In one aspect, there is provided a method of producing an immunoligand/payload conjugate, which method encompasses conjugating at least one payload to an immunoligand by means of a sequence-specific transpeptidase, or a catalytic domain thereof,
wherein the immunoligand comprised in the immunoligand/payload conjugate is at least one selected from the group consisting of an antibody, modified antibody format, antibody derivative or fragment, and/or an antibody mimetic,
wherein the sequence-specific transpeptidase is a sortase enzyme,
and wherein the payload is a toxin not exceeding a molecular weight of 2'500 Dalton.

In one aspect, there is provided an immunoligand/payload conjugate obtained with a method as described herein, which conjugate comprises

- an antibody, modified antibody format, antibody derivative or fragment, and/or an antibody mimetic and
- a toxin not exceeding a molecular weight of 2'500 Dalton.

In one aspect, there is provided the use of an immunoligand/payload conjugate according to the above mentioned claims in the manufacture of a medicament for the treatment of a human or animal subject suffering from or being at risk of developing a given pathologic condition.

In one aspect, there is provided a low molecular-weight payload modified with a Gly_n-modification, wherein, n>1, preferably n=3 or n=5, which payload is a toxin not exceeding a molecular weight of 2'500 Dalton.

In one aspect, there is provided the use of the low molecular-weight payload as disclosed herein for conjugation thereof to an immunoligand selected from the group consisting of an antibody, modified antibody format, antibody derivative or fragment, and/or an antibody mimetic.

It is thus one object of the present ~~invention~~ disclosure to provide an efficient method for conjugating immunoligands and payloads, e.g., drugs, toxins, cytokines, markers, or the like, preferably full-length monoclonal antibodies to small-molecular weight toxins, for the generation of site-specifically conjugated antibody drug conjugates (ADCs).

It is another object of the present ~~invention~~ disclosure to create immunoligand/payload conjugates, which have better efficacy and/or can be produced with higher reproducibility.

It is another object of the present ~~invention~~ disclosure to allow the conjugation of payloads to immunoligands in a site-specific and/or sequence specific manner.

It is another object of the present ~~invention~~ disclosure to create immunoligand/payload conjugates which preserve the characteristic features of its components, e.g., target affinity, target specificity, target sensitivity, solubility, pharmacological function and the like
These objects are achieved by the subject matter of the independent claims, while the dependent claims as well as the specification disclose further preferred embodiments.

Definitions

As used herein, the term "immunoligand" is meant to define an entity, an agent or a molecule that has affinity to a given target, e.g., a receptor, a cell surface protein, a cytokine or the like. Such immunoligand may optionally block or dampen agonist-mediated responses, or inhibit receptor-agonist interaction. Most importantly, however, the immonoligand may serve as a shuttle to deliver a payload to a specific site, which is defined by the target recognized by said immunoligand. Thus, an immunoligand targeting, for instance, but not limited to a receptor, delivers its payload to a site which is characterized by abundance of said receptor. Immunoligands include, but are not limited to, antibodies, antibody fragments, antibody-based binding proteins, antibody mimetics, receptors, soluble decoy receptors, scaffold proteins with affinity for a given target and ligands of receptors.

"Antibodies", also synonymously called "immunoglobulins" (Ig), are generally comprising four polypeptide chains, two heavy (H) chains and two light (L) chains, and are therefore multimeric proteins, or an equivalent Ig homologue thereof (e.g., a camelid nanobody, which comprises only a heavy chain, single domain antibodies (dAbs) which can be either

What is claimed is

1. A method of producing an immunoligand/payload conjugate, which method encompasses conjugating a payload to an immunoligand by means of a sequence-specific transpeptidase, or a catalytic domain thereof.

2. The method according to claim 1, wherein the payload and/or the immunoligand either

a) consists, entirely, of a protein or peptide
b) comprises at least one protein or peptide domain, or
c) comprises at least one peptide chain

and wherein, further, the protein or peptide or domain comprises an amino acid sequence that can be detected by the sequence-specific transpeptidase, or a catalytic domain thereof.

3. The method according to any of the aforementioned claims, wherein the immunoligand comprised in the immunoligand/payload conjugate is at least one selected from the group consisting of an antibody, modified antibody format, antibody derivative or fragment, and/or an antibody mimetic.

4. The method according to any of the aforementioned claims, wherein the immunoligand binds at least one entity selected from the group consisting of

- a receptor
- an antigen
- a growth factor,
- a cytokine, and/or
- a hormone.

5. The method according to any of the aforementioned claims, wherein at least one catalytic domain of the sequence-specific transpeptidase is fused to the N-terminus or the C-terminus of either the immunoligand or the payload.

6. The method according according to any of the aforementioned claims, wherein the sequence-specific transpepeptidase is at least one selected from the group consisting of

- a sortase enzyme, or one or more fragments or derivatives thereof
- a split-intein, or one or more fragments or derivatives thereof

7. The method according to any of the aforementioned claims, wherein the payload comprised in the immunoligand/ payload conjugate is at least one selected from the group consisting of

- a marker,
- a processing tag, and/or
- a drug.

8. The method according to any of the aforementioned claims, wherein said marker is at least one selected from the group consisting of

- a radiolabel, preferably a radioactively labelled peptide or protein
- a fluorescent label, preferably a fluorescent peptide or protein, and/or
- an enzyme label, preferably a peroxidase.

9. The method according to any of the aforementioned claims, wherein said drug is at least one selected from the group consisting of

- a cytokine
- a radioactive agent
- an anti-inflammatory drug
- a toxin, and/or
- a chemotherapeutic agent

10. The method according to any of the aforementioned claims, wherein said immunoligand comprises at least two subunits each being conjugated to a payload.

11. The method according to claim 10, wherein said immunoligand with at least two subunits is conjugated to at least two different payloads, preferably toxic payloads interfering with one or more cellular pathways.

12. The method according to claim 10 or 11, wherein said immunoligand with at least two subunits is being conjugated with at least 80% efficiency per conjugation site.

13. The method according to any of claims 10 - 12, wherein said immunoligand with at least two subunits contains a peptide spacer sequence of at least two amino acids, preferably 2-5 amino acids, appended to the C-termini of at least one of the two subunits

14. The method according to any of the aforementioned claims, which method allows a stoichiometrically defined relationship between immunoligand and payload.

15. The method according to claim 14, in which said stoichiometrically defined relationship between immunoligand and payload is achieved by removal of partially reacted C-terminally modified immunoligand substrate.

16. The method according to any of the aforementioned claims, which method allows a site-specific conjugation of a payload to the immunoligand.

17. An immunoligand/payload conjugate obtained with a method according to the above claims.

18. The immunoligand/payload conjugate according to claim 17, which is selected from the group consisting of an antibody/drug conjugate, and/or an antibody/marker conjugate.

19. Use of an immunoligand/payload conjugate according to the above mentioned claims for

- *in vitro* or *in vivo* diagnosis of a given pathologic condition
- *in vitro* or *in vivo* prediction or prognosis with respect to a given pathologic condition
- the treatment of a human or animal subject suffering from or being at risk of developing a given pathologic condition, and/or
- research and/or development purposes

20. The use according to claim 19, wherein said pathologic condition is at least one selected from the group consisting of

- Neoplastic disease

- Autoimmune disease
- Neurodegenerative disease, and/or
- Infectious disease

21. A low molecular-weight payload modified with a Gly_n-modification, wherein, n>1, preferably n=3 or n=5.

22. The low molecular-weight payload according to claim 21, wherein said payload is at least one selected from the group consisting of

- a cytokine
- a radioactive agent
- a toxin, and/or
- a chemotherapeutic agent.

23. Use of the low molecular-weight payload according to claim 21 or 22 for conjugation thereof to an immunoligand.

24. The method according to any of claims 1 - 16, in which the immunoligand-payload conjugation is performed in crude cell culture supernatant.

What is claimed is

1. A method of producing an immunoligand/payload conjugate, which method encompasses conjugating at least one payload to an immunoligand by means of a sequence-specific transpeptidase, or a catalytic domain thereof,

wherein the immunoligand comprised in the immunoligand/payload conjugate is at least one selected from the group consisting of an antibody, modified antibody format, antibody derivative or fragment, and/or an antibody mimetic,

wherein the sequence-specific transpeptidase is a sortase enzyme,

and wherein the payload is a toxin not exceeding a molecular weight of 2'500 Dalton.

2. The method according to any of the aforementioned claims, wherein the immunoligand binds at least one entity selected from the group consisting of

- a receptor
- an antigen
- a growth factor,
- a cytokine, and/or
- a hormone.

3. The method according to any of the aforementioned claims, wherein at least one catalytic domain of the sequence-specific transpeptidase is fused to the C-terminus of either the immunoligand or the payload.

4. The method according to any of the aforementioned claims, wherein said immunoligand comprises at least two subunits each being conjugated to a payload.

5. The method according to claim 4, wherein said immunoligand with at least two subunits is conjugated to at least two different payloads, at least one of which is a toxin not exceeding a molecular weight of 2'500 Dalton.

6. The method according to any of claims 4 - 5, wherein said immunoligand with at least two subunits contains a peptide spacer sequence of at least two amino acids, preferably 2-5 amino acids, appended to the C-termini of at least one of the two subunits

7. The method according to any of the aforementioned claims, which method allows a stoichiometrically defined relationship between immunoligand and payload.

8. The method according to claim 7, in which method said stoichiometrically defined relationship between immunoligand and payload is achieved by removal of partially reacted C-terminally modified immunoligand substrate.

9. The method according to claim 8, which method comprises the use of at least two affinity purification tags,

which affinity purification tags are bound to at least two different sites of the antibody, modified antibody format, antibody derivative or fragment, and/or antibody mimetic via sortase recognition motifs prior to the conjugation of the at least one small molecular toxin,

which method further comprises an affinity purification step,

in which affinity purification step conjugates which after the conjugation of at least one small molecular toxin to the antibody, modified antibody format, antibody derivative or fragment, and/or antibody mimetic, still bear affinity purification tags, and thus qualify as incomplete conjugates, are separated from the complete comnjuagtes by affinity purification.

10. The method according to any of the aforementioned claims, which method allows a site-specific conjugation of a payload to the immunoligand.

11. An immunoligand/payload conjugate obtained with a method according to the above claims, which conjugate comprises

- an antibody, modified antibody format, antibody derivative or fragment, and/or an antibody mimetic and
- a toxin not exceeding a molecular weight of 2'500 Dalton.

12. The immunoligand/payload conjugate according to claim 11, which conjugate further comprises a sortase recognition motif that has undergone transpeptidation reaction.

13. The immunoligand/payload conjugate according to claim 12, wherein the sortase recognition motif is LPXTG or NPQTN.

14. Use of an immunoligand/payload conjugate according to the above mentioned claims in the manufacture of a medicament for the treatment of a human or animal subject suffering from or being at risk of developing a given pathologic condition.

15. The use or conjugate according to claim 14, wherein said pathologic condition is at least one selected from the group consisting of

- Neoplastic disease
- Autoimmune disease
- Neurodegenerative disease, and/or
- Infectious disease

16. A low molecular-weight payload modified with a Gly_n-modification, wherein, $n>1$, preferably $n=3$ or $n=5$, which payload is a toxin not exceeding a molecular weight of 2'500 Dalton.

17. Use of the low molecular-weight payload according to claim 16 for conjugation thereof to an immunoligand selected from the group consisting of an antibody, modified antibody format, antibody derivative or fragment, and/or an antibody mimetic.

18. The method according to any of claims 1 - 10, in which the immunoligand-payload conjugation is performed in crude cell culture supernatant.