whether, for example, by one or more separate administrations, or by continuous infusion.

If another therapeutic agent is administered, it is usually administered at dosages known therefore, or optionally lowered due to combined action of the drugs or negative side effects attributable to administration of the therapeutic agent. Preparation and dosing schedules for such therapeutic agents may be used according to manufacturers' instructions or as determined empirically by the skilled practitioner.

Articles of Manufacture

The present invention also concerns an article of manufacture comprising:

- a) a packaging material;
- b) a composition according to the invention comprising an anti-CD19 antibody-drug conjugate, at least one buffer agent selected from the group consisting of acetate and histidine, sucrose, mannitol, polysorbate, wherein the pH of the composition is 5.0 to 6.5, and
- c) a label or package insert contained within said packaging material indicating that said conjugate is administered at a dose of 5 to 72 mg/m².

The present invention also concerns an article of manufacture comprising:

- a) a packaging material;
- b) a composition according to the invention comprising an anti-CD19 antibody-drug conjugate, at least one buffer agent selected from the group consisting of acetate
and histidine, sucrose, mannitol, polysorbate, wherein the pH of the composition is 5.0 to 6.5, and

c) a label or package insert contained within said packaging material indicating that said conjugate is administered for treating a B-cell diseases.

In one embodiment the composition is available as a concentrate solution for infusion at for example 3 to 5mg/ml in glass vial, such as 5ml to 40ml glass vials, for example 5, 10, 15, 20, 25, 30 ml glass vials.

In a further embodiment the label or package insert further specifies that the composition is for intravenous injection.

In a further embodiment, the invention herein also concerns a device comprising a pharmaceutical composition of the invention. Such devices may hold a liquid volume of between 0.1 ml and 2ml (single use), or 0.5 and 1.5ml. In one embodiment the volume is about 0.8 or about 1.0ml.

In one embodiment the device is for subcutaneous delivery. For subcutaneous delivery, the composition may be administered via syringe (e.g. pre-filled syringe); auto injector; injection device (e.g. the INJECT-EASE™ and GENJECT™ device); injector pen (such as the GENPEN™); or other device suitable for administering a suspension composition subcutaneously. In one embodiment the device herein is a pre-filled syringe.

In a related aspect, the invention provides a method of making an article of manufacture comprising filling a container with the pharmaceutical composition of the invention.

Embodiments of the container in the article of manufacture include: syringes (such as pre-filled syringe), auto Injectors, bottles, vials (e.g. dual chamber vials), and test tubes, etc. The container holds the suspension composition and the label on, or associated with, the container may indicate directions for use. The article of manufacture may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use as noted in the previous section.

Kit

According to another aspect of the present invention, a kit is provided; said kit comprises at least one container comprising at least one pharmaceutical composition as described above, and an injection device. In one embodiment the kit or the injection device is adapted for intramuscular or subcutaneous administration, for instance for subcutaneous administration. In one embodiment the kit further comprises instructions for administration of the composition, for example for subcutaneous administration.
According to yet another embodiment of the present invention, the use of a device comprising the pharmaceutical composition or of a kit according to the invention is provided. In a further embodiment the invention refers to the use of the device comprising the pharmaceutical composition of the invention or of a kit in context of the invention for treatment of at least one disease as described above.

Method for reducing aggregation

In view to the above, the present invention also relates to a method for reducing aggregation of an anti-CD19 antibody-drug conjugate by using a composition according to the present invention. A skilled person will understand that formulating a therapeutically active anti-CD19 antibody-drug conjugate in a composition according to the present invention which is susceptible to aggregation or less stable will lead to a reduced amount of aggregation and stabilization of the anti-CD19 antibody-drug conjugate compared to a reference composition.

Thus, in one aspect, the present invention relates to an ex vivo and/or in vitro method for reducing aggregation of an anti-CD19 antibody-drug conjugate, comprising formulating anti-CD19 antibody-drug conjugate in a composition comprising:

b) at least one buffer agent selected from the group consisting of acetate and histidine,

c) sucrose,

d) mannitol,

e) polysorbate, and

wherein the pH of the composition is 5.0 to 6.5.

In a further aspect the invention refers to a method of stabilizing an anti-CD19 antibody-drug conjugate comprising formulating an anti-CD19 antibody-drug conjugate in a composition of the invention.

An anti-CD19 antibody-drug conjugate which is "susceptible to aggregation" has been found to aggregate with other anti-CD19 antibody-drug conjugate molecule(s) for example in form of dimers, especially upon storage at increased temperatures such as 40°C for 1 week. An anti-CD19 antibody-drug conjugate that is susceptible to aggregation might be for example an anti-CD19 antibody-drug conjugate that has less than 94%, less than 93%, less than 92%, less than 90% of monomer after storage at about 40°C for one week as measured by SEC.

By "reducing, aggregation" is intended preventing or decreasing the amount of, aggregation relative to the same anti-CD19 antibody-drug conjugate formulated in a reference composition comprising 10mM citrate, 135mM NaCl at pH 5.5.
Any combination of the above embodiments makes part of the invention.

Throughout the instant application, the term "comprising" is to be interpreted as encompassing all specifically mentioned features as well optional, additional, unspecified ones. As used herein, the use of the term "comprising" also discloses the embodiment wherein no features other than the specifically mentioned features are present (i.e. "consisting of"). Furthermore the indefinite article "a" or "an" does not exclude a plurality. The mere fact that certain measures are recited in mutually different dependent claims does not indicate that a combination of these measures cannot be used to advantage.

The invention will now be described in more detail with reference to the following examples. All literature and patent documents cited herein are hereby incorporated by reference. While the invention has been illustrated and described in detail in the the foregoing description, the examples are to be considered illustrative or exemplary and not restrictive.

**BRIEF DESCRIPTION OF THE SEQUENCES**

SEQ ID NO: 1, 2 and 3 show the amino acid sequences of CDR1-L, CDR2-L and CDR3-L of the anti-CD19 antibody huB4.

SEQ ID NO: 4, 5 and 6 show the amino acid sequences of CDR1-H, CDR2-H and CDR3-H of the anti-CD19 antibody huB4.

SEQ ID NO: 7 shows the light chain sequence of the anti-CD19 antibody huB4.

SEQ ID NO: 8 shows the heavy chain sequence of the anti-CD19 antibody huB4.

**FIGURES**

**Figure 1**: Graph representing size exclusion chromatograms of SAR3419 samples comprising different amounts of Polysorbate 80. The SAR3419 samples comprise (G) no PS80, (F) 0.0005% w/v PS80, (E) 0.001% w/v PS80, (D) 0.0025% w/v PS80, (C) 0.005% w/v PS80, (B) 0.0075% w/v PS80, and (A) 0.01% w/v PS80. The samples were analysed on a Superdex 200 (Amersham Bioscience). The peak eluted at 26 and 28 min contains high molecular species, the peak eluted at 36 to 38 min contains dimers, the peak eluted at 40 to 48 min contains monomers. It can be concluded from the different chromatograms that are displayed in the graph that a low percentage of PS80 (which should not exceed 0.01%) allows to decrease the formation of visible and sub-visible particles and also to decrease the formation of soluble aggregates.

**Figure 2**: Graph representing the total free maytansinoid species in SAR 3419 samples. Total free maytansinoid species are indicated in % of total DM4. The amount of free
maytansinoid species was measured at different time points during storage of SAR3419 samples in (A) buffer comprising 5mg/ml SAR3419 in 10 mM acetate, sucrose 2.5% w/v, mannitol 3.75% w/v, PS80 0.005% w/v, pH 5.5 (B) 1 mg/ml SAR3419 in 10mM citrate, 135mM sodium chloride, pH 5.5, and (C) 5 mg/ml SAR3419 in 10mM citrate, 135mM sodium chloride, pH 5.5. The content of free maytansinoid compounds appeared to be higher in acetate formulation compared to citrate formulation.

**Figure 3**: Graph representing the dimer formation of SAR3419 in different buffers. The dimer content of different SAR3419 samples is represented in % after storage at 25°C at different time points, wherein SAR3419 is formulated in (A) 10mM citrate buffer, 135mM NaCl at pH 5.5, (B) 10mM histidine buffer, sucrose 5.0% w/v, mannitol 2.41% w/v, PS80 0.005% w/v, at pH 6, and (C) 10 mM acetate, sucrose 2.5% w/v, mannitol 3.75% w/v, PS80 0.005% w/v, pH 5.5. Acetate formulation allows to significantly limiting formation of dimers, what is of special interest for the use of the product.

**Figures 4 and 5**: Graphs representing monomer, dimer, high molecular weight and free maytansinoid content of SAR3419 samples in % after storage for 1 week at 40°C in 10mM histidine buffer, sucrose 5.0% w/v, mannitol 2.41% w/v, PS80 0.005% w/v, at different pH values. Triangles represent the content of high molecular weight species, squares represent the dimer content and dots represent the free maytansinoid content in figure 4, diamonds as shown in figure 5 represent the monomer content. Optimal pH has been selected based on a compromise between formation of two main by-products: High Molecular Weight compounds (HMW) and Free Maytansinoids compounds.

**Figures 6 and 7**: Graphs representing the particle content for SAR3149 samples in different buffers with particles having a size >10µm or >25 µm. The amount of visible and sub-visible particles were measured using HIAC/ROYCO analysis with a sample volume of 25 ml after 0, 1, 3 and 6 months storage at 5°C (figure 6) and after 0, 1, 3 and 6 months storage at 25°C (figure 7). Acetate (acetate 10mM, sucrose 2.5% w/v, mannitol 3.75% w/v, PS80 0.005% w/v, pH 5.5) and histidine (histidine 10mM, sucrose 5.0% w/v, mannitol 2.41% w/v, PS80 0.005% w/v, pH 6.0) formulations allow limiting formation of sub-visible particles after 6 months when compared to citrate formulation (citrate 10mM, 135mM NaCl, pH 5.5).
CLAIMS

1. A pharmaceutical composition comprising:
   a) an anti-CD19 antibody-drug conjugate,
   b) at least one buffer agent selected from the group consisting of acetate and histidine,
   c) sucrose,
   d) mannitol, and
   e) polysorbate,

   wherein the pH of the composition is 5.0 to 6.5.

2. The pharmaceutical composition according to claim 1, wherein the composition comprises less than 0.01% w/v of polysorbate.

3. The pharmaceutical composition according to claim 1 or 2, wherein the composition comprises 5 to 15mM of said at least one buffer agent.

4. The pharmaceutical composition according to any one of claims 1 to 3, wherein the composition comprises 8 to 12mM of said at least one buffer agent.

5. The pharmaceutical composition according to any one of claims 1 to 4, wherein the at least one buffer agent is acetate.

6. The pharmaceutical composition according to claim 5, wherein the pH of the composition comprising acetate as a buffer agent is 5.4 to 5.6.

7. The pharmaceutical composition according to any one of claims 1 to 4, wherein the at least one buffer agent is histidine.

8. The pharmaceutical composition according to claim 7, wherein the pH of the composition comprising histidine as a buffer agent is 5.9 to 6.1.

9. The pharmaceutical composition according to any one of claims 1 to 8, wherein the polysorbate is polysorbate 80.

10. The pharmaceutical composition according to any one of claims 1 to 9, wherein the composition comprises 0.0025% w/v to 0.0075% w/v polysorbate 80.
11. The pharmaceutical composition according to any one of claims 1 to 10, wherein the composition comprises 0.0045% to 0.0055% w/v polysorbate 80.

12. The pharmaceutical composition according to any one of claims 1 to 11, wherein the composition comprises 4 to 6 mg/ml anti-CD19 antibody-drug conjugate.

13. The pharmaceutical composition according to any one of claims 1 to 12, wherein the composition is a liquid composition.

14. The pharmaceutical composition according to any one of claims 1 to 13, comprising
   a) 5 mg/ml anti-CD19 antibody-drug conjugate,
   b) 10mM acetate buffer,
   c) 73mM sucrose,
   d) 206mM mannitol, and
d) 0.005% w/v polysorbate 80,
wherein the pH is 5.5.

15. The pharmaceutical composition according to any of claims 1 to 14, wherein the anti-CD19 antibody-drug conjugate comprises an anti-CD19-antibody conjugated to a maytansinoid.

16. The pharmaceutical composition according to claim 15, wherein the maytansinoid is DM4.

17. The pharmaceutical composition according to any of claims 1 to 16, wherein the anti-CD19 antibody comprises a light chain variable domain comprising CDR1-L of sequence SEQ ID NO: 1, CDR2-L of SEQ ID NO: 2 and CDR3-L of sequence SEQ ID NO: 3, and a heavy chain variable domain comprising CDR1-H of sequence SEQ ID NO: 4, CDR2-H of sequence SEQ ID NO: 5 and CDR3-H of sequence SEQ ID NO: 6.

18. The pharmaceutical composition according to any of claims 1 to 17, wherein the anti-CD19 antibody comprises a light chain consisting of the amino acid sequence SEQ ID NO: 7 and a heavy chain consisting of the amino acid sequence represented by SEQ ID NO: 8.

19. The pharmaceutical composition according to claim 15, wherein the anti-CD19 antibody is conjugated to a maytansinoid through a cleavable linker.
20. The pharmaceutical composition according to claim 19, wherein the cleavable linker is a N-succinimidyl 4-(2-pyridylthio)butanoate (SPDB) linker.

21. The pharmaceutical composition according to claim 20, wherein the anti-CD19 antibody -drug conjugate is coltuximab ravtansine.
FIG. 1
FIG. 4

FIG. 5
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K9/00 A61K47/18 A61K47/26 A61K47/48

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

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, WPI Data, CHEM ABS Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:

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Date of the actual completion of the international search: 5 August 2016

Date of mailing of the international search report: 18/08/2016

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

Authorized officer: Palma, Vera

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