NOVEL ANTIBODY FORMULATION

The present invention relates to a pharmaceutical formulation of an antibody against Epidermal Growth Factor Receptor (anti-EGFR antibody), a process for the preparation of said formulation and uses of the formulation.

In a first aspect, the invention relates to a pharmaceutical formulation comprising:
1 to 200 mg/ml of an IgG-class anti-EGFR antibody;
1 to 100 mM of a buffering agent;
0.001 to 1% (w/v) of a surfactant;
1 to 500 mM of at least one stabilizer;
at a pH in the range of from 4.0 to 7.0.

The formulation according to the invention may be provided in liquid form, lyophilized form or in liquid form reconstituted from a lyophilized form.

Unless otherwise defined in the following, terms are used herein as generally used in the art.

The term "IgG-class anti-EGFR antibody", as used herein, includes antibodies of the immunoglobulin G (IgG) class of immunoglobulins, which target the human epidermal growth factor receptor (EGFR), also known as HER-1 or ErbB-1 (Ullrich et al., Nature 309, 418-425 (1984); SwissProt Accession #P00533; secondary accession numbers: O00688, O00732, P06268, Q14225, Q68GS5, Q92795, Q9BZS2, Q9GZX1, Q9H2C9, Q9H3C9, Q9UMD7, Q9UMD8, Q9UMG5), as well as naturally-occurring isoforms and variants thereof.

Exemplary IgG-class anti-EGFR antibodies useful in the formulation according to the present invention include cetuximab/IMC-C225 (Erbitux®, described in Goldstein et al., Clin Cancer Res 1, 1311-1318 (1995)), panitumumab/ABX-EGF (Vectibix®, described in Yang et al., Cancer Res 59, 1236-1243 (1999), Yang et al., Critical Reviews in Oncology/Hematology 38, 17-23 (2001)), nimotuzumab/h-R3 (TheraCim®, described in Mateo et al., Immunotechnology 3, 71-81 (1997); Crombet-Ramos et al., Int J Cancer 101, 567-575 (2002), Boland & Bebb, Expert Opin Biol Ther 9, 1199-1206 (2009)), matuzumab/EMD 72000 (described in Bier et al., Cancer Immunol Immunother 46, 167-173 (1998), Kim, Curr Opin Mol Ther 6, 96-103 (2004)), and

zalutumumab/2F8 (described in Bleeker et al., J Immunol 173, 4699-4707 (2004), Lammerts van Bueren, PNAS 105, 6109-6114 (2008)).

Preferred IgG-class anti-EGFR antibodies useful in the formulation according to the present invention are described in WO 2006/082515 and WO 2008/017963, the entire content of which is incorporated herein by reference, and include antibodies which are characterized in that they are chimeric antibodies having the binding specificity of the rat monoclonal antibody ICR62 and that their effector functions are enhanced by altered glycosylation.

Preferred antibodies are characterized in that they comprise at least one (i.e. one, two, three, four, five, or six) complementarity determining region (CDR) of the rat ICR62 antibody, or a variant or truncated form thereof containing at least the specificity-determining residues for said CDR, and comprising a sequence derived from a heterologous polypeptide. By "specificity-determining residue" is meant those residues that are directly involved in the interaction with the antigen. Specifically, preferred antibodies comprise: (a) a heavy chain CDR1 sequence selected from a group consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, and SEQ ID NO:13; (b) a heavy chain CDR2 sequence selected from a group consisting of: SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, and SEQ ID NO:30; and (c) the heavy chain CDR3 sequence SEQ ID NO:31. Preferred antibodies further comprise: (a) a light chain CDR1 sequence selected from the group consisting of SEQ ID NO:32 and SEQ ID NO:33; (b) the light chain CDR2 sequence SEQ ID NO:34; and (c) the light chain CDR3 sequence SEQ ID NO:35.

More preferred antibodies are characterized in that they comprise at least three CDRs of the rat ICR62 antibody, or variants or truncated forms thereof containing at least the specificity-determining residues for said CDRs.

Most preferred antibodies comprise:

a) in the heavy chain variable domain a CDR1 of SEQ ID NO:1, a CDR2 of SEQ ID NO:16, and a CDR3 of SEQ ID NO:31, and

b) in the light chain variable domain a CDR1 of SEQ ID NO:33, a CDR2 of SEQ ID NO:34, and a CDR3 of SEQ ID NO:35.

Other preferred antibodies comprise the heavy chain variable domain of the rat ICR62 antibody according to SEQ ID NO:36, or a variant thereof; and a non-murine polypeptide. Further, preferred antibodies may comprise the light chain variable domain of the rat ICR62 antibody according to SEQ ID NO:37, or a variant thereof; and a non-murine polypeptide.

More preferred antibodies comprise the heavy chain variable domain of SEQ ID NO:38 and the light chain variable domain of SEQ ID NO:39.

Preferred antibodies are primatized or, more preferred, humanized antibodies.

A "humanized" antibody refers to a chimeric antibody comprising amino acid residues from non-human hypervariable regions (HVRs) and amino acid residues from human framework regions (FRs). A humanized antibody comprises substantially typically two variable domains, in which all or substantially all of the HVRs (e.g., complementarity determining regions (CDRs)) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization. Humanization may be achieved by various methods known in the art, including, but not limited to, (a) grafting the entire non-human variable domains onto human constant regions to generate chimeric antibodies, (b) grafting only the non-human (e.g., donor antibody) CDRs onto human (e.g., recipient antibody) framework and constant regions with or without retention of critical framework residues (e.g., those that are important for retaining good antigen binding affinity or antibody functions), (c) grafting only the non-human specificity-determining regions (SDRs or a-CDRs; the residues critical for the antibody-antigen interaction) onto human framework and constant regions, or (d) transplanting the entire non-human variable domains, but "cloaking" them with a human-like section by replacement of surface residues.

Preferably, the antibodies useful in the formulation according to the present invention comprise a human Fc region. More preferably, the human heavy chain constant region is Ig gamma-1, as set forth in SEQ ID NO:40, i.e. the antibody is of human IgG1 subclass.

Preferred antibodies have been glycoengineered to have an altered oligosaccharide structure in the Fc region.

Specifically, preferred antibodies have an increased proportion of non-fucosylated oligosaccharides in the Fc region as compared to non-glycoengineered antibodies. Preferably, the percentage of non-fucosylated oligosaccharides is at least 20%, more preferably at least 50, at

least 70%, most preferably at least 75%. The non-fucosylated oligosaccharides may be of the hybrid or complex type.

Preferred antibodies may also have an increased proportion of bisected oligosaccharides in the Fc region. Preferably, the percentage of bisected oligosaccharides in the Fc region of the antibody is at least 50%, more preferably, at least 60%, at least 70%, at least 80%, or at least 90%, and most preferably at least 90-95% of the total oligosaccharides.

Particularly preferred antibodies have an increased proportion of bisected, non-fucosylated oligosaccharides in the Fc region. The bisected, non-fucosylated oligosaccharides may be either hybrid or complex. Specifically, antibodies are preferred in which at least 15%, more preferably at least 20%, more preferably at least 25%, more preferably at least 30%, more preferably at least 35% of the oligosaccharides in the Fc region of the antibody are bisected, non-fucosylated.

The term "glycoengineered", as used herein, includes any manipulation of the glycosylation pattern of a naturally occurring or recombinant protein, polypeptide or a fragment thereof. Glycoengineering includes metabolic engineering of the glycosylation machinery of a cell, including genetic manipulations of the oligosaccharide synthesis pathways to achieve altered glycosylation of glycoproteins expressed in these cells. Furthermore, glycoengineering includes the effects of mutations and cell environment on glycosylation. In particular, glycoengineering can result in altered glycosyltransferase activity in a cell, such as altered glucosaminyltransferase and/or fucosyltransferase activity.

The relative amount of non-fucosylated and/or bispecific is the percentage of carbohydrate structures lacking fucose and/or having a bisecting GlcNAc residue, related to all glycostructures identified in an N-Glycosidase F treated protein sample by MALDI-TOF MS.

Preferred antibodies are also characterized in that they have been glycoengineered to have increased effector function and/or increased Fc receptor binding affinity. The term "effector function", as used herein, refers to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody. Preferably, the increased effector function is one or more of the following: increased Fc-mediated cellular cytotoxicity (including increased antibody-dependent cellular cytotoxicity (ADCC)), increased antibody-dependent cellular phagocytosis (ADCP), increased cytokine secretion, increased immune-complex-mediated antigen uptake by antigen-presenting cells, increased binding to natural killer (NK) cells, increased binding to macrophages, increased binding to monocytes, increased binding to polymorphonuclear cells, increased direct signaling inducing apoptosis, increased crosslinking of target-bound antibodies, increased dendritic cell

maturation, or increased T cell priming. The increased Fc receptor binding affinity is preferably increased binding to a Fc activating receptor, most preferably increased binding to FcγRIIIa.

The most preferred IgG-class anti-EGFR antibody useful in the formulations according to the invention is characterized in that it comprises the heavy chain variable domain of SEQ ID NO:38 and the light chain variable domain of SEQ ID NO:39, is humanized, and comprises the human heavy chain constant region Ig gamma-1, as set forth in SEQ ID NO:40. This antibody is termed "hu-ICR62 IgG1 anti-EGFR mAb". hu-ICR62 IgG1 anti-EGFR mAb may or may not be glycoengineered as described above, to have an increased proportion of non-fucosylated oligosaccharides in the Fc region as compared to non-glycoengineered antibodies.

The antibodies useful in the formulations according to the invention are preferably produced by recombinant means, e.g. by those described in WO 2006/082515 and WO 2008/017963. Such methods are widely known in the art and comprise protein expression in prokaryotic or eukaryotic cells with subsequent isolation of the antibody polypeptide and usually purification to a pharmaceutically acceptable purity. For the protein expression, nucleic acids encoding light and heavy chains or fragments thereof are inserted into suitable expression vectors by standard methods. Expression is performed in appropriate host cells, which include cultured cells, preferably cultured mammalian cells such as CHO cells, HEK 293 cells, HEK293-EBNA cells, BHK cells, NS0 cells, SP2/0 cells, YO myeloma cells, P3X63 mouse myeloma cells, PER cells, PER.C6 cells or hybridoma cells, *E. coli* cells, yeast cells, insect cells and plant cells, but also cells comprised within a transgenic animal, transgenic plant or cultured plant or animal tissue. Preferred host cells are CHO cells. The host cells used to express the antibodies may have been manipulated to have altered levels glycosyltransferase activity, such as altered glucosaminyltransferase and/or fucosyltransferase activity, to produce antibodies with an altered glycosylation pattern. Preferably, the host cells have been manipulated to express increased levels of one or more polypeptides having β(1,4)-N-acetylglucosaminyltransferase III (GnTIII) activity, and optionally one or more polypeptides having mannosidase II (ManII) activity. The polypeptide having GnTIII activity may be a fusion polypeptide comprising the catalytic domain of GnTIII and the Golgi localization domain of a heterologous Golgi resident polypeptide, preferably the Golgi localization domain of mannosidase II. The glycoengineering methods that can be employed with the IgG-class anti-EGFR antibodies useful in the present invention are described in WO 2006/082515, WO 2008/017963, U.S. Pat. No. 6,602,684, EP 1071700, WO 1999/54342, U.S. Pat. Appl. Publ. No. 2004/0241817, EP 1587921, WO 2004/065540, Umaña et

al., Nature Biotechnol 17, 176-180 (1999), and Ferrara et al., Biotechn Bioeng 93, 851-861 (2006), the entire contents of each of which are incorporated herein by reference in their entirety.

The antibody is recovered from the cells (supernatant or cells after lysis) by standard techniques, e.g. Protein A affinity chromatography, size exclusion chromatography, and others well known in the art, e.g. as described in WO 2006/082515 and WO 2008/017963.

For the formulation according to the present invention the antibody is used at a concentration of about 1 to about 200 mg/ml, preferably about 1 to about 100 mg/ml, more preferably about 10 to about 75 mg/ml, and most preferably about 20 to about 50 mg/ml.

The term "buffering agent" as used herein denotes a pharmaceutically acceptable excipient, which stabilizes the pH of a pharmaceutical preparation. Suitable buffers are well known in the art and can be found in the literature. For example, citrate salts, acetate salts, histidine salts, succinate salts, malate salts, phosphate salts or lactate salts, and/or the respective free acids or bases thereof, as well as mixtures of the various salts and/or acids and bases thereof can be employed. Preferred pharmaceutically acceptable buffers comprise but are not limited to histidine buffers, citrate buffers, succinate buffers, acetate buffers and phosphate buffers. Preferred buffers are acetate buffers, most preferred is sodium acetate buffer. Other preferred buffers are histidine buffers, i.e. buffers having histidine, generally L-histidine, as buffering agent. Most preferred is L-histidine/HCl buffer, comprising L-histidine or mixtures of L-histidine and L-histidine hydrochloride and pH adjustment achieved with hydrochloric acid. Unless otherwise indicated, the term "L-histidine" when used herein to describe a buffering agent, refers to L-histidine/HCl buffer. L-histidine/HCl buffer can be prepared by dissolving suitable amounts of L-histidine and L-histidine hydrochloride in water, or by dissolving a suitable amount of L-histidine in water and adjusting the pH to the desired value by addition of hydrochloric acid. The abovementioned buffers are generally used at a concentration of about 1 mM to about 100 mM, preferably of about 10 mM to about 50 mM, more preferably of about 15 to 30 mM, and most preferably of 20 mM. Regardless of the buffer used, the pH can be adjusted to a value in the range from about 4.0 to about 7.0, preferably about 5.0 to about 6.0, and most preferably about 5.5, with an acid or a base known in the art, e.g. hydrochloric acid, acetic acid, phosphoric acid, sulfuric acid and citric acid, sodium hydroxide and potassium hydroxide.

The term "surfactant" as used herein denotes a pharmaceutically acceptable, surface-active agent. Preferably, a non-ionic surfactant is used. Examples of pharmaceutically acceptable surfactants

include, but are not limited to, polyoxyethylen-sorbitan fatty acid esters (Tween), polyoxyethylene alkyl ethers (Brij), alkylphenylpolyoxyethylene ethers (Triton X), polyoxyethylene-polyoxypropylene copolymers (Poloxamer, Pluronic), and sodium dodecyl sulphate (SDS). Preferred polyoxyethylene-sorbitan fatty acid esters are polysorbate 20 (polyoxyethylene sorbitan monolaureate, sold under the trademark Tween 20™) and polysorbate 80 (polyoxyethylene sorbitan monooleate, sold under the trademark Tween 80™). Preferred polyethylene-polypropylene copolymers are those sold under the names Pluronic® F68 or Poloxamer 188™. Preferred polyoxyethylene alkyl ethers are those sold under the trademark Brij™. Preferred alkylphenylpolyoxyethylene ethers are sold under the tradename Triton X, most preferred is *p-tert*-octylphenoxy polyethoxyethanol (sold under the tradename Triton X-100™). When polysorbate 20 (Tween 20™) and polysorbate 80 (Tween 80™) are used, they are generally used at a concentration range of about 0.001 to about 1%, preferably of about 0.01 to about 0.1%, and more preferably of about 0.02% to about 0.05%. In the formulation of the invention, the concentration of the surfactant is described as a percentage, expressed in weight/volume (w/v).

The term "stabilizer" as used herein denotes a pharmaceutically acceptable excipient, which protects the active pharmaceutical ingredient and/or the formulation from chemical and/or physical degradation during manufacturing, storage and application. Stabilizers include but are not limited to saccharides, amino acids, polyols, e.g. mannitol, sorbitol, xylitol, dextran, glycerol, arabitol, propylene glycol, polyethylene glycol, cyclodextrines, e.g. hydroxypropyl-β-cyclodextrine, sulfobutylethyl-β-cyclodextrine, β-cyclodextrine, polyethylenglycols, e.g. PEG 3000, PEG 3350, PEG 4000, PEG 6000, albumines, e.g. human serum albumin (HSA), bovine serum albumin (BSA), salts, e.g. sodium chloride, magnesium chloride, calcium chloride, chelators, e.g. EDTA as hereafter defined. As mentioned hereinabove, stabilizers can be present in the formulation in an amount of about 1 to about 500 mM, preferably in an amount of about 10 to about 300 mM and more preferably in an amount of about 120 mM to about 300 mM. More than one stabilizer, selected from the same or from different groups, can be present in the formulation.

The term "saccharide" as used herein includes monosaccharides and oligosaccharides. A monosaccharide is a monomeric carbohydrate which is not hydrolysable by acids, including simple sugars and their derivatives, e.g. aminosugars. Saccharides are usually in their D conformation. Examples of monosaccharides include glucose, fructose, galactose, mannose,

sorbose, ribose, deoxyribose, neuraminic acid. An oligosaccharide is a carbohydrate consisting of more than one monomeric saccharide unit connected via glycosidic bond(s) either branched or in a linear chain. The monomeric saccharide units within an oligosaccharide can be identical or different. Depending on the number of monomeric saccharide units the oligosaccharide is a di-, tri-, tetra- penta- and so forth saccharide. In contrast to polysaccharides the monosaccharides and oligosaccharides are water soluble. Examples of oligosaccharides include sucrose, trehalose, lactose, maltose and raffinose. Preferred saccharides are sucrose and trehalose (i.e. α,α-D-trehalose), most preferred is sucrose. Trehalose is available as trehalose dihydrate. Sacchrides can be present in the formulation in an amount of about 100 to about 500 mM, preferably in an amount of about 200 to about 300 mM, most preferably in an amount of about 240 mM.

The term "amino acid" as used herein denotes a pharmaceutically acceptable organic molecule possessing an amino moiety located at α-position to a carboxylic group. Examples of amino acids include but are not limited to arginine, glycine, ornithine, lysine, histidine, glutamic acid, asparagic acid, isoleucine, leucine, alanine, phenylalanine, tyrosine, tryptophane, methionine, serine, proline. The amino acid employed is preferably in each case the L-form. Basic amino acids, such as arginine, histidine, or lysine, are preferably employed in the form of their inorganic salts (advantageously in the form of the hydrochloric acid salts, i.e. as amino acid hydrochlorides). Preferred amino acids are arginine hydrochloride and methionine. Methionine is preferably used at a concentration of about 10 to about 25 mM, most preferably about 10 mM. Arginine hydrochloride is preferably used at a concentration of about 100 to about 200 mM, most preferably at a concentration of about 155 mM.

A subgroup within the stabilizers are lyoprotectants. The term "lyoprotectant" denotes pharmaceutically acceptable excipients, which protect the labile active ingredient (e.g. a protein) against destabilizing conditions during the lyophilisation process, subsequent storage and reconstitution. Lyoprotectants comprise but are not limited to the group consisting of saccharides, polyols (such as e.g. sugar alcohols) and amino acids. Preferred lyoprotectants can be selected from the group consisting of saccharides such as sucrose, trehalose, lactose, glucose, mannose, maltose, galactose, fructose, sorbose, raffinose, neuraminic acid, amino sugars such as glucosamine, galactosamine, N-methylglucosamine ("Meglumine"), polyols such as mannitol and sorbitol, and amino acids such as arginine and glycine or mixtures thereof. Lyoprotectants are generally used in an amount of about 10 to 500mM, preferably in an amount of about 10 to about 300 mM and more preferably in an amount of about 100 to about 300 mM.

A subgroup within the stabilizers are antioxidants. The term "antioxidant" denotes pharmaceutically acceptable excipients, which prevent oxidation of the active pharmaceutical ingredient. Antioxidants comprise but are not limited to ascorbic acid, gluthathione, cysteine, methionine, citric acid, EDTA. Antioxidants can be used in an amount of about 0.01 to about 100 mM, preferably in an amount of about 5 to about 50 mM and more preferably in an amount of about 5 to about 25 mM.

The formulations according to the invention may also comprise one or more tonicity agents. The term "tonicity agents" denotes pharmaceutically acceptable excipients used to modulate the tonicity of the formulation. The formulation can be hypotonic, isotonic or hypertonic. Isotonicity in general relates to the osmotic pressure of a solution, usually relative to that of human blood serum (around 250-350 mOsmol/kg). The formulation according to the invention can be hypotonic, isotonic or hypertonic but will preferably be isotonic. An isotonic formulation is liquid or liquid reconstituted from a solid form, e.g. from a lyophilized form, and denotes a solution having the same tonicity as some other solution with which it is compared, such as physiologic salt solution and the blood serum. Suitable tonicity agents comprise but are not limited to sodium chloride, potassium chloride, glycerine and any component from the group of amino acids or sugars, in particular glucose. Tonicity agents are generally used in an amount of about 5 mM to about 500 mM.

Within the stabilizers and tonicity agents there is a group of compounds which can function in both ways, i.e. they can at the same time be a stabilizer and a tonicity agent. Examples thereof can be found in the group of sugars, amino acids, polyols, cyclodextrines, polyethyleneglycols and salts. An example for a sugar which can at the same time be a stabilizer and a tonicity agent is trehalose.

The formulations may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of presence of microorganisms may be ensured both by sterilization procedures, and by the inclusion of various antibacterial and antifungal agents, e.g. paraben, chlorobutanol, phenol, sorbic acid, and the like. Preservatives are generally used in an amount of about 0.001 to about 2% (w/v). Preservatives comprise but are not limited to ethanol, benzyl alcohol, phenol, m-cresol, p-chlor-m-cresol, methyl or propyl parabens, benzalkonium chloride.

In a first aspect the present invention relates to a pharmaceutical formulation comprising:

1 to 200 mg/ml of an IgG-class anti-EGFR antibody;
1 to 100 mM of a buffering agent;
0.001 to 1% (w/v) of a surfactant;
1 to 500 mM of at least one stabilizer
at a pH in the range of from 4.0 to 7.0.

In a preferred embodiment, the concentration of the IgG-class anti-EGFR antibody comprised in the formulation according to the invention is in the range of 1 to 100 mg/ml, preferably 10 to 75 mg/ml, most preferably 20 to 50 mg/ml. Particularly preferred is a concentration of 25 mg/ml.

In another preferred embodiment, the buffering agent comprised in the formulation according to the invention is a histidine buffer, preferably a L-histidine/HCl buffer, or an acetate buffer, preferably a sodium acetate buffer. Particularly preferred is a L-histidine/HCl buffer (i.e. L-histidine as the buffering agent).

Preferably, the buffering agent is at a concentration of 10 to 50 mM, more preferably 15 to 30 mM, most preferably 20 mM.

Preferably, the buffering agent provides a pH of 5.0 to 6.0, more preferably 5.5 ± 0.3.

In a preferred embodiment, the surfactant comprised in the formulation according to the invention is a polysorbate, preferably polysorbate 20 or polysorbate 80, most preferably polysorbate 80.

Preferably, the surfactant is at a concentration of 0.01 to 0.1%, more preferably 0.02 to 0.05% (w/v), most preferably 0.02 to 0.03 %.

In yet another preferred embodiment, the at least one stabilizer comprised in the formulation according to the invention is selected from the group of salts, preferably sodium chloride, saccharides, preferably trehalose dihydrate or sucrose, and amino acids, preferably arginine hydrochloride.

Preferably, the at least one stabilizer is at a concentration of 120 to 300 mM.

In a preferred embodiment, the formulation according to the invention comprises a first stabilizer selected from the group of salts, saccharides and amino acids, and methionine as a second stabilizer.

In a preferred embodiment, the first stabilizer is at a concentration of 120 to 300 mM, and the second stabilizer methionine is present at a concentration of 5 to 25 mM.

In a particularly preferred embodiment, the formulation according to the invention comprises a saccharide, preferably trehalose dihydrate or sucrose, most preferably sucrose, as a first stabilizer, and methionine as a second stabilizer. The saccharide is preferably at a concentration of about 240 mM, and methionine is preferably at a concentration of about 10 mM.

In another preferred embodiment, the IgG-class anti-EGFR antibody comprised in the formulation according to the invention is a humanized antibody and comprises
a) in the heavy chain variable domain a CDR1 of SEQ ID NO:1, a CDR2 of SEQ ID NO:16 and a CDR3 of SEQ ID NO:31, and
b) in the light chain variable domain a CDR1 of SEQ ID NO:33, a CDR2 of SEQ ID NO:34 and a CDR3 of SEQ ID NO:35.

In a particularly preferred embodiment, the IgG-class anti-EGFR antibody comprised in the formulation according to the invention is hu-ICR62 IgG1 anti-EGFR mAb.

In certain embodiments, the IgG-class anti-EGFR antibody comprised in the formulation according to the invention has been glycoengineered to have an increased proportion, preferably at least 20%, at least 50% or at least 70%, of non-fucosylated oligosaccharides it its Fc region, as compared to the non-glycoengineered antibody.

In a preferred embodiment, the formulation according to the invention comprises:
10 to 50 mg/ml of an IgG-class anti-EGFR antibody;
15 to 30 mM of a buffering agent, selected from L-histidine and sodium acetate;
0.02 to 0.05% (w/v) of a surfactant, selected from polysorbate 20 and polysorbate 80;
120 to 300 mM of at least one stabilizer, selected from trehalose dihydrate, sucrose, arginine hydrochloride and sodium chloride;
optionally, 5 to 25 mM of methionine as a second stabilizer;
at a pH of 5.5 ± 0.3.

In a particularly preferred embodiment, the formulation according to the invention comprises:
10 to 50 mg/ml of hu-ICR62 IgG1 anti-EGFR mAb;
15 to 30 mM L-histidine;
0.02 to 0.05% (w/v) polysorbate 80;

120 to 300 mM of at least one stabilizer, selected from trehalose dihydrate, sucrose, and arginine hydrochloride;
optionally, 5 to 25 mM of methionine as a second stabilizer;
at a pH of 5.5 ± 0.3.

In yet another preferred embodiment, the formulation according to the invention comprises:
10 to 50 mg/ml IgG-class anti-EGFR antibody, preferably hu-ICR62 IgG1 anti-EGFR mAb, 20 mM L-histidine, 240 mM trehalose dihydrate, 0.02 to 0.03% (w/v) polysorbate 80, at pH 5.5;
or
10 to 50 mg/ml IgG-class anti-EGFR antibody, preferably hu-ICR62 IgG1 anti-EGFR mAb, 20 mM L-histidine, 155 mM arginine hydrochloride, 0.02% (w/v) polysorbate 80, at pH 5.5;
or
10 to 50 mg/ml IgG-class anti-EGFR antibody, preferably hu-ICR62 IgG1 anti-EGFR mAb, 20 mM L-histidine, 240 mM trehalose dihydrate, 0.02 to 0.03% (w/v) polysorbate 80, 10 mM methionine at pH 5.5;
or
10 to 50 mg/ml IgG-class anti-EGFR antibody, preferably hu-ICR62 IgG1 anti-EGFR mAb, 20 mM L-histidine, 240 mM sucrose, 0.02 to 0.03% (w/v) polysorbate 80, at pH 5.5;
or
10 to 50 mg/ml IgG-class anti-EGFR antibody, preferably hu-ICR62 IgG1 anti-EGFR mAb, 20 mM L-histidine, 240 mM sucrose, 0.02 to 0.03% (w/v) polysorbate 80, 10 mM methionine at pH 5.5.

In a particularly preferred embodiment, the formulation according to the invention comprises:
20 to 50 mg/ml IgG-class anti-EGFR antibody, preferably hu-ICR62 IgG1 anti-EGFR mAb;
20 mM L-histidine;
0.02 to 0.03% (w/v) polysorbate 80;
240 mM of a first stabilizer, wherein said first stabilizer is a saccharide selected from trehalose dihydrate and sucrose, preferably sucrose;
10 mM of methionine as a second stabilizer;
at a pH of 5.5 ± 0.3.

In certain embodiments, the formulation according to the invention does not comprise sodium chloride. In certain embodiments, the formulation does not comprise a divalent cation. In certain embodiments, the formulation does not comprise lactobionic acid. In certain embodiments, the

formulation does not comprise a polyol. In certain embodiments, the formulation does not comprise a dextran.

The formulation according to the invention can be in a liquid form, in a lyophilized form or in a liquid form reconstituted from a lyophilized form. In certain embodiments, the formulation is in a liquid form.

The term "liquid" as used herein in connection with the formulation according to the invention denotes a formulation which is liquid at a temperature of at least about 2 to about 8°C under atmospheric pressure.

The term "lyophilized" as used herein in connection with the formulation according to the invention denotes a formulation which is manufactured by freeze-drying methods known in the art *per se*. The solvent (e.g. water) is removed by freezing followed by sublimation of the ice under vacuum and desorption of residual water at elevated temperature. The lyophilizate usually has a residual moisture of about 0.1 to 5% (w/w) and is present as a powder or a physically stable cake. The lyophilizate is characterized by a fast dissolution after addition of a reconstitution medium.

The term "reconstituted form" as used herein in connection with the formulation according to the invention denotes a formulation which is lyophilized and re-dissolved by addition of reconstitution medium. Suitable reconstitution media comprise but are not limited to water for injection (WFI), bacteriostatic water for injection (BWFI), sodium chloride solutions (e.g. 0.9% (w/v) NaCl), glucose solutions (e.g. 5% glucose), surfactant-containing solutions (e.g. 0.01% polysorbate 20), pH-buffered solutions (eg. phosphate-buffered solutions).

The formulation according to the invention is physiologically well tolerated, can be prepared easily, can be dispensed precisely and is stable with respect to decomposition products and aggregates over the duration of storage, during repeated freezing and thawing cycles and mechanical stress. It is stable at refrigerator temperatures (2-8°C) over a period of more than 1 year.

The invention further comprises a process for the preparation of the formulations according to the invention. Said process comprises buffer-exchanging the IgG-class anti-EGFR antibody against a diafiltration buffer containing the anticipated buffer composition, and, where required, concentration of the antibody by diafiltration, followed by adding the excipients (e.g. trehalose

dihydrate, sucrose, arginine, sodium chloride, methionine) as stock solutions to the antibody solution, followed by adding the surfactant as stock solution to the antibody/excipient solution, and finally adjusting the antibody concentration to the desired final concentration using buffer solution, whereby also the final excipient and surfactant concentrations are reached.

Alternatively, the excipients can also be added as solids to the starting solution comprising the IgG-class anti-EGFR antibody. If the IgG-class anti-EGFR antibody is in the form of a solid, e.g. a lyophilizate, the formulation according to the invention can be prepared by firstly dissolving the antibody in water or buffer solution, optionally comprising one or more of the excipients, and subsequently adding the further excipients as stock solutions or solids. The IgG-class anti-EGFR antibody can advantageously also be dissolved directly in a solution comprising all further excipients. One or more of the excipients present in the formulation according to the invention may already be added during or at the end of the process for the preparation of the IgG-class anti-EGFR antibody, e.g. by dissolving the IgG-class anti-EGFR antibody directly in a solution comprising one, more than one or preferably all of the excipients of the formulation in the final step of the purification carried out after the preparation of the antibody. If the solution comprising the antibody and the excipients does not yet have the desired pH, this is adjusted by addition of an acid or base, preferably using the acid or base already present in the buffer system. This is followed by sterile filtration.

The invention further comprises the use of the formulations according to the invention for the preparation of a medicament useful for treating diseases, particularly cell proliferation disorders, wherein EGFR is expressed, particularly wherein EGFR is abnormally expressed (e.g., overexpressed) compared to normal tissue of the same cell type. Such disorders include different types of cancer, such as cancers of the bladder, brain, head and neck, pancreas, lung, breast, ovary, colon, prostate, skin, and kidney. EGFR expression levels may be determined by methods known in the art and those described in WO 2006/082515 and WO 2008/017963 (e.g., via immunohistochemistry assay, immunofluorescence assay, immunoenzyme assay, ELISA, flow cytometry, radioimmunoassay, Western blot, ligand binding, kinase activity, etc.).

A composition of the present invention can be administered by a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results.

To administer a composition of the invention by certain routes of administration, it may be necessary to dilute the composition in a diluent. Pharmaceutically acceptable diluents include saline, glucose, Ringer and aqueous buffer solutions.

Preferably, the formulation according to the invention is administered by intravenous (i.v.), subcutaneous (s.c.) or any other parental administration means such as those known in the pharmaceutical art.

The phrases "parenteral administration" and "administered parenterally" as used herein mean modes of administration other than enteral and topical administration, usually by injection, and include, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

The composition must be sterile and fluid to the extent that the composition is deliverable by syringe or an infusion system. In addition to water, the carrier can be an isotonic buffered saline solution, ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof.

The formulation according to the invention can be prepared by methods known in the art, e.g. ultrafiltration-diafiltration, dialysis, addition and mixing, lyophilisation, reconstitution, and combinations thereof. Examples of preparations of formulations according to the invention can be found hereinafter.

The examples explain the invention in more detail but should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.

Examples

The IgG-class anti-EGFR antibody formulations according to the invention were developed based on the experimental results as provided below using the general preparatory and analytical methods and assays as outlined below.

Example 1: Preparation of the components for the formulation

The IgG-class anti-EGFR antibody hu-ICR62 IgG1 anti-EGFR mAb was manufactured by techniques generally known from the production of recombinant proteins. Techniques to manufacture this antibody are described in WO 2006/082515 and WO 2008/017963. Briefly, a genetically engineered Chinese hamster ovary cell line (CHO) prepared as described in WO 2006/082515 and WO 2008/017963 was expanded in cell culture from a master cell bank. The hu-ICR62 IgG1 anti-EGFR mAb was purified from the conditioned cell culture medium using protein A affinity chromatography on a MabSelect SuRe™ column (GE), followed by cation exchange chromatography on a Capto S™ column (GE) and a final anion exchange chromatographic step on a Capto Q™ column (GE). Viruses were removed by nanofiltration using a Viresolve® Pro membrane (Millipore) and the antibody was concentrated and transferred into the desired buffer by diafiltration. For preparing the formulations in accordance with these examples the hu-ICR62 IgG1 anti-EGFR mAb antibody was provided at a concentration of approx. 20 mg/ml in a 20 mM histidine buffer (a L-histidine/HCl buffer) at a pH of approximately 6.0.

The excipients of the formulation in accordance with the present invention are widely used in the practice and known to the person skilled in the art. There is therefore no need to explain them here in detail.

Liquid drug product formulations according to the invention were developed as follows.

Example 2: Preparation of the liquid formulations

For the preparation of the liquid formulations hu-ICR62 IgG1 anti-EGFR mAb was buffer-exchanged against a diafiltration buffer containing the anticipated buffer composition and where required, concentrated by diafiltration to an antibody concentration of approx. 50 - 80 mg/ml. After completion of the diafiltration operation, the excipients (e.g. trehalose, sodium chloride, methionine) were added as stock solutions to the antibody solution. The surfactant was then added as a 50 to 200-fold stock solution. Finally, the protein concentration was adjusted with a

buffer to the final hu-ICR62 IgG1 anti-EGFR mAb antibody concentration of approx. 25 mg/ml or approx. 50 mg/ml.

All formulations were sterile-filtered through 0.22 µm low protein binding filters and aseptically filled into sterile 6 ml glass vials closed with ETFE (copolymer of ethylene and tetrafluoroethylene)-coated rubber stoppers and aluminium crimp caps. The fill volume was approx. 2.4 ml. These formulations were stored at different ICH climate conditions (5°C, 25°C and 40°C) for different intervals of time and stressed by shaking (1 week at a shaking frequency of 200 min^{-1} at 5°C and 25°C) and freeze-thaw stress methods. The samples were analyzed before and after applying the stress tests by the following analytical methods:

1) UV spectrophotometry;
2) Size Exclusion Chromatography (SEC);
3) Ion Exchange Chromatography (IEC);
4) measurement of the turbidity of the solution;
5) inspection for visible particles.

UV spectroscopy, used for determination of protein content, was performed on a Perkin Elmer λ35 UV spectrophotometer in a wavelength range from 240 nm to 400 nm. Neat protein samples were diluted to approx. 0.5 mg/ml with the corresponding formulation buffer. The protein concentration was calculated according to Equation 1.

Equation 1: $$\text{Protein content} = \frac{(A_{280nm} - A_{320nm}) \times dil.factor}{\varepsilon\left[\frac{cm^2}{mg}\right] \times d_{cm}}$$

The UV light absorption at 280 nm was corrected for light scattering at 320 nm and multiplied with the dilution factor, which was determined from the weighed masses and densities of the neat sample and the dilution buffer. The numerator was divided by the product of the cuvette's path length d and the extinction coefficient ε.

Size Exclusion Chromatography (SEC) was used to detect soluble high molecular weight species (aggregates) and low molecular weight hydrolysis products (LMW) in the formulations. The method was performed on a Waters Alliance 2695 HPLC instrument with a Waters W2487 Dual Absorbance Detector and equipped with a TosoHaas TSK Gel G3000SWXL column. Intact monomer, aggregates and hydrolysis products were separated by an isocratic elution profile, using 200 mM sodium phosphate, pH 7.0 as mobile phase, and were detected at a wavelength of 280 nm.

Ion Exchange Chromatography (IEC) was performed to detect chemical degradation products altering the net charge of hu-ICR62 IgG1 anti-EGFR mAb in the formulations. The method used a suitable HPLC instrument equipped with a UV detector (detection wavelength 280nm) and a Dionex ProPac WCX-10 column (4mm x 250mm). 10 mM sodium phosphate buffer pH 6.0 in water and 10 mM sodium phosphate buffer pH 6.0 + 750 mM NaCl were used as mobile phases A and B, respectively, with a flow rate of 1.0mL/min.

For the determination of the turbidity, opalescence was measured in FTU (turbidity units) using a HACH 2100AN turbidimeter at room temperature.

Samples were analyzed for visible particles by using a Seidenader V90-T visual inspection instrument.

The results of the stability testing for the Formulations A to L are provided in Table1 added below.

The results show, that for obtaining maximum antibody stability and antibody formulations free from particles, the use of L-histidine/HCl buffer is more favorable than the use of sodium acetate buffer, while saccharides such as trehalose dihydrate and sucrose are the most favorable stabilizers, and polysorbate 80 is the most favorable surfactant.

Table 1: Composition and stability data of liquid anti-EGFR antibody drug product formulations according to this invention

Formulation A is a liquid formulation with the composition 25 mg/ml hu-ICR62 IgG1 anti-EGFR mAb, 20 mM sodium acetate pH 5.3, 140 mM sodium chloride, 0.02% polysorbate 20.

Storage condition	Storage Time	Protein concentration (mg/ml)	Size Exclusion-HPLC			Ion Exchange-HPLC			Turbidity (FTU)	Visible particles
			HMW (%)	Monomer (%)	LMW (%)	Acidic Peak (%)	Main Peak (%)	Basic Peak (%)		
-	Initial	25.6	1.4	98.6	0	35.6	60.2	4.3	8.9	Free from particles
Shaking 5°C	1 week	25.7	1.5	98.4	0.1	36.3	59.5	4.2	8.9	Free from particles
Shaking 25°C	1 week	25.6	1.7	98.2	0.1	36.7	58.1	5.2	8.8	Free from particles
Freezing/ Thawing	(5 cycles)	25.6	1.8	98.1	0.1	36.6	59.1	4.4	9.6	Free from particles
2-8°C	9 weeks	25.5	1.7	98.2	0.1	36.2	59.1	4.7	8.9	Free from particles
	32 weeks	25.3	2.0	97.9	0.1	32.1	64.5	3.4	9.8	With many particles
	51 weeks	25.4	2.2	97.7	0.1	35.6	59.1	5.3	10.0	With many particles
	74 weeks	nd	2.3	97.6	0.1	37.2	57.8	5.1	10.2	With many particles
25°C	9 weeks	nd	2.2	97.5	0.3	41.4	53.3	5.3	10.0	Free from particles
	32 weeks	nd	2.6	95.0	2.4	52.0	43.3	4.7	9.8	Free from particles
	51 weeks	nd	2.9	93.0	4.1	59.5	35.2	5.3	9.7	Free from particles
	74 weeks	nd	3.1	91.8	5.1	66.1	28.7	5.2	9.9	With many particles
40°C	9 weeks	25.4	2.9	95.7	1.4	nd	nd	nd	9.4	Free from particles
	32 weeks	nd	5.3	82.1	12.6	nd	nd	nd	10.7	Free from particles
	51 weeks	nd	8.0	74.4	17.6	nd	nd	nd	11.8	Free from particles
	74 weeks	nd	12.1	66.8	21.1	nd	nd	nd	13.8	With many particles

Formulation B is a liquid formulation with the composition 25 mg/ml hu-ICR62 IgG1 anti-EGFR mAb, 20 mM sodium acetate pH 5.3, 240 mM trehalose dihydrate, 0.02% polysorbate 20.

Storage condition	Storage Time	Protein concentration (mg/ml)	Size Exclusion-HPLC			Ion Exchange-HPLC			Turbidity (FTU)	Visible particles
			HMW (%)	Monomer (%)	LMW (%)	Acidic Peak (%)	Main Peak (%)	Basic Peak (%)		
-	Initial	25.6	1.2	98.8	0	36.1	60.0	3.9	2.7	Free from particles
Shaking 5°C	1 week	25.7	1.2	98.7	0.1	36.6	59.7	3.7	2.9	Free from particles
Shaking 25°C	1 week	25.8	1.3	98.6	0.1	37.0	58.7	4.2	2.7	Practically free from particles
Freezing/ Thawing	(5 cycles)	26.2	1.2	98.8	0	37.1	58.9	4.0	2.7	Practically free from particles
2-8°C	9 weeks	25.8	1.3	98.6	0.1	36.9	59.0	4.1	3.1	Free from particles
	32 weeks	25.6	1.4	98.5	0.1	34.1	62.8	3.1	3.1	With many particles
	51 weeks	25.4	1.5	98.4	0.1	37.0	58.5	4.5	3.5	With many particles
	74 weeks	nd	1.5	98.4	0.1	39.0	56.9	4.2	4.3	With many particles
25°C	9 weeks	nd	1.4	98.4	0.2	43.0	52.7	4.3	2.8	Free from particles
	32 weeks	nd	1.6	95.8	2.6	55.9	41.3	2.8	3.5	Free from particles
	51 weeks	nd	1.7	94.6	3.7	64.0	32.3	3.7	3.5	Practically free from particles
	74 weeks	nd	1.8	93.7	4.5	71.0	25.5	3.6	3.6	With many particles
40°C	9 weeks	25.7	1.5	97.4	1.1	nd	nd	nd	3.0	Practically free from particles
	32 weeks	nd	2.5	87.0	10.5	nd	nd	nd	3.7	Free from particles
	51 weeks	nd	4.1	80.6	15.3	nd	nd	nd	3.7	Practically free from particles
	74 weeks	nd	6.3	74.6	19.1	nd	nd	nd	3.9	With many particles

Formulation C is a liquid formulation with the composition 25 mg/ml hu-ICR62 IgG1 anti-EGFR mAb, 20 mM sodium acetate pH 5.3, 155 mM arginine hydrochloride, 0.02% polysorbate 20.

Storage condition	Storage Time	Protein concentration (mg/ml)	Size Exclusion-HPLC			Ion Exchange-HPLC			Turbidity (FTU)	Visible particles
			HMW (%)	Monomer (%)	LMW (%)	Acidic Peak (%)	Main Peak (%)	Basic Peak (%)		
-	Initial	25.7	1.2	98.8	0	35.8	60.4	3.7	7.0	Free from particles
Shaking 5°C	1 week	25.9	1.2	98.7	0.1	36.2	59.7	4.1	7.4	Free from particles
Shaking 25°C	1 week	25.9	1.3	98.6	0.1	36.2	58.9	4.9	7.3	Practically free from particles
Freezing/ Thawing	(5 cycles)	25.9	1.2	98.7	0.1	36.7	59.1	4.2	7.7	Practically free from particles
2-8°C	9 weeks	25.3	1.3	98.6	0.1	35.9	59.7	4.4	7.5	Free from particles
	32 weeks	25.6	1.5	98.4	0.1	33.1	62.9	4.0	8.2	With many particles
	51 weeks	25.5	1.6	98.3	0.1	35.0	59.8	5.2	8.8	With many particles
	74 weeks	nd	1.7	98.2	0.1	36.7	58.3	5.0	8.1	With many particles
25°C	9 weeks	nd	1.5	98.2	0.3	41.0	53.9	5.1	8.0	Free from particles
	32 weeks	nd	1.7	95.7	2.6	52.0	43.7	4.3	10.0	Free from particles
	51 weeks	nd	1.8	93.9	4.3	59.3	35.5	5.2	8.0	Free from particles
	74 weeks	nd	1.9	93.0	5.1	66.3	28.7	5.0	8.2	Free from particles
40°C	9 weeks	25.2	1.9	96.6	1.5	nd	nd	nd	8.1	Free from particles
	32 weeks	nd	4.0	82.9	13.1	nd	nd	nd	8.6	Free from particles
	51 weeks	nd	7.3	73.6	19.1	nd	nd	nd	10.1	Free from particles
	74 weeks	nd	11.7	66.0	22.3	nd	nd	nd	13.4	With many particles

Formulation D is a liquid formulation with the composition 25 mg/ml hu-ICR62 IgG1 anti-EGFR mAb, 20 mM L-histidine pH 5.5, 140 mM sodium chloride, 0.02% polysorbate 80.

Storage condition	Storage Time	Protein concentration (mg/ml)	Size Exclusion-HPLC			Ion Exchange-HPLC			Turbidity (FTU)	Visible particles
			HMW (%)	Monomer (%)	LMW (%)	Acidic Peak (%)	Main Peak (%)	Basic Peak (%)		
-	Initial	25.5	1.3	98.7	0	36.3	59.8	3.9	8.6	Free from particles
Shaking 5°C	1 week	25.4	1.4	98.5	0.1	36.6	59.5	4.0	8.8	Free from particles
Shaking 25°C	1 week	25.4	1.5	98.4	0.1	36.9	58.4	4.8	9.2	Practically free from particles
Freezing/ Thawing	(5 cycles)	25.4	1.5	98.4	0.1	36.8	59.1	4.2	8.9	Free from particles
2-8°C	9 weeks	25.0	1.5	98.4	0.1	36.9	58.9	4.2	9.0	Free from particles
	32 weeks	25.3	1.8	98.1	0.1	34.7	61.9	3.4	9.6	Practically free from particles
	51 weeks	25.4	1.9	98.0	0.1	37.4	58.0	4.5	10.1	Free from particles
	74 weeks	nd	1.9	98.0	0.1	39.7	56.0	4.3	10.0	Free from particles
25°C	9 weeks	nd	1.8	98.0	0.2	43.3	52.1	4.6	9.0	Free from particles
	32 weeks	nd	2.2	94.9	2.9	58.1	38.1	3.6	9.7	Free from particles
	51 weeks	nd	2.4	93.2	4.4	65.5	30.0	4.5	9.3	With many particles
	74 weeks	nd	2.5	92.4	5.1	73.5	22.6	3.9	10.7	Practically free from particles
40°C	9 weeks	25.24	2.7	95.7	1.6	nd	nd	nd	9.6	Free from particles
	32 weeks	nd	5.8	81.3	12.9	nd	nd	nd	11.8	Free from particles
	51 weeks	nd	9.1	72.2	18.7	nd	nd	nd	13.1	Free from particles
	74 weeks	nd	13.5	64.6	21.9	nd	nd	nd	16.7	With many particles

Formulation E is a liquid formulation with the composition 25 mg/ml hu-ICR62 IgG1 anti-EGFR mAb, 20 mM L-histidine pH 5.5, 240 mM trehalose dihydrate, 0.02% polysorbate 80.

Storage condition	Storage Time	Protein concentration (mg/ml)	Size Exclusion-HPLC			Ion Exchange-HPLC			Turbidity (FTU)	Visible particles
			HMW (%)	Monomer (%)	LMW (%)	Acidic Peak (%)	Main Peak (%)	Basic Peak (%)		
-	Initial	25.7	1.1	98.9	0	36.4	60.0	4.0	2.9	Free from particles
Shaking 5°C	1 week	25.6	1.2	98.7	0.1	36.9	59.2	3.9	2.9	Free from particles
Shaking 25°C	1 week	25.6	1.2	98.7	0.1	37.3	58.7	4.1	3.0	Free from particles
Freezing/ Thawing	(5 cycles)	25.7	1.2	98.8	0	37.1	59.2	3.7	3.0	Practically free from particles
2-8°C	9 weeks	25.6	1.3	98.6	0.1	37.4	58.8	3.9	3.0	Free from particles
	32 weeks	25.6	1.4	98.5	0.1	35.4	61.6	3.1	3.4	Free from particles
	51 weeks	25.6	1.4	98.5	0.1	38.4	57.6	4.0	3.3	Free from particles
	74 weeks	nd	1.4	98.5	0.1	40.1	56.0	3.9	3.7	Free from particles
25°C	9 weeks	nd	1.3	98.5	0.2	44.1	52.0	3.9	3.2	Free from particles
	32 weeks	nd	1.4	95.7	2.9	59.5	37.7	2.8	3.9	Free from particles
	51 weeks	nd	1.6	93.9	4.5	67.5	29.1	3.4	3.7	Free from particles
	74 weeks	nd	1.5	93.2	5.3	74.9	22.2	2.9	3.8	Free from particles
40°C	9 weeks	25.8	1.4	97.2	1.4	nd	nd	nd	3.3	Free from particles
	32 weeks	nd	3.4	84.9	11.7	nd	nd	nd	4.3	Practically free from particles
	51 weeks	nd	6.2	76.6	17.2	nd	nd	nd	4.8	Practically free from particles
	74 weeks	nd	11.6	65.5	22.9	nd	nd	nd	5.4	With many particles

Formulation F is a liquid formulation with the composition 25 mg/ml hu-ICR62 IgG1 anti-EGFR mAb, 20 mM L-histidine pH 5.5, 155mM arginine hydrochloride, 0.02% polysorbate 80.

Storage condition	Storage Time	Protein concentration (mg/ml)	Size Exclusion-HPLC			Ion Exchange-HPLC			Turbidity (FTU)	Visible particles
			HMW (%)	Monomer (%)	LMW (%)	Acidic Peak (%)	Main Peak (%)	Basic Peak (%)		
-	Initial	25.3	1.2	98.8	0	36.5	59.9	3.7	7.8	Free from particles
Shaking 5°C	1 week	25.6	1.2	98.7	0.1	36.6	59.4	4.0	7.6	Free from particles
Shaking 25°C	1 week	25.6	1.3	98.6	0.1	36.8	58.5	4.7	7.4	Free from particles
Freezing/ Thawing	(5 cycles)	25.6	1.2	98.7	0.1	37.2	58.8	4.0	7.8	Practically free from particles
2-8°C	9 weeks	25.0	1.4	98.5	0.1	36.8	59.2	3.7	7.4	Free from particles
	32 weeks	25.3	1.5	98.4	0.1	35.3	61.5	3.2	8.4	Free from particles
	51 weeks	25.4	1.6	98.3	0.1	37.2	58.3	4.5	8.1	Free from particles
	74 weeks	nd	1.6	98.3	0.1	39.3	56.4	4.3	8.0	Free from particles
25°C	9 weeks	nd	1.4	98.3	0.3	42.9	52.6	4.5	8.0	Free from particles
	32 weeks	nd	1.6	95.4	3.0	57.9	38.4	3.7	8.1	Free from particles
	51 weeks	nd	1.7	93.8	4.5	66.2	29.9	3.8	8.2	With many particles
	74 weeks	nd	1.7	93.0	5.3	72.9	23.4	3.7	8.8	Free from particles
40°C	9 weeks	25.2	2.0	96.2	1.8	nd	nd	nd	8.3	Free from particles
	32 weeks	nd	4.3	82.6	13.1	nd	nd	nd	9.7	Practically free from particles
	51 weeks	nd	7.3	73.3	19.4	nd	nd	nd	10.8	Practically free from particles
	74 weeks	nd	11.6	65.5	22.9	nd	nd	nd	12.7	With many particles

Formulation G is a liquid formulation with the composition 50 mg/ml hu-ICR62 IgG1 anti-EGFR mAb, 20 mM L-histidine pH 5.5, 240 mM trehalose dihydrate, 0.03% polysorbate 80.

Storage condition	Storage Time	Protein concentration (mg/ml)	Size Exclusion-HPLC			Ion Exchange-HPLC			Turbidity (FTU)	Visible particles
			HMW (%)	Monomer (%)	LMW (%)	Acidic Peak (%)	Main Peak (%)	Basic Peak (%)		
-	Initial	58.7	1.6	98.3	0.1	34.7	56.1	9.3	4.1	Practically free from particles
Shaking 5°C	1 week	58.6	1.6	98.3	0.1	34.8	56.0	9.2	4.7	Free from particles
Shaking 25°C	1 week	58.5	1.7	98.2	0.1	35.3	55.6	9.1	4.3	Free from particles
Freezing/ Thawing	(5 cycles)	58.4	1.6	98.3	0.1	34.9	56.0	9.1	4.0	Free from particles
2-8°C	16 weeks	58.3	1.8	98	0.2	35.6	55.1	9.3	4.0	Free from particles
	29weeks	nd	1.9	97.9	0.2	36.5	54.0	9.5	4.3	Free from particles
25°C	16 weeks	nd	1.8	97.7	0.5	35.6	55.1	9.3	3.8	Free from particles
	29 weeks	nd	1.9	97.4	0.7	52.5	40.3	7.3	4.3	Free from particles
40°C	16 weeks	nd	3.3	86.7	10.0	nd	nd	nd	4.6	Free from particles

Formulation H is a liquid formulation with the composition 50 mg/ml hu-ICR62 IgG1 anti-EGFR mAb, 20 mM L-histidine pH 5.5, 240 mM trehalose dihydrate, 0.03% polysorbate 80, 10 mM methionine.

Storage condition	Storage Time	Protein concentration (mg/ml)	Size Exclusion-HPLC			Ion Exchange-HPLC			Turbidity (FTU)	Visible particles
			HMW (%)	Monomer (%)	LMW (%)	Acidic Peak (%)	Main Peak (%)	Basic Peak (%)		
-	Initial	58.4	1.6	98.3	0.1	34.6	56.2	9.2	3.8	Free from particles
Shaking 5°C	1 week	60.4	1.6	98.3	0.1	35.0	56.2	8.9	4.2	Free from particles
Shaking 25°C	1 week	60.0	1.5	98.4	0.1	35.3	55.8	8.9	4.2	Free from particles
Freezing/ Thawing	(5 cycles)	60.2	1.5	98.4	0.1	34.8	56.3	8.9	4.4	Free from particles
2-8°C	16 weeks	58.4	1.7	98.1	0.2	35.6	55.2	9.2	4.0	Free from particles
	29weeks	nd	1.7	98.1	0.2	36.2	54.5	9.3	3.9	Free from particles
25°C	16 weeks	nd	1.7	97.8	0.5	44.6	47.6	7.9	4.1	Practically free from particles
	29 weeks	nd	1.8	97.5	0.7	51.6	41.3	7.1	4.2	Free from particles
40°C	16 weeks	nd	2.6	88	9.4	nd	nd	nd	4.9	Free from particles

Formulation I is a liquid formulation with the composition 25 mg/ml hu-ICR62 IgG1 anti-EGFR mAb, 20 mM L-histidine pH 5.5, 240 mM trehalose dihydrate, 0.02% polysorbate 80, 10 mM methionine.

Storage condition	Storage Time	Protein concentration (mg/ml)	Size Exclusion-HPLC			Ion Exchange-HPLC			Turbidity (FTU)	Visible particles
			HMW (%)	Monomer (%)	LMW (%)	Acidic Peak (%)	Main Peak (%)	Basic Peak (%)		
-	Initial	24.1	1.3	98.5	0.1	49.0	47.9	3.0	3.6	Free from particles
Shaking 5°C	1 week	24.2	1.3	98.5	0.1	49.5	47.2	3.3	3.4	Practically free from particles
Shaking 25°C	1 week	24.3	1.3	98.5	0.2	49.9	46.6	3.5	3.4	Practically free from particles
Freezing/ Thawing	(5 cycles)	24.1	1.3	98.5	0.2	49.4	47.1	3.5	3.5	Free from particles
2-8°C	4 weeks	nd	Nd	nd	nd	nd	nd	nd	nd	nd
25°C	4 weeks	nd	1.3	98.5	0.2	51.6	45.0	3.4	3.6	Free from particles
40°C	4 weeks	nd	1.0	98.3	0.7	69.0	27.7	3.4	3.5	Free from particles
2-8°C	12 weeks	nd	1.3	98.5	0.2	48.9	47.4	3.7	3.7	Free from particles
25°C	12 weeks	nd	1.3	98.4	0.3	54.9	41.5	3.7	3.7	Free from particles
40°C	12 weeks	nd	1.1	93.9	5.1	84.6	12.8	2.6	4.0	Free from particles
2-8°C	24 weeks	nd	1.3	98.5	0.2	50.2	46.2	3.6	3.3	Free from particles
25°C	24 weeks	nd	1.3	98.3	0.4	61.0	35.67	3.4	3.4	Free from particles
40°C	24 weeks	nd	1.3	89.3	9.4	nd	nd	nd	3.5	Free from particles
2-8°C	36 weeks	nd	1.3	98.5	0.2	50.2	46.5	3.4	3.2	Practically free from particles
25°C	36 weeks	nd	1.2	98.2	0.6	65.0	32.1	2.9	3.3	Free from particles
2-8°C	55 weeks	nd	1.3	98.5	0.2	50.1	46.3	3.6	3.6	Free from particles
25°C	55 weeks	nd	1.2	98.0	0.8	70.6	26.3	3.1	3.8	Free from particles

Formulation J is a liquid formulation with the composition 25 mg/ml hu-ICR62 IgG1 anti-EGFR mAb, 20 mM L-histidine pH 5.5, 240 mM sucrose, 0.02% polysorbate 80, 10 mM methionine.

Storage condition	Storage Time	Protein concentration (mg/ml)	Size Exclusion-HPLC			Ion Exchange-HPLC			Turbidity (FTU)	Visible particles
			HMW (%)	Monomer (%)	LMW (%)	Acidic Peak (%)	Main Peak (%)	Basic Peak (%)		
-	Initial	24.3	1.3	98.5	0.1	49.2	47.8	3.1	3.5	Free from particles
Shaking 5°C	1 week	24.2	1.3	98.5	0.2	49.4	47.1	3.5	3.7	Practically free from particles
Shaking 25°C	1 week	24.2	1.3	98.6	0.2	49.9	46.6	3.6	4.0	Practically free from particles
Freezing/ Thawing	(5 cycles)	24.1	1.3	98.5	0.2	49.3	48.6	2.1	3.6	Practically free from particles
2-8°C	4 weeks	nd	nd	nd	nd	nd	nd	nd	nd	nd
25°C	4 weeks	nd	1.3	98.5	0.2	51.3	45.3	3.3	3.4	Free from particles
40°C	4 weeks	nd	1.0	98.3	0.7	69.2	27.4	3.4	3.6	Free from particles
2-8°C	12 weeks	nd	1.3	98.5	0.2	49.1	47.0	3.9	3.8	Practically free from particles
25°C	12 weeks	nd	1.2	98.5	0.3	54.7	41.6	3.7	4.0	Practically free from particles
40°C	12 weeks	nd	1.1	93.6	5.4	85.2	12.1	2.7	3.8	Free from particles
2-8°C	24 weeks	nd	1.3	98.5	0.2	50.2	46.2	3.6	3.4	Free from particles
25°C	24 weeks	nd	1.2	98.3	0.4	60.8	35.8	3.4	3.4	Free from particles
40°C	24 weeks	nd	1.5	89.1	9.4	nd	nd	nd	3.9	Free from particles
2-8°C	36 weeks	nd	1.3	98.5	0.2	50.2	46.2	3.6	3.1	Free from particles
25°C	36 weeks	nd	1.2	98.2	0.6	64.8	32.2	3.0	3.5	Free from particles
2-8°C	55 weeks	nd	1.3	98.5	0.2	50.2	46.3	3.5	3.7	Free from particles
25°C	55 weeks	nd	1.2	98.0	0.8	71.2	26.2	2.7	4.0	Free from particles

Formulation K is a liquid formulation with the composition 25 mg/ml hu-ICR62 IgG1 anti-EGFR mAb, 20 mM L-histidine pH 5.5, 240 mM trehalose dihydrate, 0.02% polysorbate 80.

Storage condition	Storage Time	Protein concentration (mg/ml)	Size Exclusion-HPLC			Ion Exchange-HPLC			Turbidity (FTU)	Visible particles
			HMW (%)	Monomer (%)	LMW (%)	Acidic Peak (%)	Main Peak (%)	Basic Peak (%)		
-	Initial	24.2	1.3	98.5	0.1	49.4	47.5	3.2	3.6	Free from particles
Shaking 5°C	1 week	24.2	1.3	98.5	0.2	49.4	47.1	3.5	3.7	Practically free from particles
Shaking 25°C	1 week	24.2	1.3	98.5	0.2	50.0	46.5	3.5	3.7	Free from particles
Freezing/ Thawing	(5 cycles)	23.9	1.3	98.5	0.2	49.6	47.0	3.4	3.5	Practically free from particles
2-8°C	4 weeks	nd	nd	nd	nd	nd	nd	nd	nd	nd
25°C	4 weeks	nd	1.3	98.5	0.2	51.8	44.8	3.4	3.6	Free from particles
40°C	4 weeks	nd	1.1	98.2	0.7	69.1	27.4	3.5	3.6	Free from particles
2-8°C	12 weeks	nd	1.3	98.5	0.2	49.3	47.0	3.8	3.8	Free from particles
25°C	12 weeks	nd	1.3	98.4	0.3	54.9	41.3	3.8	3.5	Practically free from particles
40°C	12 weeks	nd	1.4	92.9	5.7	85.4	11.7	3.0	4.2	Free from particles
2-8°C	24 weeks	nd	1.4	98.5	0.2	50.2	46.2	3.6	3.5	Free from particles
25°C	24 weeks	nd	1.3	98.2	0.4	61.5	35.2	3.3	3.4	Free from particles
40°C	24 weeks	nd	2.1	88.6	9.3	nd	nd	nd	3.8	Free from particles
2-8°C	36 weeks	nd	1.3	98.5	0.2	50.0	46.6	3.4	3.6	Free from particles
25°C	36 weeks	nd	1.3	98.0	0.7	66.2	30.8	3.0	3.4	Free from particles
2-8°C	55 weeks	nd	1.3	98.5	0.2	50.2	46.2	3.6	3.6	Free from particles
25°C	55 weeks	nd	1.5	97.7	0.9	71.8	25.0	3.1	3.8	Free from particles

Formulation L is a liquid formulation with the composition 25 mg/ml hu-ICR62 IgG1 anti-EGFR mAb, 20 mM L-histidine pH 5.5, 240 mM sucrose, 0.02% polysorbate 80.

Storage condition	Storage Time	Protein concentration (mg/ml)	Size Exclusion-HPLC			Ion Exchange-HPLC			Turbidity (FTU)	Visible particles
			HMW (%)	Monomer (%)	LMW (%)	Acidic Peak (%)	Main Peak (%)	Basic Peak (%)		
-	Initial	24.2	1.3	98.5	0.1	49.6	47.2	3.2	3.6	Free from particles
Shaking 5°C	1 week	24.1	1.3	98.5	0.2	49.5	47.0	3.5	3.8	Practically free from particles
Shaking 25°C	1 week	24.2	1.3	98.5	0.2	50.0	46.4	3.6	3.5	Practically free from particles
Freezing/ Thawing	(5 cycles)	24.4	1.3	98.5	0.2	49.5	47.0	3.5	3.6	Practically free from particles
2-8°C	4 weeks	nd	nd	nd	nd	nd	nd	nd	nd	nd
25°C	4 weeks	nd	1.3	98.5	0.2	51.5	44.9	3.6	3.6	Free from particles
40°C	4 weeks	nd	1.1	98.2	0.7	69.5	27.3	3.3	3.8	Free from particles
2-8°C	12 weeks	nd	1.3	98.5	0.2	49.3	46.9	3.8	3.8	Free from particles
25°C	12 weeks	nd	1.3	98.4	0.3	54.8	41.4	3.8	3.9	Practically free from particles
40°C	12 weeks	nd	1.5	92.7	5.8	86.0	11.1	2.9	4.0	Free from particles
2-8°C	24 weeks	nd	1.4	98.5	0.2	50.0	46.4	3.6	3.5	Free from particles
25°C	24 weeks	nd	1.4	98.2	0.5	61.6	35.1	3.3	3.5	Free from particles
40°C	24 weeks	nd	2.5	88.1	9.4	nd	nd	nd	3.9	Free from particles
2-8°C	36 weeks	nd	1.3	98.5	0.2	50.1	46.4	3.5	3.4	Practically free from particles
25°C	36 weeks	nd	1.3	98.0	0.7	65.9	30.8	3.3	3.3	Free from particles
2-8°C	55 weeks	nd	1.3	98.5	0.2	50.5	46.0	3.4	3.5	Free from particles
25°C	55 weeks	nd	1.4	97.8	0.9	71.8	25.0	2.0	3.7	Free from particles

Claims

1. A pharmaceutical formulation comprising:
1 to 200 mg/ml of an IgG-class anti-EGFR antibody;
1 to 100 mM of a buffering agent;
0.001 to 1% (w/v) of a surfactant;
1 to 500 mM of at least one stabilizer
at a pH in the range of from 4.0 to 7.0.

2. The formulation according to claim 1, wherein the IgG-class anti-EGFR antibody concentration is in the range of 1 to 100 mg/ml.

3. The formulation according to claim 1 or 2, wherein the buffering agent is a histidine buffer or an acetate buffer.

4. The formulation according to any one of claims 1 to 3, wherein the buffering agent is at a concentration of 10 to 50 mM.

5. The formulation according to any one of claims 1 to 4, wherein the buffering agent provides a pH of 5.0 to 6.0.

6. The formulation according to any one of claims 1 to 5, wherein the surfactant is a polysorbate.

7. The formulation according to any one of claims 1 to 6, wherein the surfactant is at a concentration of 0.01 to 0.1% (w/v).

8. The formulation according to any one of claims 1 to 7, wherein at least one stabilizer is selected from the group of salts, saccharides, and amino acids.

9. The formulation according to any one of claims 1 to 8, wherein the at least one stabilizer is at a concentration of 120 to 300 mM.

10. The formulation according to any one of claims 1 to 9, comprising a first stabilizer selected from the group of salts, saccharides and amino acids, and methionine as a second stabilizer.

11. The formulation according to claim 10, wherein the first stabilizer is at a concentration of 120 to 300 mM, and the second stabilizer methionine is at a concentration of 5 to 25 mM.

12. The formulation according to any one of claims 1 to 11, which comprises:
10 to 50 mg/ml of an IgG-class anti-EGFR antibody;
15 to 30 mM of a buffering agent, selected from L-histidine and sodium acetate;
0.02 to 0.05% (w/v) of a surfactant, selected from polysorbate 20 and polysorbate 80;
120 to 300 mM of at least one stabilizer, selected from trehalose dihydrate, sucrose, arginine hydrochloride and sodium chloride;
optionally 5 to 25 mM of methionine as a second stabilizer;
at a pH of 5.5 ± 0.3.

13. The formulation according to any one of claims 1 to 12, which comprises:
10 to 50 mg/ml IgG-class anti-EGFR antibody, 20 mM L-histidine, 240 mM trehalose dihydrate, 0.02 to 0.03% (w/v) polysorbate 80, at pH 5.5;
or
10 to 50 mg/ml IgG-class anti-EGFR antibody, 20 mM L-histidine, 155 mM arginine hydrochloride, 0.02% (w/v) polysorbate 80, at pH 5.5;
or
10 to 50 mg/ml IgG-class anti-EGFR antibody, 20 mM L-histidine, 240 mM trehalose dihydrate, 0.02 to 0.03% (w/v) polysorbate 80, 10 mM methionine at pH 5.5;
or
10 to 50 mg/ml IgG-class anti-EGFR antibody, 20 mM L-histidine, 240 mM sucrose, 0.02 to 0.03% (w/v) polysorbate 80, at pH 5.5.
or
10 to 50 mg/ml IgG-class anti-EGFR antibody, 20 mM L-histidine, 240 mM sucrose, 0.02 to 0.03% (w/v) polysorbate 80, 10 mM methionine, at pH 5.5.

14. The formulation according to any one of claims 1 to 13, which comprises
20 to 50 mg/ml of hu-ICR62 IgG1 anti-EGFR mAb;
20 mM L-histidine;
0.02 to 0.03% (w/v) polysorbate 80;
240 mM of a first stabilizer, wherein said first stabilizer is a saccharide selected from trehalose dihydrate and sucrose;
10 mM of methionine as a second stabilizer;
at a pH of 5.5 ± 0.3.

15. The formulation according to any one of claims 1 to 14, wherein the IgG-class anti-EGFR antibody is a humanized antibody and comprises

a) in the heavy chain variable domain a CDR1 of SEQ ID NO:1, a CDR2 of SEQ ID NO:16 and a CDR3 of SEQ ID NO:31, and

b) in the light chain variable domain a CDR1 of SEQ ID NO:33, a CDR2 of SEQ ID NO:34 and a CDR3 of SEQ ID No:35.

16. The formulation according to any one of claims 1 to 15, wherein the IgG-class anti-EGFR antibody is hu-ICR62 IgG1 anti-EGFR mAb.

17. The formulation according to any one of claims 1 to 16, wherein the IgG-class anti-EGFR antibody has been glycoengineered to have an increased proportion of non-fucosylated oligosaccharides it its Fc region, as compared to the non-glycoengineered antibody.

18. The formulation according to any one of claims 1 to 17, which is in a liquid form, in a lyophilized form or in a liquid form reconstituted from a lyophilized form.

19. Use of a formulation according to any one of claims 1 to 18 for the preparation of a medicament useful for treating cancer.

20. The invention as hereinbefore described.