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.

In one aspect, there is provided a method of producing an immunoligand/payload conjugate, the method comprising

a) providing an immunoligand selected from the group consisting of:

(i) an antibody,

(ii) an antibody-based binding protein being a protein containing at least one antibody-derived V_H, V_L, or C_H immunoglobulin domain,

(iii) an antibody fragment binding to a receptor, antigen, growth factor, cytokine and/or hormone, and/or

(iv) an antibody mimetic selected from the group consisting of DARPins, C-type lectins, A-domain proteins of *S. aureus,* transferrins, lipocalins, 10th type III domains of fibronectin, Kunitz domain protease inhibitors, affilins, gamma crystallin derived binders, cysteine knots or knottins, thioredoxin A scaffold based binders, nucleic acid aptamers, artificial antibodies produced by molecular imprinting of polymers, and stradobodies,

and

b) enzymatically conjugating at least one payload to the immunoligand by a sequence-specific sortase or a catalytic domain thereof, wherein the payload is a toxin having a molecular weight not exceeding 2,500 Dalton,

wherein either

- the immunoligand comprises a sortase recognition motif and the toxin is modified with a Gly_n-modification, wherein $n > 1$, or
- the toxin comprises a sortase recognition motif and the immunoligand is modified with a Gly_n-modification, wherein $n > 1$,

thereby producing the immunoligand/payload conjugate.

In one aspect, there is provided an immunoligand/payload conjugate produced/obtained/obtainable by a method comprising

a) providing an immunoligand selected from the group consisting of:

(i) an antibody,

(ii) an antibody-based binding protein being a protein containing at least one antibody-derived V_H, V_L, or C_H immunoglobulin domain,

(iii) an antibody fragment binding to a receptor, antigen, growth factor, cytokine and/or hormone, and/or

(iv) an antibody mimetic selected from the group consisting of DARPins, C-type lectins, A-domain proteins of *S. aureus*, transferrins, lipocalins, 10th type III

(v) domains of fibronectin, Kunitz domain protease inhibitors, affilins, gamma crystallin derived binders, cysteine knots or knottins, thioredoxin A scaffold based binders, nucleic acid aptamers, artificial antibodies produced by molecular imprinting of polymers, and stradobodies, and,

b) enzymatically conjugating at least one payload to the immunoligand by a sequence-specific sortase or a catalytic domain thereof, wherein the payload is a toxin having a molecular weight not exceeding 2,500 Dalton, wherein either

- the immunoligand comprises a sortase recognition motif and the toxin is modified with a Gly_n-modification, wherein $n > 1$, or
- the toxin comprises a sortase recognition motif and the immunoligand is modified with a Gly_n-modification, wherein $n > 1$.

In one aspect, there is provided an immunoligand/payload conjugate comprising

a) an immunoligand selected from the group consisting of:

(i) an antibody,
(ii) an antibody-based binding protein being a protein containing at least one antibody-derived V_H, V_L, or C_H immunoglobulin domain,
(iii) an antibody fragment binding to a receptor, antigen, growth factor, cytokine and/or hormone, and/or
(iv) an antibody mimetic selected from the group consisting of DARPins, C-type lectins, A-domain proteins of *S. aureus*, transferrins, lipocalins, 10th type III domains of fibronectin, Kunitz domain protease inhibitors, affilins, gamma crystallin derived binders, cysteine knots or knottins, thioredoxin A scaffold based binders, nucleic acid aptamers, artificial antibodies produced by molecular imprinting of polymers, and stradobodies, and,

b) at least one a payload being a toxin having a molecular weight not exceeding 2,500 Dalton

wherein the conjugate further comprises at least one linker that conjugates a payload to the immunoligand, which linker

a) comprises a peptide motif that is a sortase recognition motif or a derivative thereof, and
b) is conjugated to the C-terminus of at least one peptide chain of the immunoligand..

In one aspect, there is provided a low molecular-weight payload, wherein the payload is a toxin having a molecular weight not exceeding 2,500 Dalton, and the toxin is modified with a Gly_n-modification, wherein $n > 1$.

It is thus one object of the present 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 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 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 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, the method comprising

a) providing an immunoligand selected from the group consisting of:

(i) an antibody,

(ii) an antibody-based binding protein being a protein containing at least one antibody-derived V_H, V_L, or C_H immunoglobulin domain,

(iii) an antibody fragment binding to a receptor, antigen, growth factor, cytokine and/or hormone, and/or

(iv) an antibody mimetic selected from the group consisting of DARPins, C-type lectins, A-domain proteins of *S. aureus,* transferrins, lipocalins, 10th type III domains of fibronectin, Kunitz domain protease inhibitors, affilins, gamma crystallin derived binders, cysteine knots or knottins, thioredoxin A scaffold based binders, nucleic acid aptamers, artificial antibodies produced by molecular imprinting of polymers, and stradobodies,

and

b) enzymatically conjugating at least one payload to the immunoligand by a sequence-specific sortase or a catalytic domain thereof, wherein the payload is a toxin having a molecular weight not exceeding 2,500 Dalton,

wherein either

- the immunoligand comprises a sortase recognition motif and the toxin is modified with a Gly_n-modification, wherein $n > 1$, or
- the toxin comprises a sortase recognition motif and the immunoligand is modified with a Gly_n-modification, wherein $n > 1$,

thereby producing the immunoligand/payload conjugate.

2. The method of claim 1, wherein the immunoligand binds at least one entity selected from the group consisting of a receptor, an antigen, a growth factor, a cytokine, and a hormone.

3. The method of claim 1, wherein at least one catalytic domain of the sortase is fused to the C-terminus of either the immunoligand or the payload.

4. The method of claim 1, wherein comprising conjugating said immunoligand to at least two different payloads, at least one of which is the toxin, and wherein said immunoligand has at least two subunits each being conjugated to a payload.

5. The method of claim 1, wherein said immunoligand comprises two subunits, at least one of which has a peptide spacer appended to the C-terminus thereof, said spacer comprises at least two amino acids.

6. The method of claim 5, wherein the spacer is 2-5 amino acids.

7. The method of claim 1, wherein the ratio of immunoligand and payload is stoichiometrically defined.

8. The method of claim 7, wherein said method comprises removal of partially reacted C-terminally modified immunoligand substrate.

9. The method of claim 1, wherein the method comprises an affinity purification step to separate incomplete conjugates from complete conjugates using an affinity purification tag which is attached to the immunoligand via a sortase recognition motif.

10. The method of claim 9, wherein the immunoligand contains at least two affinity purification tags.

11. The method of claim 1, wherein the conjugation of the payload to the immunoligand is site-specific.

12. The method of claim 1, wherein the conjugation comprises a transpeptidation of the sortase recognition motif.

13. The method of claim 1, wherein the sortase recognition motif is LPXTG (SEQ ID NO:27) or NPQTN (SEQ ID NO:28).

14. The method of claim 1, wherein the conjugation is performed in crude cell culture supernatant.

15. An immunoligand/payload conjugate produced/obtained/obtainable by a method comprising

a) providing an immunoligand selected from the group consisting of:

(i) an antibody,

(ii) an antibody-based binding protein being a protein containing at least one antibody-derived V_H, V_L, or C_H immunoglobulin domain,

(iii) an antibody fragment binding to a receptor, antigen, growth factor, cytokine and/or hormone, and/or

(iv) an antibody mimetic selected from the group consisting of DARPins, C-type lectins, A-domain proteins of *S. aureus*, transferrins, lipocalins, 10th type III domains of fibronectin, Kunitz domain protease inhibitors, affilins, gamma crystallin derived binders, cysteine knots or knottins, thioredoxin A scaffold based binders, nucleic acid aptamers, artificial antibodies produced by molecular imprinting of polymers, and stradobodies, and,

b) enzymatically conjugating at least one payload to the immunoligand by a sequence-specific sortase or a catalytic domain thereof, wherein the payload is a toxin having a molecular weight not exceeding 2,500 Dalton, wherein either

- the immunoligand comprises a sortase recognition motif and the toxin is modified with a Gly_n-modification, wherein $n > 1$, or
- the toxin comprises a sortase recognition motif and the immunoligand is modified with a Gly_n-modification, wherein $n > 1$.

16. The immunoligand/payload conjugate of claim 15, wherein the immunoligand binds at least one entity selected from the group consisting of a receptor, an antigen, a growth factor, a cytokine, and a hormone.

17. The immunoligand/payload conjugate of claim 15, wherein the immunoligand is conjugated to at least two different payloads, at least one of which is the toxin, and wherein said immunoligand has at least two subunits each being conjugated to a payload.

18. The immunoligand/payload conjugate of claim 15, wherein the immunoligand comprises two subunits, at least one of which has a peptide spacer appended to the C-terminus thereof, said spacer comprises at least two amino acids.

19. The immunoligand/payload conjugate of claim 18, wherein the spacer is 2-5 amino acids.

20. The immunoligand/payload conjugate of claim 15, wherein the ratio of immunoligand and payload is stoichiometrically defined.

21. The immunoligand/payload conjugate of claim 15, wherein the conjugation of the payload to the immunoligand is site-specific.

22. The immunoligand/payload conjugate of claim 15, wherein the sortase recognition motif is LPXTG (SEQ ID NO:27) or NPQTN (SEQ ID NO:28).

23. An immunoligand/payload conjugate comprising

a) an immunoligand selected from the group consisting of:

(i) an antibody,

(ii) an antibody-based binding protein being a protein containing at least one antibody-derived V_H, V_L, or C_H immunoglobulin domain,

(iii) an antibody fragment binding to a receptor, antigen, growth factor, cytokine and/or hormone, and/or

(iv) an antibody mimetic selected from the group consisting of DARPins, C-type lectins, A-domain proteins of *S. aureus*, transferrins, lipocalins, 10th type III domains of fibronectin, Kunitz domain protease inhibitors, affilins, gamma crystallin derived binders, cysteine knots or knottins, thioredoxin A scaffold based binders, nucleic acid aptamers, artificial antibodies produced by molecular imprinting of polymers, and stradobodies, and,

b) at least one a payload being a toxin having a molecular weight not exceeding 2,500 Dalton

wherein the conjugate further comprises at least one linker that conjugates a payload to the immunoligand, which linker

a) comprises a peptide motif that is a sortase recognition motif or a derivative thereof, and

b) is conjugated to the C-terminus of at least one peptide chain of the immunoligand.

24. The immunoligand/payload conjugate according to claim 23, wherein, in the derivative of the sortase recognition motif, the C-terminal amino acid residue of the original sortase recognition motif is replaced by a G residue.

25. The immunoligand/payload conjugate according to claim 24, wherein the derivative of the sortase recognition motif is $-LPXTG_n-$ or $-NPQTG_n-$.

26. The immunoligand/payload conjugate of claim 23, wherein the immunoligand binds at least one entity selected from the group consisting of a receptor, an antigen, a growth factor, a cytokine, and a hormone.

27. The immunoligand/payload conjugate of claim 23, wherein the immunoligand is conjugated to at least two different payloads, at least one of which is the toxin, and wherein said immunoligand has at least two subunits each being conjugated to a payload.

28. The immunoligand/payload conjugate of claim 23, wherein the immunoligand comprises two subunits, at least one of which has a peptide spacer appended to the C-terminus thereof, said spacer comprises at least two amino acids.

29. The immunoligand/payload conjugate of claim 18, wherein the spacer is 2-5 amino acids.

30. The immunoligand/payload conjugate of claim 23, wherein the ratio of immunoligand and payload is stoichiometrically defined.

31. The immunoligand/payload conjugate of claim 23, wherein the conjugation of the payload to the immunoligand is site-specific.

32. A low molecular-weight payload, wherein the payload is a toxin having a molecular weight not exceeding 2,500 Dalton, and the toxin is modified with a Gly_n-modification, wherein $n > 1$.

33. The low molecular-weight payload of claim 32, wherein the payload is selected from Maytansine, Monomethyl Auristatin and Alpha-amanitin or derivatives thereof.

34. Use of an immunoligand/payload conjugate of claim 15 or claim 23 in the manufacture of a medicament for treating a human or animal subject suffering from or being at risk of developing a pathologic condition.

35. The use of claim 34, wherein the pathologic condition is selected from the group consisting of neoplastic disease, autoimmune disease, neurodegenerative disease, and infectious disease.