SUMMARY

[**0006**] The present technology is directed to an antibody-urease conjugate. The present technology provides for a pharmaceutical composition comprising a pharmaceutically acceptable aqueous solution suitable for intravenous injection and an antibody-urease conjugate substantially free of urease, free of unconjugated antibody, and/or free of non-aqueous HPLC solvents.

[0006a] In one aspect, there is provided a pharmaceutical composition comprising a pharmaceutically acceptable aqueous solution suitable for intravenous injection and a single domain antibody-urease conjugate, wherein the conjugate has a conjugation ratio of 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 single domain antibody moieties per urease moiety and said conjugate is substantially free of unconjugated urease.

[0006b] In one aspect, there is provided a method of preparing a composition comprising a single domain antibody-urease conjugate and no more than about 5% w/w of unconjugated urease based on the weight of the single domain antibody-urease conjugate, which method comprises (1) combining an activated single domain antibody and urease in a solvent in which the activated single domain antibody and urease substantially do not react to form a reaction mixture wherein the distribution of the activated single domain antibody and urease in the solvent is uniform; and

(2) increasing pH of the mixture of (1) to about 8-9 such that the activated single domain antibody readily reacts with the urease to form the single domain antibody-urease conjugate having 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 single domain antibody moieties per urease moiety; and

(3) optionally purifying the single domain antibody-urease conjugate by a purification step, wherein said conjugate is substantially free of unconjugated urease, and

wherein the method does not comprise a chromatographic purification step.

[0006c] In one aspect, there is provided a method of increasing antibody binding affinity to a tumor antigen, the method comprising conjugating a plurality of single domain antibody molecules to a urease molecule to form an antibody-urease conjugate having a conjugation ratio of 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 single domain antibody molecules per urease molecule, wherein the single domain antibody-urease conjugate has a binding affinity to the tumor antigen at least about100 times higher than an un-conjugated antibody.

[0006d] In one aspect, there is a kit comprising the composition as disclosed herein and instructions for use of the composition.

[**0007**] In some embodiments, the conjugate has a conjugation ratio of 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 antibody moieties per urease moiety. In some embodiments, the conjugate has a conjugation ratio of about 6 or more antibody moieties per urease moiety. In some embodiments, the conjugate has a conjugation ratio of 6, 7, 8, 9, 10, 11, or 12 antibody moieties per urease moiety. In

some embodiments, the conjugate has a conjugation ratio of 8, 9, 10, 11, or 12 antibody moieties per urease moiety. In some embodiments, the conjugate has an average conjugation ratio of about 6 or more antibody moieties per urease moiety. In some embodiments, the conjugate has an average conjugation ratio of about 8-11 antibody moieties per urease moiety. In some embodiments, the urease is a Jack bean urease.

[0008] In some embodiments, the antibody is a humanized or non-human antibody. In some embodiments, the molecular weight of the antibody is from about 5 kDa to about 200 kDa. In some embodiments, the molecular weight of the antibody is from about 5 kDa to about 50 kDa. In some embodiments, the antibody is a single domain antibody. In some embodiments, the single domain antibody has a size of no more than 110 amino acid residues, or from about 90 to 130 amino acid residues. In some embodiments, the molecular weight of the single domain antibody is from about 10 kDa to about 50 kDa. In some embodiments, the molecular weight of the single domain antibody is from about 12 kDa to about 15 kDa. In some embodiments, the antibody has specificity to an antigen a tumor cell. In some embodiments, the antibody has specificity to a tumor antigen expressed by non-small cell lung carcinoma. In some embodiments, the antibody has specificity to CEACAM6.

[0009] In some embodiments, the antibody has a binding affinity to CEACAM6 with a K_d value of higher than about 1×10^{-6} M. In some embodiments, the conjugate has a binding affinity to CEACAM6 with a K_d value of no more than about 1×10^{-8} M. In some embodiments, the conjugate has a binding affinity to CEACAM6 with a K_d value of no more than about 1×10^{-10} M. In some embodiments, the conjugate has a binding affinity to CEACAM6 with an IC_{50} value

of no more than about 5 nM. In some embodiments, the IC_{50} value is about 3 nM to about 5 nM. In some embodiments, the IC_{50} value is about 3.22 nM. In some embodiments, the conjugate binds to CEACAM6 with an IC_{50} value of about 10 μg/mL to about 30 μg/mL. In some embodiments, the conjugate binds to CEACAM6 with an IC_{50} value of about 20 μg/mL.

[0010] In some embodiments, the antibody comprises a polypeptide comprising an amino acid sequence of SEQ ID NO. 1. In some embodiments, the antibody comprises a polypeptide comprising at least one modification to the amino acid sequence of SEQ ID NO. 1.

[0011] The present technology provides for a method of treating cancer in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the composition provided herein, thereby treating cancer in the subject.

[0012] In some embodiments, the cancer is one or more of non-small cell lung carcinoma, breast, pancreatic, ovarian, lung, colon cancer, or a combination thereof. In some embodiments, the cancer is non-small cell lung carcinoma. In some embodiments, the subject is a human.

[0013] The present technology provides for a method of preparing a composition comprising an antibody-urease conjugate substantially free of urease, which method comprises combining activated antibody and urease in an aqueous buffer having a pH of about 6.0-7.0, such as about 6.5, adjusting the pH to 8.0-9.0, such as about 8.3 to form the antibody-urease conjugate, and purifying the antibody-urease conjugate, wherein the method does not comprise a chromatographic purification step, such as commonly used chromatographic methods for protein purifications, including size exclusion chromatography (SEC), ion exchange chromatography, affinity chromatography, immobilized metal affinity chromatography, immunoaffinity chromatography, liquid-solid adsorption chromatography, hydrophobic interaction chromatography (HIC), revered phase chromatography (RPC), and high performance liquid chromatography (HPLC), etc. In some embodiments, antibody-urease conjugate is purified by ultradiafiltration.

[0014] In some embodiments, the antibody-urease conjugate has a conjugation ratio of 8-11 antibody moieties per urease moiety. In some embodiments, the buffer having a pH of about 6.5 is a sodium acetate buffer. In some embodiments, the pH is adjusted to about 8.3 by a method comprising addition of a sodium borate solution.

[0015] The present technology provides for a method of increasing antibody binding affinity to a tumor antigen, comprising conjugating a plurality of the antibody molecules to a urease molecule to form an antibody-urease conjugate, wherein the conjugate has a binding affinity to the tumor antigen at least about 100 times higher than the un-conjugated antibody.

[0016] In some embodiments, the antibody is a humanized or non-human antibody. In some embodiments, the antibody is a single domain antibody. In some embodiments, the tumor antigen is expressed by non-small cell lung carcinoma. In some embodiments, the antibody has specificity to CEACAM6.

[0017] The present technology further provides for a kit comprising the composition provided herein and instructions for use of the composition.

[0018] These and other aspects of the disclosure are further described below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] FIG. 1A-B depicts exemplifying binding and cytotoxicity studies of L-DOS47 in cancer cell lines. (A) Direct binding of L-DOS47 to five cancer cell lines: BxPC-3, Capan-1, ZR-75-30, LS174T, and MDA-MB231. The binding signal was represented by the amount of ammonia generated upon incubation with 20mM urea. Good L-DOS47 binding was observed in BxPC-3 (●), Capan-1 (▼), and ZR-75-30 (◆) cells, whereas moderate binding was observed in LS174T cells (▲) and no binding was found in MDA-MB231 cells (■). In addition, no binding was observed with the unconjugated DOS47 control on corresponding cell lines (data not shown), suggesting that L-DOS47 binding was specific and was contributed by the antibody moiety. (B) L-DOS47 induced cytotoxicity on the cancer cell lines upon addition of 20mM urea. No effects was observed in MDA-MB231 cells (■), which agreed with the binding study. BxPC-3 (●) and ZR-75-30 (◆) were highly susceptible to L-DOS47, whereas only moderate effects were found in Capan-1 (▼) and LS174T cells (▲).

[0020] FIG. 2A-B depicts exemplifying direct and competitive binding of L-DOS47, DOS47, and AFAIKL2 antibody to BxPC-3 cells. (A) Binding of ruthenium-tagged L-DOS47, AFAIKL2 antibody, and unconjugated DOS47 to BxPC-3 cells. The electrochemiluminescence assay provides a direct measurement of L-DOS47 and AFAIKL2 antibody binding to BxPC-3 cells. Weak binding signal was observed with the AFAIKL2

What is claimed is:

1. A pharmaceutical composition comprising a pharmaceutically acceptable aqueous solution suitable for intravenous injection and a single domain antibody-urease conjugate, wherein the conjugate has a conjugation ratio of 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 single domain antibody moieties per urease moiety and said conjugate is substantially free of unconjugated urease.

2. The pharmaceutical composition of claim 1, wherein the unconjugated urease is less than 5% w/w.

3. The pharmaceutical composition of claim 1 or 2, wherein said composition is substantially free of non-aqueous HPLC solvents and further wherein the pH is about 6.8.

4. The pharmaceutical composition of claim 1 or 2, wherein the single domain antibody-urease conjugate has an average conjugation ratio of about 6 or more single domain antibody moieties per urease moiety or an average conjugation ratio of about 8 to 11 single domain antibody moieties per urease moiety.

5. The pharmaceutical composition of any one of claims 1 to 3, wherein the urease is a Jack bean urease.

6. The pharmaceutical composition of any one of claims 1 to 4, wherein the single domain antibody is a humanized or non-human antibody.

7. The pharmaceutical composition of any one of claims 1 to 6, wherein the single domain antibody has specificity to a tumor antigen expressed by non-small cell lung carcinoma.

8. The pharmaceutical composition of any one of claims 1 to 7, wherein the single domain antibody has specificity to CEACAM6.

9. The pharmaceutical composition of any one of claims 1 to 8, wherein the single domain antibody has a binding affinity to CEACAM6 with a K_d value of higher than about 1×10^{-6} M, or the conjugate has a binding affinity to CEACAM6 with a K_d value of no more than about 1×10^{-8} M, or the conjugate has a binding affinity to CEACAM6 with a K_d value of no more than about 1×10^{-10} M, or the conjugate has a binding affinity to CEACAM6 with an IC_{50} value of no more than about 5 nM.

10. The pharmaceutical composition of claim 9, wherein the IC_{50} value is about 3.22 nM or the conjugate binds to CEACAM6 with an IC_{50} value of about 20 μg/mL.

11. The pharmaceutical composition of any one of claims 1 to 10, wherein the single domain antibody comprises a polypeptide comprising an amino acid sequence of SEQ ID NO. 1 or the single domain antibody comprises a polypeptide comprising at least one amino acid modification to the amino acid sequence of SEQ ID NO. 1, wherein said amino acid modification retains the functional property of said polypeptide.

12. A method of preparing a composition comprising a single domain antibody-urease conjugate and no more than about 5% w/w of unconjugated urease based on the weight of the single domain antibody-urease conjugate, which method comprises:

(1) combining an activated single domain antibody and urease in a solvent in which the activated single domain antibody and urease substantially do not react to form a reaction mixture wherein the distribution of the activated single domain antibody and urease in the solvent is uniform; and

(2) increasing pH of the mixture of (1) to about 8-9 such that the activated single domain antibody readily reacts with the urease to form the single domain antibody-urease conjugate having 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 single domain antibody moieties per urease moiety; and

(3) optionally purifying the single domain antibody-urease conjugate by a purification step, wherein said conjugate is substantially free of unconjugated urease, and

wherein the method does not comprise a chromatographic purification step.

13. The method of claim 12, wherein the composition comprises about 10-20% unconjugated antibody by total protein weight prior to the purification step and/or the composition comprises about less than 1% unconjugated antibody by total protein weight after the purification step.

14. A method of increasing antibody binding affinity to a tumor antigen, the method comprising conjugating a plurality of single domain antibody molecules to a urease molecule to form a single domain antibody-urease conjugate having a conjugation ratio of 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 single domain antibody molecules per urease molecule, wherein the single domain antibody-urease conjugate has a binding affinity to the tumor antigen at least about 100 times higher than an un-conjugated antibody.

15. The method of claim 14, wherein the urease is a Jack bean urease.

16. The method of claim 14 or 15, wherein the single domain antibody is a humanized or non-human antibody.

17. The method of claim 14, 15 or 16, wherein the tumor antigen is CEACAM6 and said single domain antibody has specificity to said CEACAM6.

18. The method of any one of claims 14 to 17, wherein the tumor antigen is expressed by non-small cell lung carcinoma.

19. The method of any one of claims 14 to 18, wherein the single domain antibody-urease conjugate has a binding affinity to CEACAM6 with a K_d value of higher than about 1×10^{-6} M, or the single domain antibody-urease conjugate binds to CEACAM6 with a K_d value of no more than about 1×10^{-8} M, or the single domain antibody-urease conjugate binds to CEACAM6 with a K_d value of no more than about 1×10^{-10} M, or the single domain antibody-urease conjugate binds to CEACAM6 with an IC_{50} value of no more than about 5 nM, or the single domain antibody-urease conjugate binds to CEACAM6 with an IC_{50} value of about 3.22 nM, or the single domain antibody-urease conjugate binds to CEACAM6 with an IC_{50} value of about 20 μg/mL.

20. A kit comprising the composition of any one of claims 1 to 11 and instructions for use of the composition.