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(54) **Title**: A NOVEL STABLE FORMULATION

(57) **Abstract**: The present invention relates to a stable formulation particularly suitable for the anti-mesothelin immunoconjugate MF-T-SPDB-DM4. The described stable aqueous formulation comprising MF-T-SPDB-DM4 is directly suitable for therapeutic applications and for subsequent lyophilization. The lyophilized powder can be reconstituted with water to create a reconstituted solution which is again suitable for therapeutic applications. It is a further object to provide a stable reconstituted protein formulation which is suitable for therapeutic administrations.

A NOVEL STABLE FORMULATION

FIELD OF THE INVENTION

The present invention relates to a stable formulation particularly suitable for the anti-mesothelin immunoconjugate MF-T-SPDB-DM4. The described stable aqueous formulation comprising MF-T-SPDB-DM4 is directly suitable for therapeutic applications and for subsequent lyophilization. The lyophilized powder can be reconstituted with water to create a reconstituted solution which is again suitable for therapeutic applications. It is a further object to provide a stable reconstituted protein formulation which is suitable for therapeutic administrations.

BACKGROUND OF THE INVENTION

Antibody-based therapy is proving very effective in the treatment of various cancers, including solid tumors. Central to the development of a successful antibody-based therapy is the isolation of antibodies against cell-surface proteins found to be preferentially expressed on tumor cells. The mesothelin precursor polypeptide is a glycophosphatidylinositol (GPI)-anchored, glycosylated cell surface protein that is proteolytically cleaved to a 30 kDa N-terminal secreted polypeptide and a 40 kDa, C-terminal polypeptide, which predominantly occurs in the membrane-bound, GPI-anchored form (Chang, K. and I. Pastan, Proc. Natl. Acad. Sei. U S A, (1996) 93(1):136), and which is named mesothelin herein. Mesothelin is preferentially expressed by certain tumor cells, particularly mesothelioma cells, pancreatic tumor cells and ovarian carcinoma cells, while its expression is limited in normal tissue, making it an attractive target for the development of tumor therapy (Argani, P. et al., Clin. Cancer Res. (2001) 7(12): 3862; Hassan, R., et a/., Clin. Cancer Res. (2004) 10(12 Pt 1):3937).

Anti-mesothelin antibodies including MF-T, antigen-binding antibody fragments, and variants of these antibodies have been described in WO2009/068204. The described antibodies have special features making them very suitable for a use as immunoconjugates. An immunoconjugate is composed of an antibody specifically recognizing a target cell antigen, such as a tumor cell antigen, and one or several covalently linked molecules of a

drug, particularly a cytotoxic drug such as a maytansinoid. Immunoconjugates composed of anti-mesothelin antibodies, antibody fragments, and variants of these antibodies and fragments linked to a chemotherapeutic agent, e.g. maytansinoids, or derivatives thereof have been described in WO2010/124797. A special preferred embodiment of an anti-mesothelin immunoconjugate is MF-T-SPDB-DM4 described in WO2010/124797 in detail.

Unlike traditional organic and inorganic drugs antibody based drugs are larger and more complex. This makes it more difficult to develop formulations which preserve the antibody based drugs in its biologically active form and prevent degradation. Degradation can take place due to chemical instability (resulting in a new chemical entity) or physical instability. The conjugation of drugs, especially cytotoxic drugs, which are often hydrophobic, small molecules, to hydrophilic monoclonal antibodies, introduces additional instability to immunoconjugates. Particle formation in protein pharmaceuticals, in particular, can destabilize the pharmaceutical compound, thus making the formulation less potent or even harmful for clinical use. For example, particles in injected pharmaceutical formulations can cause significant injury in patients. In addition, formation of aggregates is a major degradation pathway of protein pharmaceuticals (Chari et al., Pharm Res. 20, 1325-1336 (2003)), and may lead to undesirable effects such as immunogenicity. Chemical instability of immunoconjugates can result in the generation of free cytotoxic drugs, which can lead to toxic side effects.

A suitable formulation for an immunoconjugate prevents chemical and physical instability over a long time period. Suitable stable liquid or lyophilized formulations for maytansinoid containing immunoconjugates have been described for example in WO2004/004639, WO2004/110498, and WO2007/019232. WO2007/019232 describes a liquid immunoconjugate formulation comprising several excipients, wherein the formulation is a buffered aqueous solution. WO2004/110498 provides a liquid and a lyophilized composition comprising an antibody chemically coupled to a maytansinoid. WO2004/004639 describes suitable formulations for the immunoconjugate huC242-DM1.

Nevertheless the general message from all of these publications is that each immunoconjugate, here MF-T-SPDB-DM4, is a special combination of an antibody, linker and a cytotoxic drug. This combination results in certain physicochemical properties which need an unpredictable solution for a suitable formulation which is provided in this invention.

BRIEF SUMMARY OF THE INVENTION

The invention provides a formulation / composition suitable for therapeutic applications comprising (i) MF-T-SPDB-DM4, (ii) a buffering system preferentially comprising the amino acids L-Histidine and Glycine, (iii) a cryoprotectant, and optionally (iv) a surfactant, wherein the composition has a pH of 5.0 to 8.0.

The invention also provides a lyophilized composition comprising (i) MF-T-SPDB-DM4, (ii) a buffering system preferentially comprising the amino acids L-Histidine and Glycine, (iii) a cryoprotectant, and optionally (iv) a surfactant, wherein the composition has a pH of 5.0 to 8.0 when reconstituted with water. The reconstituted solution of this lyophilized composition is suitable for therapeutic applications.

DETAILED DESCRIPTION OF THE INVENTION

This invention relates to the pharmaceutically suitable formulation of the immunoconjugate MF-T-SPDB-DM4 wherein the formulation comprises (i) MF-T-SPDB-DM4, (ii) a buffering system preferentially comprising the amino acids L-Histidine and Glycine, (iii) a cryoprotectant, and optionally (iv) a surfactant, wherein the composition has a pH of 5.0 to 8.0. The invention is also related to lyophilized composition comprising (i) MF-T-SPDB-DM4, (ii) a buffering system preferentially comprising the amino acids L-Histidine and Glycine, (iii) a cryoprotectant, and optionally (iv) a surfactant, wherein the composition has a pH of 5.0 to 8.0 when reconstituted with water.

The immunoconjugate MF-T-SPDB-DM4 is known in the art (WO2010/124797). MF-T-SPDB-DM4 is an immunoconjugate comprising the antibody MF-T chemically coupled to a maytansinoid (described in WO2009/068204).

Degradation of immunoconjugates, here MF-T-SPDB-DM4, is an undesired effect for pharmaceutical applications. The efficacy or availability of the drug can change dramatically. Degradation can take place due to chemical instability (resulting in a new chemical entity) or physical instability. Chemical instability can result from e.g. deamidation, hydrolysis, oxidation or disulfide exchange. Physical instability can result from e.g. aggregation or

adsorption. The conjugation of drugs, especially cytotoxic drugs, which are often hydrophobic, small molecules, to hydrophilic monoclonal antibodies, introduces additional instability to immunoconjugates. Particle formation in protein pharmaceuticals, in particular, can destabilize the pharmaceutical compound, thus making the formulation less potent or even harmful for clinical use. For example, particles in injected pharmaceutical formulations can cause significant injury in patients. In addition, formation of aggregates is a major degradation pathway of protein, and may lead to undesirable effects such as immunogenicity.

So it is an object of this invention to provide pharmaceutically suitable formulations of the immunoconjugate MF-T-SPDB-DM4 which prevent the formation of “high molecular weight aggregates”. The term "high molecular weight aggregates" or “HMW”, as used herein, refers to aggregates comprising two or more immunoconjugate molecules.

It is a further object of this invention to provide pharmaceutically suitable formulations of the immunoconjugate MF-T-SPDB-DM4 which prevent chemical instability. Chemical instability of immunoconjugates can result in the generation of free cytotoxic drugs (here free DM4 maytansinoid species), which may lead to toxic side effects. So in addition the presence or generation of any kind of “low molecular weight” (LMW) fragments should be minimized or avoided.

The present invention is based on the finding that a composition containing MF-T-SPDB-DM4 was achieved, which allow for a long shelf live as liquid formulation as well as lyophilized composition. This formulation allows for long term storage as lyophilized composition (long shelf live) and after reconstitution for the possibility to store the not used portion over a long time period as aqueous solution.

A typical shelf life for the immunoconjugate compositions of the present invention is about 1 to 5 years, preferably 1 to 4 years, more preferably 2 to 4 years, at 4°C.

This kind of formulations could be achieved by inclusion of excipients that inhibit or reduce aggregation and particle formation. Formulations to stabilize immunoconjugates are known in the art. Suitable stable liquid or lyophilized formulations for maytansinoid containing immunoconjugates have been described for example in WO2004/004639, WO2004/110498, and WO2007/019232. WO2007/019232 describes a liquid

immunoconjugate formulation comprising: an immunoconjugate and one or more excipients selected from the group consisting of: sucrose, polysorbate 20, polysorbate 80, cyclodextrin, dextrose, glycerol, polyethylene glycol, mannitol, sodium chloride, and an amino acid, wherein the formulation is a buffered aqueous solution having a pH of 4.5 to 7.6. WO2004/110498 provides a liquid and a lyophilized composition comprising an antibody chemically coupled to a maytansinoid. WO2004/004639 describes suitable formulations for the immunoconjugate huC242-DM1. But it is also well known that each immunoconjugate, here MF-T-SPDB-DM4, is a special combination of an antibody, linker and a cytotoxic drug, resulting in unpredictable formulation problems.

The present invention also relates to the administration of pharmaceutical compositions. Such administration is accomplished orally or parenterally. For immunoconjugates parenteral routes of administration are more preferred. Methods of parenteral delivery include topical, intra-arterial (directly to the tumor), intramuscular, subcutaneous, intramedullary, intrathecal, intraventricular, intravenous, intraperitoneal, or intranasal administration. In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Ed. Maack Publishing Co, Easton, Pa.).

Formulations of the invention may be administered using an injector, a pump, a syringe, or any other devices known in the art as well as by gravity. A needle or a catheter may be used for introducing the formulations of the present invention into the body of a patient via certain parenteral routes.

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose, i.e. treatment of a particular disease. The determination of an effective dose is well within the capability of those skilled in the art.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., neoplastic cells, or in animal models, usually mice, rabbits, dogs, pigs or monkeys. The animal model is also used to achieve a desirable concentration range

and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

The compositions of this invention are formulated to be acceptable in a therapeutic application. “Therapeutic application” refers to treatments involving administration to a subject in need of treatment a therapeutically effective amount of the immunoconjugate MF-T-SPDB-DM4. A “therapeutically effective amount” hereby is defined as the amount of the immunoconjugate MF-T-SPDB-DM4 that is of sufficient quantity to reduce proliferation of mesothelin positive cell or to reduce size of a mesothelin expressing tumor in a treated area of a subject - either as a single dose or according to a multiple dose regimen, alone or in combination with other agents, which leads to the alleviation of an adverse condition, yet which amount is toxicologically tolerable. The subject may be a human or non-human animal (e.g., rabbit, rat, mouse, dog, monkey or other lower-order primate).

The exact dosage is chosen by the individual physician in view of the patient to be treated. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Additional factors that may be taken into account include the severity of the disease state, e.g., tumor size and location; age, weight and gender of the patient; diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long acting pharmaceutical compositions might be administered for example every 3 to 4 days, every week, once every two weeks, or once every three weeks, depending on half-life and clearance rate of the particular formulation.

The term "pharmaceutical formulation" or “formulation” refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

The present invention also provides for a lyophilized powder of the liquid formulation. “Lyophilized” means that the composition has been freeze-dried under vacuum. During lyophilization the liquid formulation is frozen and the solutes are separated from the solvent. The solvent is removed by sublimation (i.e., primary drying) and next by

desorption (i.e., secondary drying). Lyophilization results in a cake or powder which can be stored over a long time period. Prior to administration the lyophilized composition is reconstituted in solvent, preferentially sterile water for injection. The term "reconstituted formulation", as used herein, refers to such a lyophilized composition after solubilization.

Lyophilization methods are well known in the art (e.g. Wang, W., Int. J . Pharm., 203, 1-60 (2000)). The inventive lyophilized composition was achieved with two different lyophilization protocols as described in the examples in detail.

In order to prevent degradation during the lyophilization process the inventive liquid composition comprises a cryoprotectant. The term "cryoprotectant", as used herein, refers to an excipient that protects unstable molecules during freezing. Suitable cryoprotectants known is the art are ingredients including but not limited to polyethylene glycol (PEG), dextran, glycerol, glucose, trehalose, and sucrose. Most preferably, the cryoprotectant is sucrose. When the cryoprotectant is sucrose the liquid composition prior lyophilization comprises 2 to 15% sucrose, more preferably 5 to 10% sucrose. Most preferably, the liquid composition prior lyophilization comprises 5% sucrose.

The lyophilized composition can further contain a bulking agent, preferably a crystallizable bulking agent. Bulking agents typically are used in the art to provide structure and weight to the "cake" produced as a result of lyophilization. Any suitable bulking agent known in the art may be used in connection with the inventive lyophilized composition. Suitable bulking agents include, for example, mannitol, dextran, and glycine.

The inventive composition optionally comprises a surfactant. The term "surfactant", as used herein, refers to all detergents comprising a hydrophilic and a hydrophobic portion and includes non-ionic, cationic, anionic, and zwitterionic detergents. For the described composition a non-ionic surfactant is preferred. Preferred detergents are for example polysorbate 80 (also known as Tween 80, or polyoxyethylene (20) sorbitan monooleate) or polysorbate 20 (also known as Tween 20, or polyoxyethylene (20) sorbitan monolaurate). Most preferably the surfactant is polysorbate 80. The surfactant can be used at a concentration of 0.001% to 0.2%.

The term "buffer", as used herein, refers to a buffered solution, which pH changes only marginally after addition of acidic or basic substances. Buffered solutions contain a

mixture of a weak acid and its corresponding base, or a weak base and its corresponding acid, respectively. The term "buffering system", as used herein, refers to a mixture of one or more of the aforementioned acids and bases. A preferred buffering system of this invention contains one or more amino acids. Most preferably the buffering system comprises a mixture of L-Histidine and Glycine.

An embodiment of the invention is an immunoconjugate formulation comprising MF-T-SPDB-DM4 and one or more excipients selected from the group consisting of an amino acid, a cryoprotectant, and a surfactant wherein the composition has a pH of 5.0 to 8.0, more preferably of pH of 5.5 to 7.3, more preferably of pH 5.5.

An embodiment of the invention is an immunoconjugate formulation comprising MF-T-SPDB-DM4 and one or more excipients selected from the group consisting of L-Histidine, Glycine, a cryoprotectant, and a surfactant wherein the composition has a pH of 5.0 to 8.0, more preferably of pH of 5.5 to 7.3, more preferably of pH 5.5.

An embodiment of the invention is an immunoconjugate formulation comprising approximately 0.1 mg/ml, approximately 0.2 mg/ml, approximately 0.3 mg/ml, approximately 0.4 mg/ml, approximately 0.5 mg/ml, approximately 0.6 mg/ml, approximately 0.7 mg/ml, approximately 0.8 mg/ml, approximately 0.9 mg/ml, approximately 1.0 mg/ml, approximately 1.5 mg/ml, approximately 2.0 mg/ml, approximately 2.5 mg/ml, approximately 3.0 mg/ml, approximately 4.0 mg/ml, approximately 5.0 mg/ml, approximately 6.0 mg/ml, approximately 7.0 mg/ml, approximately 8.0 mg/ml, approximately 9.0 mg/ml, or approximately 10.0 mg/ml MF-T-SPDB-DM4.

An embodiment of the invention is an immunoconjugate formulation comprising 0.5 mg/ml, 0.6 mg/ml, 0.7 mg/ml, 0.8 mg/ml, 0.9 mg/ml, 1.0 mg/ml, 1.5 mg/ml, 2.0 mg/ml, 2.5 mg/ml, 3.0 mg/ml, 4.0 mg/ml, 5.0 mg/ml, 6.0 mg/ml, 7.0 mg/ml, 8.0 mg/ml, 9.0 mg/ml, or 10.0 mg/ml MF-T-SPDB-DM4.

An embodiment of the invention is an immunoconjugate formulation comprising 5.0 mg/ml, 10.0 mg/ml, 20 mg/ml, or 50 mg/ml MF-T-SPDB-DM4.

An embodiment of the invention is an immunoconjugate formulation comprising 1 mg/ml to 50 mg/ml, more preferably 2 mg/ml to 20 mg/ml, and even more preferably 5 mg/ml to 10 mg/ml MF-T-SPDB-DM4.

A preferred embodiment of the invention is an immunoconjugate formulation comprising 5 mg/ml MF-T-SPDB-DM4.

An embodiment of the invention is an immunoconjugate formulation comprising MF-T-SPDB-DM4 and a buffering system. In a preferred embodiment the buffering system comprises the amino acids L-Histidine and Glycine. In a preferred embodiment the amino acids L-Histidine and Glycine have a concentration each of 5 mM to 250 mM. More preferred are concentrations for L-Histidine and Glycine each of 10 mM to 150 mM. Most preferred is a mixture comprising 10 mM L-Histidine and 130 mM Glycine.

An embodiment of the invention is an immunoconjugate formulation comprising MF-T-SPDB-DM4, a buffering system, and a cryoprotectant. In a preferred embodiment the cryoprotectant is selected from the group consisting of polyethylene glycol (PEG), dextran, glycerol, glucose, trehalose, and sucrose. Most preferably, the cryoprotectant is sucrose. When the cryoprotectant is sucrose the liquid composition or the liquid composition prior lyophilization comprises 2 to 15 % sucrose, more preferably 5 to 10% sucrose. Most preferably, the liquid composition or the liquid composition prior lyophilization comprises 5% sucrose.

An embodiment of the invention is an immunoconjugate formulation comprising MF-T-SPDB-DM4, a buffering system, a cryoprotectant, and a surfactant. In a preferred embodiment the surfactant is a non-ionic surfactant. In a more preferred embodiment the surfactant is selected from the group consisting of polysorbate 80 and polysorbate 20. The most preferred surfactant is polysorbate 80. The surfactant has a concentration of 0.001% to 0.1%. Most preferred is a composition comprising 0.01% polysorbate 80.

A preferred embodiment of the invention is an immunoconjugate formulation comprising 1mg/ml to 20 mg/ml MF-T-SPDB-DM4, 10 mM to 15 mM L-Histidine, 100 mM to 250 mM Glycine, 5% to 15% sucrose, 0.001% to 0.1% polysorbate 80, wherein the formulation is a buffered aqueous solution having a pH of 5.0 to 8.0, more preferably of pH 5.5 to pH 7.3, most preferably of pH 5.5.

A highly preferred embodiment of the invention is an immunoconjugate formulation comprising MF-T-SPDB-DM4, 10 mM L-Histidine, 130 mM Glycine, 5% sucrose, and 0.01% polysorbate 80, at a pH of 5.5.

A most preferred embodiment of the invention is an immunoconjugate formulation comprising 5 mg/ml MF-T-SPDB-DM4, 10 mM L-Histidine, 130 mM Glycine, 5% sucrose, and 0.01% polysorbate 80, at a pH of 5.5.

An embodiment of the invention is a lyophilized composition obtained by lyophilization of the liquid immunoconjugate formulation according to this invention, or obtainable by lyophilization of a liquid immunoconjugate formulation according to this invention.

A preferred embodiment of the invention is a lyophilized composition obtained by freeze-drying of a liquid immunoconjugate formulation according to this invention.

A preferred embodiment of the invention is a lyophilized composition obtained by freeze-drying of a liquid immunoconjugate formulation according to this invention using methods explained in example 6.

A highly preferred embodiment of the invention is a lyophilized composition obtained by freeze-drying of the liquid immunoconjugate formulation comprising 5 mg/ml MF-T-SPDB-DM4, 10 mM L-Histidine, 130 mM Glycine, 5% sucrose, and 0.01% polysorbate 80, at a pH of 5.5, or obtainable by freeze-drying of the liquid immunoconjugate formulation comprising 5 mg/ml MF-T-SPDB-DM4, 10 mM L-Histidine, 130 mM Glycine, 5% sucrose, and 0.01% polysorbate 80 at a pH of 5.5.

An embodiment of this invention is a liquid immunoconjugate formulation obtained by reconstitution of a lyophilized composition according to this invention (called reconstituted formulation). In a preferred embodiment of this invention the lyophilized composition is reconstituted in water, preferentially in sterile water for injection.

Thus, in accordance with the invention, the contents of a lyophilized composition that is to be reconstituted to contain 5 mg/ml MF-T-SPDB-DM4 comprises 0.31 mg L-Histidine, 1.95 mg Glycine, 9.99 mg sucrose, and 0.02 mg polysorbate 80 per mg of the

immunoconjugate MF-T-SPDB-DM4. Once reconstituted with water, such a lyophilized composition has a pH of about 5.5.

If not otherwise stated all % values in this case are % weight per volume.

The present invention is further described by the following examples, which are illustrative of the process and should not be construed as limiting the invention. The process parameters given below can be adopted and adapted by the skilled person to suit the particular need.

EXAMPLE 1

This example shows how different liquid compositions containing MF-T-SPDB-DM4 were produced. These compositions were subsequently analyzed as described in the following examples.

The immunoconjugate MF-T-SPDB-DM4 was prepared as described in WO2010/124797. To study several formulation compositions the solution comprising the immunoconjugate MF-T-SPDB-DM4 must be changed in a defined way. Solutions comprising MF-T-SPDB-DM4 were injected onto a preparative size exclusion column filled with Sephadex G25 connected to an ÄKTA explorer (GE Healthcare). Applied solutions were eluted with the final buffer solution of interest. The size of the column enabled a complete buffer exchange so that the collected elution fraction consisted of MF-T-SPDB-DM4 in the buffer solution of interest. The protein concentration was adjusted via ultrafiltration using a Vivapore Cell (10/20, 7500 MWCO; Sartorius).

EXAMPLE 2

This example shows the effect of several buffer systems on aggregation assessed by visual appearance as well as by dynamic light scattering (DLS) and on immunoconjugate stability measured with differential scanning calorimetry (DSC) for MF-T-SPDB-DM4.

Buffer ingredients as well as the pH have a strong influence to inhibit or to reduce the formation of visible and sub-visible particles. Visible particles can be observed by visual inspection. Sub-visible aggregation is detectable via dynamic light scattering (DLS).

A higher melting temperature T_m measured with DSC is a strong indication for increased immunoconjugate stability.

The MF-T-SPDB-DM4 conjugate was formulated at approximately 5.0 mg/ml in:

(1) 100 mM potassium phosphate pH 7.5
(2) 10 mM L-Histidine, 130 mM Glycine pH 7.3
(3) 20 mM sodium citrate, 10% trehalose pH 6.6
(4) 10 mM L-Histidine, 130 mM Glycine pH 5.5

(5) 0.9% NaCl

Table 1: Influence of several buffer systems on aggregation behavior and stability

Composition	**1**	**2**	**3**	**4**	**5**
Buffer	KPi	His-Gly	Citrate	His-Gly	-
pH	7.5	7.3	6.6	5.5	-
Visual inspection	ok	ok	ok	ok	ok
DLS d_H [nm]	20	19	19	18	20
DSC T_{m1} [°C]	70.1	73.6	70.9	73.4	69.0

The buffering system has a strong influence on the immunoconjugate stability indicated by the melting temperature T_{m1}, whilst the particle formation and aggregation was not affected. Preferred for MF-T-SPDB-DM4 are as depicted in Table 1 buffering systems containing L-Histidine and Glycine showing an increased T_{m1} of approximately 3°C.

EXAMPLE 3

This example shows the effect of several buffer systems on aggregation assessed by visual appearance, by dynamic light scattering (DLS), and by size exclusion chromatography (SEC) in the following three experiments:

a. 48 hours storing experiment at 20°C
b. Shaking stress test for 24 hours at 20°C
c. Inverted shaking stress test with stopper contact for 24 hours at 20°C

These experiments try to assess the immunoconjugate stability at non optimal treatment (storage) conditions.

The MF-T-SPDB-DM4 conjugate was formulated at approximately 5.0 mg/ml in:

(1) 100 mM potassium phosphate pH 7.5

(2) 10 mM L-Histidine, 130 mM Glycine pH 7.3

(3) 20 mM sodium citrate, 10% trehalose pH 6.6

(4) 10 mM L-Histidine, 130 mM Glycine pH 5.5

(5) 0.9% NaCl

Table 2: Influence of several buffer systems on the aggregation behavior at non optimal treatment conditions.

Composition		1	2	3	4	5
Buffer		KPi	His-Gly	Citrate	His-Gly	-
pH		7.5	7.3	6.6	5.5	-
48h/20°C	visual	ok	ok	ok	ok	ok
Storing test	DLS d_H [nm]	22	20	14	16	16
	c_{Ab} [mg/ml]	5.7	3.9	4.9	4.5	5.0
	c_{Ab} [%]	102.5	102.7	102.5	99.8	99.2
	DM4/Ab Ratio	2,9	2,9	2,9	2,7	2,9
	Monomer [%]	92.8	95.8	93.3	95.2	***83.3***
	LMW1+2 [%]	0.4	0.4	1.6	0.6	***11.8***
	HMW Dimer [%]	6.8	3.8	***5.1***	4.2	4.8
Shaking test	visual	***aggregates***	ok	ok	ok	ok
	DLS d_H [nm]	12	14	12	18	17
	c_{Ab} [mg/ml]	***4.0***	4.0	5.0	4.7	5.1
	c_{Ab} [%]	***72.5***	105.1	104.6	103.8	102.8
	DM4/Ab Ratio	2,6	2,8	2,9	2,6	2,9
	Monomer [%]	-	95.5	93.3	95.1	***83.0***
	LMW 1+2 [%]	-	0.7	1.6	0.7	***12.3***
	HMW Dimer [%]	-	3.8	***5.1***	4.2	4.8
Inv.shaking	visual	***aggregates***	ok	ok	ok	ok
(stopper	DLS d_H [nm]	17	11	12	13	22
contact)	c_{Ab} [mg/ml]	***4.3***	***3.5***	4.8	4.5	5.0
	c_{Ab} [%]	***77.2***	***92.0***	101.5	99.6	99.6
	DM4/Ab Ratio	2,6	2,8	2,9	2,7	2,9
	Monomer [%]	-	95.7	93.0	95.0	***82.7***
	LMW1+2 [%]	-	0.6	1.6	0.5	***12.3***
	HMW Dimer [%]	-	3.8	***5.4***	4.1	5.0

The buffering conditions have a strong effect on the aggregation behavior at non optimal treatment (storage) conditions, which was not obvious in example 2. In example 2 it could only be observed that the buffering systems containing L-Histidine and Glycine show an increased T_{m1} indicating higher immunoconjugate stability.

As shown in Table 2 composition 1 and composition 5 have a strong behavior to form aggregates. For composition 1 the tendency to aggregate is already obvious by visual inspection in the shaking test as well as in the inverted shaking experiment. For composition 5 size exclusion chromatography (SEC) results show a dramatic immunoconjugate monomer decrease indicating aggregation. Composition 3 shows an increase of immunoconjugate dimer formation and therefore reveals the tendency for aggregation.

Surprisingly the composition 2 containing L-Histidine and Glycine at pH 7.3 shows a loss of protein concentration in the inverted shaking (stopper contact) experiment. The relation of protein concentration before and after the shaking experiment (cAb [%]) is only 92% compared to 99.6% at pH 5.5. This loss of protein might be due to an increased adsorption to the stopper at pH 7.3 compared to pH 5.5. This is even more surprising as a decrease of immunoconjugate stability assed by DSC and an increased aggregation behavior could not be observed.

This experiment shows that compositions for MF-T-SPDB-DM4 containing L-Histidine and Glycine are preferred and compositions for MF-T-SPDB-DM4 containing L-Histidine and Glycine at pH 5.5 are highly preferred.

EXAMPLE 4

This example shows the effect of polysorbate 80 on protein aggregation assessed by visual inspection and DLS.

The MF-T-SPDB-DM4 conjugate was formulated at approximately 5.0 mg/ml in:

10 mM L-Histidine, 130 mM Glycine pH 5.5, containing 0.0 to 0.1 % polysorbate 80

Table 3: Influence of polysorbate 80 on protein aggregation

Conditions	polysorbate 80 [%]	Visual inspection	DLS d_H [nm]	$Span_{25-75}$	C_{Ab} [mg/ml]
Without agitation	0.000	clear	8	0.41	5.09
	0.001	clear	17	0.38	5.11
	0.005	clear	15	0.45	5.09
	0.010	clear	8	0.25	5.09
	0.100	clear	17	0.37	4.61
Inv. shaking (stopper contact)	0.000	clear	17	0.39	5.04
	0.001	clear	16	0.45	5.19
	0.005	clear	18	0.45	5.06
	0.010	clear	19	0.33	4.93
	0.100	clear	22	0.39	4.62

The addition of polysorbate 80 does not have any negative effect in the preferred puffer system up to a concentration of 0.1% (see Table 3). The addition of a detergent is generally favored in order to avoid uncontrolled adsorption during storage and application. A polysorbate 80 concentration of about 0.01% is preferred.

EXAMPLE 5

This example shows the stability of preferred liquid formulations over a 14 day time period. In addition two sucrose concentrations were checked for protein aggregation assessed by visual inspection, DLS and size exclusion chromatography (SEC).

Sucrose is a very powerful and one of the commonly used lyoprotectants. For a formulation suitable for liquid storage as well as lyophilization the influence of sucrose on the MF-T-SPDB-DM4 immunoconjugate was assessed.

The MF-T-SPDB-DM4 conjugate was formulated at approximately 5.0 mg/ml in:

(1) 10% sucrose, 0.01% polysorbate 80, 10 mM L-Histidine, 130 mM Glycine pH 5.5

(2) 5% sucrose, 0.01% polysorbate 80, 10 mM L-Histidine, 130 mM Glycine pH 5.5

Table 4: Influence of sucrose on protein aggregation

Composition		Time [d]	Storage temperature	Visual inspection	DLS d_H [nm]	C_{Ab} [mg/ml]	SEC HMW [area%]	SEC Monomer [area%]	SEC LMW [area%]
1	10% sucrose	start	-	clear	15	4.99	4.32	95.64	0.04
		14	2 - 8°C	clear	22.5	4.93	4.28	95.45	0.27
		14	25°C	clear	20.2	4.98	3.79	95.80	0.40
		14	40°C	clear	17.7	5.05	6.12	93.1	0.78
2	5% sucrose	start	-	clear	19	4.93	3.6	96.4	0.0
		14	2 - 8°C	clear	20	5.2	3.7	96.1	0.2
		14	25°C	clear	22	5.33	3.9	95.7	0.4
		14	40°C	clear	19	5.25	5.3	93.8	0.9

Over a 14 day time period compositions containing the MF-T-SPDB-DM4 immunoconjugate are stable. These are compositions suitable for lyophilization as shown in example 6 and example 8. No aggregation could be observed by visual inspection, DLS and SEC. The monomer content is very stable. Even at elevated temperatures of 40°C the liquid formulation does not show aggregation over a 14 day time period.

Compositions containing 5 to 10% of sucrose are preferred formulations. Compositions containing 5% of sucrose are highly preferred due to a lower tonicity.

This experiment also shows that a reconstituted solution after lyophilization can be stored over a long time period without aggregation.

EXAMPLE 6

This example shows the robustness of lyophilization and the suitability of the liquid composition for lyophilization. In addition the results achieved after reconstitution of the lyophilized samples clearly show that the composition in addition allows for a lyophilized formulation.

The MF-T-SPDB-DM4 conjugate was formulated at 5.0 mg/ml in the highly preferred embodiment:

10 mM L-Histidine, 130 mM Glycine, 5% sucrose, 0.01% polysorbate 80, pH 5.5

Two lyophilization methods have been used: conditions 1: a quite harsh method taking in total approximately 62.5 hours; conditions 2: a more gentle method taking in total approximately 95.5 hours. The details of the lyophilization cycles have been summarized in Table 5.

Table 5: Parameters of the used lyophilization cycles

		Conditions 1			**Conditions 2**		
		Time [hh:mm]	Temp [°C]	Pressure [mbar]	Time [hh:mm]	Temp [°C]	Pressure [mbar]
	Loading	00:01	5.0		00:01	20.0	
	Freezing	02:00	-50.0		00:30	-5.0	
	Freezing				01:00	-5.0	
	Freezing				01:00	-45.0	
	Freezing				03:30	-45.0	
	Evacuation	00:30	-50.0	0.130	00:30	-45.0	0.100
	Prim. drying	02:00	-50.0	0.130	00:01	-45.0	0.100
	Prim. drying	00:30	-50.0	0.066	01:00	-10.0	0.100
	Prim. drying	02:00	0	0.066	75:00	-10.0	0.100
	Prim. drying	16:40	0	0.066			
	Prim. drying	01:30	0	0.053			
	Sec. drying	03:00	30.0	0.053	01:00	40.0	0.050
	Sec. drying	36:40	30.0	0.053	12:00	40.0	0.050
Summary	Loading	00:01			00:01		
	Freezing	02:00			06:30		
	Primary drying	20:40			76:01		
	Secondary drying	39:40			13:00		
	Total	**62:21**			**95:32**		

The lyophilization methods described above result in a white cake or powder, which can be reconstituted rapidly (around 1 min) in water. If reconstituted at the same protein concentration as before lyophilization (5 mg/ml) a clear solution without any particles was

observed. No aggregation or hints of aggregation were detected (see Table 6). The SEC monomer content is in the same range as with no lyophilization (see Table 4 for comparison).

In depth analysis of the reconstituted solution included detection of non-visible particles via HIAC method, in addition the more sensitive MFI (micro flow imaging) method was deployed. The particles / container (volume 12.5 ml) are well within generally accepted criteria. This is an important requirement for the suitability as a pharmaceutical product.

In Table 6 properties of the samples after reconstitution with water for injection have been summarized.

Table 6: Characterization of lyophilized batches after reconstitution with water [final volume 12.5 ml / container]

Batch	**Batch 1**	**Batch 2**	**Batch 3**
Lyophilization	Conditions 1	Conditions 2	Conditions 2
Reconstitution time	62 sec	41 sec	33 sec
Visual Inspection	clear	clear	clear
SEC, Monomer [area%]	92.4	92.2	91.8
SEC, LMW, [area%]	1.0	1.4	1.4
pH	5.5	5.7	5.7
Protein concentration	5.0 mg/ml	5.0 mg/ml	4.9 mg/ml
	particles/ container	particles/ container	particles/ container
Non visible particles HIAC method. ≥ 2µm	1812	734	520
Non visible particles. HIAC method. ≥ 5µm	340	173	115
Non visible particles. HIAC method. ≥ 10µm	90	38	38
Non visible particles. HIAC method. ≥ 50µm	9	4	1
Non visible particles. MFI method. ≥ 2µm	3185	2137	2258
Non visible particles. MFI method. ≥ 5µm	540	192	359
Non visible particles. MFI method. ≥ 10µm	148	55	71
Non visible particles. MFI method. ≥ 25µm	28	9	0

EXAMPLE 7

This example shows the chemical stability of the immunoconjugate MF-T-SPDB-DM4 in several preferred liquid formulations over a 14 day time period. Two different sucrose concentrations were used.

The MF-T-SPDB-DM4 conjugate was formulated at approximately 5.0 mg/ml in:

(1) 10% sucrose, 0.01% polysorbate 80, 10 mM L-Histidine, 130 mM Glycine pH 5.5

(2) 5% sucrose, 0.01% polysorbate 80, 10 mM L-Histidine, 130 mM Glycine pH 5.5

MF-T-SPDB-DM4 is stable at different storage conditions over 14 days long time period (see Table 7). Even at elevated temperatures of 25°C or 40°C only small amounts of free toxophor species (free DM4-linker, free DM4) are detectable. So the preferred compositions are physically as well as chemically stable

Table 7: Chemical stability of immunoconjugate MF-T-SPDB-DM4 in preferred formulations

Composition		Time [d]	Storage temperature	Visual inspection	C_{Ab} [mg/ ml]	DM4/Ab ratio	free DM4-linker [µg/ ml]	free DM4 [µg/ ml]
1	10% sucrose	start	-	clear	4.99	2.7	-	-
		14	2 - 8°C	clear	4.93	2.7	0.003	<0.002
		14	25°C	clear	4.98	2.7	0.106	<0.002
		14	40°C	clear	5.05	2.4	0.349	0.004
2	5% sucrose	start	-	clear	4.93	2.4	-	-
		14	2 - 8°C	clear	5.2	2.8	0.021	0.002
		14	25°C	clear	5.33	2.7	0.110	0.002
		14	40°C	clear	5.25	2.5	0.313	0.004

EXAMPLE 8

This example shows the long term stability of the immunoconjugate MF-T-SPDB-DM4 in a preferred lyophilized composition.

The MF-T-SPDB-DM4 conjugate was formulated at approximately 5.0 mg/ml in 10 mM L-Histidine, 130 mM Glycine, 5% sucrose, 0.01% polysorbate 80, pH 5.5. The liquid formulation was lyophilized as described in example 6. The lyophilized composition to be reconstituted to contain 5 mg/ml MF-T-SPDB-DM4 comprises therefore 0.31 mg L-Histidine, 1.95 mg Glycine, 9.99 mg sucrose, and 0.02 mg polysorbate 80 per mg of the immunoconjugate MF-T-SPDB-DM4. Once reconstituted with water, such a lyophilized composition has a pH of about 5.5.

The lyophilized composition was stored for a time period of 24 month at 2 to 8°C. At certain time points (3, 6, 9, 12, 18, and 24 month) samples were reconstituted with sterile water.

After reconstitution (final volume 12.5 ml / vial) the liquid composition was analyzed for usability in pharmaceutical applications. In addition to the assays described above which analyze the physical stability (aggregation, particle formation, etc.) and chemical stability (free DM4 species; DM4/antibody ratio) also the potency as immunoconjugate was analyzed in a cell based bioassay.

As shown in table 8 all relevant parameters are stable for a 24 month time period. All measured parameters including biological potency of the immunoconjugate show that the preferred formulations enable a long shelf live.

Table 8: Long term stability (up to 24 months) of immunoconjugate MF-T-SPDB-DM4 in preferred lyophilized formulation. Test results after reconstitution with sterile water.

	Storage Time (months)						
Test	**0**	**3**	**6**	**9**	**12**	**18**	**24**
Reconstitution time [sec]	28	40	39	56	48	46	40
Protein conc. [mg/ml]	4.8	5.2	4.9	4.9	4.9	5.0	4.8
pH-value	5.6	5.5	5.6	5.5	5.6	5.5	5.7
Visible particles	free	free	free	free	free	free	free
HIAC, particles >= 25 µm per vial	5	-	-	-	0	-	21
HIAC, particles >= 10 µm per vial	21	-	-	-	15	-	9
Monomer SEC-HPLC [%]	92.1	91.6	92.1	92.6	92.4	90.5	90.9
LMW SEC-HPLC [%]	n.d.	n.d.	n.d.	0.3	0.3	2.2	1.3
Free DM4 species [µg/mg]	0.17	0.17	0.19	0.18	0.20	0.18	0.15
DM4/Ab ratio	3.0	3.1	3.1	3.0	3.1	3.1	3.2
Potency (cell based bioassay) [%]	55	66	51	69	57	76	50

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USED METHODS

Determination of protein concentrations and determination of maytansinoid/antibody ratio (DM4/Ab ratio)

Protein concentrations and the maytansinoid/antibody ratio (DM4/Ab ratio) were determined via measurement of the absorption at two wavelength (280 nm and 252 nm) using a Nanodrop 2000 (Thermo Scientific). Each solution was measured twice on three independent samples.

Samples were diluted with formulation buffer to obtain a UV-absorption at 280 nm in the range of 0.6 to 1.2. The sample was measured at 252 nm and 280 nm using the formulation buffer as blank. The calculations are shown below.

Calculations:

Concentration of DM4 [M]

$$[DM4] = \frac{[(A_{252} \times DF) - (A_{280} \times DF \times AB_{\varepsilon 252/\varepsilon 280})]}{[5323 \times (4{,}89 - AB_{\varepsilon 252/\varepsilon 280})]}$$

A_{252} = Extinction 252 nm

DF = dilution factor

A_{280} = Extinction 280 nm

$AB_{\varepsilon 252/\varepsilon 280}$ = Ratio of extinction coefficients of antibody 0.35

$AB_{\varepsilon 252}$ = 68614 [M^{-1} $(cm)^{-1}$]

$AB_{\varepsilon 280}$ = 195413 [M^{-1} $(cm)^{-1}$]

$DM4_{\varepsilon 280}$ = 5323 [M^{-1} $(cm)^{-1}$]

$DM4_{\varepsilon 252}$ = 26010 [M^{-1} $(cm)^{-1}$]

$DM4_{\varepsilon 252/\varepsilon 280}$ = Ratio of extinction coefficients of DM4 (4.89)

Concentration of antibody [M]

$$[AB] = \frac{[(A_{280} \times DF) - (5323 \times [conc.of\ DM4])]}{AB_{\varepsilon 280}}$$

$DM4_{\varepsilon 280}$ = 5323 [M^{-1} $(cm)^{-1}$]

$AB_{\varepsilon 280}$ = 195413 [M^{-1} $(cm)^{-1}$]

The DM4/AB ratio

$$= \frac{[DM4]}{[AB]}$$

The concentration of antibody [mg/mL]

$$= [AB] \times MW_{AB}$$

[AB] = Molar concentration of antibody

MW_{AB} = 146278 g/mol

Visual inspection

For visual appearance testing solutions containing MF-T-SPDB-DM4 were inspected using a dark background for particles or turbidity. A clear solution after buffer exchange or stress testing is a sign of minor presence or absence of dimer and/or oligomer formation, whereas a visible turbidity of the solution correlates with a high content of dimers and oligomers.

Dynamic light scattering (DLS)

Dynamic light scattering is a method for analyzing the scattered light generated by a laser. The light scattered by a solubilized or suspended probe can be used to calculate the hydrodynamic radius (d_H [nm]). An increasing hydrodynamic radius is an indication for aggregation of the immunoconjugate. The hydrodynamic radius via DLS was determined using a Horiba LB 550 (Retsch Technology). A measured d_H greater than 25nm was seen as critical. d_H values between 16 nm and 25 nm indicated a well behaved immunoconjugate.

Differential scanning calorimetry, (DSC)

Protein stability can be measured via determination of the melting temperature. With differential scanning calorimetry (DSC) the melting temperature (T_m) of MF-T-SPDB-DM4 in several solutions was measured. Samples were heated from 20°C to 105°C and the melting temperature (T_m) was determined using a VP-DSC calorimeter (GE Healthcare).

Shaking stress test

Immunoconjugates like MF-T-SPDB-DM4 are very sensitive towards mechanical agitation, which take place during production, filling, or transport. Upon a certain intensity of movement the immunoconjugates start to aggregate and to denature. During the shaking

stress test liquid compositions containing MF-T-SPDB-DM4 were analyzed for aggregation and denaturation under controlled conditions.

MF-T-SPDB-DM4 containing samples were stressed on a lab shaker (IKA, HS 260) in a temperature controlled chamber (MMM, FrioCell 200). The critical quality parameter aggregation (via visual inspection and DLS) was measured after 24 hours at 20°C and 300 rpm.

Size exclusion chromatography, SEC

For the determination of monomer and dimer contents as well as the low molecular weight fragments (LMW) and high molecular weight (HMW) contents a size exclusion chromatography (SEC) was performed using an HPLC system. MF-T-SPDB-DM4 was detected using a fluorescence detector and quantified using the area percent method. Standard HPLC SEC columns for proteins were used, e.g. Tosoh Biosep TSK gel G3000 SWXL 5 µm, 300mm, Length x 7.8mm i.D.

Detection of non-visible particles HIAC and MFI

For the detection and counting of non-visible particles the HIAC and MFI methods were applied. For MFI (Micro Flow Imaging) measurements a Micro-Flow Imaging™ DPA 4200 system (Brightwell Technologies Inc.) was used in accordance with the supplier's instructions. HIAC measurements were performed using an HIAC 9703+ Liquid Particle Counter (HACH Lange) in combination with a HRLD-150 13-150 µm sensor in accordance with the supplier's instructions.

Detection of free maytansinoid with HPLC

The free maytansinoid was determined using an HPLC method. A Supelcosil LC-HISEP, 50 mm x 4.6 mm, 5 µm (Sigma-Aldrich) or an equivalent column was used in combination with a Zorbax Eclipse XDB-C18, 50 mm x 2.1 mm, 3.5 µm (Agilent) or an equivalent column. The columns were used with an Agilent HPLC system. The free maytansinoid was separated

from protein by HISEP chromatography and subsequently quantified by reversed phase high performance chromatography (RP-HPLC). The columns were used at 35°C. As solvents a mobile phase A (0.1 % TFA in water) and a mobile phase B (0.08 % TFA in acetonitrile) were used. Maytansinoid (DM4) and all other components content is calculated by the Empower calc. routine using linear regression analysis of the rutin hydrate calibration curve in µg/mL. To correct the different correlation factors of rutin hydrate and DM4, the results of DM4 and all other components have to be multiplied by the factor 1.05497.

Cell based potency assay (bioassay)

The biological activity of the immunoconjugate MF-T-SPDB-DM4 was tested using a cell based potency assay as described in WO2010/124797 in detail. In brief, mesothelin transfected HT29-cells were seeded into 96-well plates and incubated with a serial dilution of MF-T-SPDB-DM4 over 4 hours. After this incubation with the immunoconjugate the cells were washed carefully with medium and incubated for additional 68 to 96 hours. After this time cellular proliferation was quantified using a standard method (e.g. WST-1 Cell Proliferation Reagent, Roche). The activity ratio compared to a standard value is evaluated and reported.

CLAIMS

We claim:

1. An immunoconjugate formulation comprising:
    a. MF-T-SPDB-DM4; and
    b. one or more excipients selected from the group consisting of an amino acid, polysorbate 80, and sucrose;

    wherein the formulation is a buffered aqueous solution having a pH of 5.0 to 8.0

2. The formulation of claim 1 comprising:
    a. 1mg/ml to 20 mg/ml MF-T-SPDB-DM4
    b. 10 mM to 15 mM L-Histidine
    c. 100 mM to 250 mM Glycine
    d. 5% to 15% sucrose
    e. 0.001% to 0.1% polysorbate 80
3. The formulation of claim 1 comprising:
    a. 5 mg/ml MF-T-SPDB-DM4,
    b. 10 mM L-Histidine,
    c. 130 mM Glycine
    d. 5% sucrose, and
    e. 0.01% polysorbate 80
4. The formulation of claim 3, wherein the formulation is a buffered aqueous solution having a pH of 5.5
5. A lyophilized composition obtained by freeze-drying of a liquid immunoconjugate formulation according to claims 1 to 4.
6. An immunoconjugate formulation obtained by reconstitution of the lyophilized composition of claim 5 in solution
7. An immunoconjugate formulation obtained by reconstitution of the lyophilized composition of claim 5 in water
8. The lyophilized composition of claim 5 that is to be reconstituted to contain 5 mg/ml MF-T-SPDB-DM4 comprises 0.31 mg L-Histidine, 1.95 mg Glycine, 9.99 mg sucrose, and 0.02 mg polysorbate 80 per mg of the immunoconjugate MF-T-

SPDB-DM4. Once reconstituted with water, such a lyophilized composition has a pH of about 5.5.

9. An immunoconjugate formulation according to any one of the preceding claims for use in a therapeutic application.

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2014/072558

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K47/18 A61K47/26 A61K47/48 A61K9/00 A61K9/08
A61K9/19
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, EMBASE, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/124797 A1 (BAYER SCHERING PHARMA AG [DE]; BERHOERSTER KERSTIN [DE]; HEISLER IRING) 4 November 2010 (2010-11-04) cited in the application	1,9
Y	page 24, lines 1-11, 20, 27-29; example 1	2-8

Y	US 2004/241174 A1 (AMPHLETT GODFREY [US] ET AL) 2 December 2004 (2004-12-02) paragraphs [0002], [0007], [0034], [0035]	2-8

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 January 2015	20/01/2015

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
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