SUMMARY OF DISCLOSURE

The present disclosure provides formulations comprising antibody-drug conjugates (ADCs) and methods relating to the use of such conjugates to treat disorders requiring treatment with an anti-epidermal growth factor receptor (anti-EGFR) ADC, such as cancer.

The disclosure provides a formulation comprising an anti-Epidermal Growth Factor Receptor (EGFR) antibody drug conjugate (ADC), a sugar, histidine, and a surfactant, wherein the formulation has a pH of about 5 - 7, and wherein the anti-EGFR ADC comprises an anti-EGFR antibody, or antigen-binding portion thereof, conjugated to an auristatin.

The disclosure provides a formulation comprising an anti-Epidermal Growth Factor Receptor (EGFR) antibody drug conjugate (ADC), a sugar, a succinate or citrate and/or phosphate buffer, and a surfactant, wherein the formulation has a pH of about 5 - 7, and wherein the anti-EGFR ADC comprises an anti-EGFR antibody, or antigen-binding portion thereof, conjugated to an auristatin. In one example, the formulation is lyophilized.

In one aspect, provide herein is a lyophilized formulation comprising an anti-Epidermal Growth Factor Receptor (EGFR) antibody drug conjugate (ADC), a sugar, histidine, and a surfactant, wherein said anti-EGFR ADC comprises an anti-EGFR antibody conjugated to an auristatin, wherein the anti-EGFR antibody comprises a heavy chain variable region comprising complementarity domain regions (CDRs) comprising the amino acid sequences set forth in SEQ ID NOS: 15, 16, and 17, and comprises a light chain variable region comprising CDRs comprising the amino acid sequences set forth in SEQ ID NOS: 20, 21, and 22.

In one aspect, provided herein is a lyophilized formulation comprising an anti-EGFR ADC comprising an anti-EGFR antibody conjugated to monomethylauristatin F (MMAF), wherein said ADC comprises a heavy chain variable region comprising complementarity domain regions (CDRs) comprising the amino acid sequences set forth in SEQ ID NOS: 15, 16, and 17, and comprises a light chain variable region comprising CDRs comprising the amino acid sequences set forth in SEQ ID NOS: 20, 21, and 22,
sucrose, histidine, and polysorbate 80, wherein the formulation comprises an ADC mixture having an average DAR of about 3 or an ADC mixture having a DAR of about 2-4.

In one example, the formulation comprises a surfactant which is a polysorbate, *e.g.,* polysorbate 80, or a poloxamer. In one example, the formulation comprises 0.05-0.15 mg/ml of a polysorbate.

In another example, the formulation comprises about 60-80 mg/ml of a sugar. In one example, the sugar is selected from the group consisting of mannitol, sorbitol, sucrose, and trehalose.

In one example, the formulation of the disclosure comprises about 5-25 mM histidine.

In another further example, the formulation is lyophilized. In one example, the formulation is lyophilized and the sugar is sucrose. In another example of the disclosure, the lyophilized formulation does not comprise lactobionic acid. In another example of the disclosure, the lyophilized formulation does not comprise a sugar acid.

In one example, the formulation of the disclosure is aqueous, and comprises about 1-40 mg/ml of an anti-EGFR ADC.

In a further example, the formulation of the disclosure comprises 1-150 mg of the anti-EGFR ADC. In one example, the formulation of the disclosure comprises 90-110 mg of an anti-EGFR ADC.

In one example, the formulation of the disclosure has a pH of about 5 to 7. In a further example, the formulation has a pH of about 5.5 to 6.5.

In one example, the auristatin is Monomethylauristatin F (MMAF). In one example, the formulation of the disclosure comprises an anti-EGFR ADC comprising an anti-EGFR antibody, or antigen-binding portion thereof, conjugated to Monomethylauristatin F (MMAF). In another example, the MMAF is conjugated to the antibody with a maleimidocaproyl linker.

The disclosure further provides a lyophilized formulation comprising an anti-Epidermal Growth Factor Receptor (EGFR) antibody drug conjugate (ADC) comprising an anti-EGFR antibody, or antigen-binding portion thereof, conjugated to an auristatin, a sugar, *e.g.,* sucrose, a surfactant, *e.g.,* a polysorbate, such as polysorbate 80, and histidine. In one example, the lyophilized formulation comprises 1-20 mg of histidine, about 320-410 mg of a sugar, about 0.1 to 0.9 mg of a surfactant, and about 1-150 mg of the anti-EGFR ADC.

The disclosure also provides an aqueous formulation comprising about 1 - 100 mg/ml of an anti-Epidermal Growth Factor Receptor (EGFR) antibody drug conjugate (ADC) comprising an anti-EGFR antibody, or antigen-binding portion thereof, conjugated to an auristatin, about 1 -

10 mg/mL histidine, about 50 -90 mg/ml of a sugar, *e.g.,* sucrose, and about 0.01 - 0.2 mg/ml of a surfactant, *e.g.,* polysorbate 80.

In one example, the formulation of the disclosure comprises an anti-EGFR ADC wherein the anti-EGFR antibody is humanized.

In another example, the formulation of the disclosure comprises an anti-EGFR ADC wherein the anti-EGFR antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 13, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 18.

In another example, the formulation of the disclosure comprises an anti-EGFR ADC wherein the anti-EGFR antibody comprises a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 13, a heavy chain constant region having the amino acid sequence as set forth in SEQ ID NO: 14, a light chain variable region having the amino acid sequence set forth in SEQ ID NO: 18, and a light chain constant region having the amino acid sequence as set forth in SEQ ID NO: 19,

In a further example, the formulation of the disclosure comprises an anti-EGFR ADC wherein the antibody comprises a heavy chain variable region comprising complementarity domain regions (CDRs) comprising the amino acid sequences set forth in SEQ ID NOS: 15, 16, and 17, and comprises a light chain variable region comprising CDRs comprising the amino acid sequences set forth in SEQ ID NOS: 20, 21, and 22.

In another example of the disclosure, the anti-EGFR ADC comprises CDRs (*i.e.,* light chain CDR1, CDR2, and CDR3) described in the light chain variable region set forth in the amino acid sequence of SEQ ID NO: 18, and CDRs (*i.e.,* heavy chain CDR1, CDR2, and CDR3) described in the amino acid sequence of SEQ ID NO: 13.

The disclosure further provides a lyophilized formulation comprising sucrose, polysorbate 80, and histidine, and wherein the antibody comprises a heavy chain variable region comprising complementarity domain regions (CDRs) comprising the amino acid sequences set forth in SEQ ID NOS: 15, 16, and 17, and comprises a light chain variable region comprising CDRs comprising the amino acid sequences set forth in SEQ ID NOS: 20, 21, and 22. In one example, the formulation comprises 1-110 mg of the anti-EGFR ADC.

In one example, the formulation of the disclosure contains an ADC mixture comprising anti-EGFR ADCs having an average DAR of about 3 or an ADC mixture comprising anti-EGFR ADCs having a DAR of about 2-4.

In one example, the formulation of the disclosure comprises an anti-EGFR ADC comprising an anti-EGFR antibody, or antigen-binding portion thereof, conjugated to monomethyl auristatin F (MMAF), wherein said ADC 1-MMAF comprises a heavy chain variable region comprising complementarity domain regions (CDRs) comprising the amino acid sequences set forth in SEQ ID NOS: 15, 16, and 17, and comprises a light chain variable region comprising CDRs comprising the amino acid sequences set forth in SEQ ID NOS: 20, 21, and 22, sucrose, histidine, and polysorbate 80, wherein the formulation comprises an ADC mixture having an average DAR of about 3 or an ADC mixture having a DAR of about 2-4. In a further example of the disclosure, the formulation is lyophilized. In yet a further disclosure, the anti-EGFR antibody is linked to MMAF via a maleimidocaproyl linker. In still a further example, the anti-EGFR antibody, or antigen-binding portion thereof, comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 13, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 18.

In one example, the formulation of the disclosure comprises an anti-EGFR ADC comprising an anti-EGFR antibody, or antigen-binding portion thereof, conjugated to monomethyl auristatin E (MMAE), wherein said ADC 1-MMAE comprises a heavy chain variable region comprising complementarity domain regions (CDRs) comprising the amino acid sequences set forth in SEQ ID NOS: 15, 16, and 17, and comprises a light chain variable region comprising CDRs comprising the amino acid sequences set forth in SEQ ID NOS: 20, 21, and 22, sucrose, histidine, and polysorbate 80, wherein the formulation comprises an ADC mixture having an average DAR of about 3 or an ADC mixture having a DAR of about 2-4. In a further example of the disclosure, the formulation is lyophilized. In still a further example, the anti-EGFR antibody, or antigen-binding portion thereof, comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 13, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 18.

In one example, the formulation of the disclosure is a pharmaceutical formulation.

In one aspect, provided herein is a method of preparing a formulation, wherein the method comprises lyophilizing an aqueous formulation having a pH ranging from about 5 to 7

and comprising 1-20 mg of histidine, about 320-410 mg of the sugar, about 0.1 to 0.9 mg of the surfactant, and about 1-150 mg of the anti-EGFR ADC.

In one aspect, provided herein is the formulation as described herein for use in therapy.

In one aspect, provided herein is a use of the formulation as described herein in the manufacture of a medicament for treating a disorder requiring treatment with the anti-EGFR ADC. The disclosure further provides a method for treating a subject comprising administering a therapeutically effective amount of an anti-EGFR ADC in the formulations described herein to a subject, wherein the subject has a disorder requiring treatment with the anti-EGFR ADC. In one example, the disorder requiring treatment with the anti-EGFR ADC is cancer, *e.g.,* glioblastoma, non-small cell lung cancer, lung cancer, colon cancer, head and neck cancer, breast cancer, squamous cell tumors, anal cancer, skin cancer, and vulvar cancer.

In one example, the formulation of the disclosure is used to treat solid tumors likely to over-express the Epidermal Growth Factor Receptor (EGFR) or squamous non-small cell lung cancer (NSCLC).

In one example, the formulation of the disclosure is used to treat a cancer selected from the group consisting of squamous tumors (including, squamous tumors of the lung, head and neck, cervical, etc.), glioblastoma, glioma, non-small cell lung cancer, lung cancer, colon cancer, head and neck cancer, breast cancer, squamous cell tumors, anal cancer, skin cancer, and vulvar cancer.

In one example, the formulation of the disclosure is used to treat glioblastoma multiforme.

In one example, the formulation of the disclosure is used to treat a solid tumor having overexpression of EGFR. In one example, the formulation of the

disclosure is used to treat a subject having an advanced solid tumor likely to overexpress EGFR.

In one example, the formulations of the disclosure are administered intravenously.

In one aspect, provided herein are kits comprising the formulations described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a diagram that shows the structure of MMAE coupled to an antibody of interest (mAb) by a linker (GN Linkers + Auristatin: vcMMAE).

Figure 2 is a diagram that shows the structure of MMAF coupled to an antibody of interest (mAb) by a linker (Linkers + Auristatin: mcMMAF).

Figure 3 (A and B) graphically depict the onset of unfolding temperature as determined with dynamic scanning fluorescence (A) and differential scanning calorimetry (B). ♦ antibody 1; ■ Antibody Drug Conjugate (ADC) 1- MMAF; ▲ ADC 1- MMAE.

Figure 4 (A and B) graphically depicts the presence of unstructured elements in antibody 1, ADC 1- MMAF and ADC 1- MMAE using Fourier Transform Infrared Spectroscopy (FTIR) (A) and near UV- Circular Dichroism (CD) (B). ♦ antibody 1; ■ ADC 1- MMAF; ▲ ADC 1- MMAE.

Figure 5 graphically depicts the aggregation propensity of ADC 1- MMAE at low concentrations, compared to ADC 1- MMAF and antibody 1.

Figure 6 graphically depicts the aggregation propensity of ADC 1- MMAE at high concentrations, compared to ADC 1- MMAF and antibody 1.

Figure 7 shows a schematic of the serum stability assay, and describes the *in vitro* serum stability of a number of molecules, including antibody 1, the ADC 1- MMAF and ADC 1- MMAE.

Figure 8 (A and B) graphically depicts freeze/thaw stability, indicated by percentage of monomer present, as determined by SEC among antibody 1, ADC 1- MMAF and ADC 1-

CLAIMS

1. A lyophilized formulation comprising an anti-Epidermal Growth Factor Receptor (EGFR) antibody drug conjugate (ADC), a sugar, histidine, and a surfactant, wherein said anti-EGFR ADC comprises an anti-EGFR antibody conjugated to an auristatin, wherein the anti-EGFR antibody comprises a heavy chain variable region comprising complementarity domain regions (CDRs) comprising the amino acid sequences set forth in SEQ ID NOS: 15, 16, and 17, and comprises a light chain variable region comprising CDRs comprising the amino acid sequences set forth in SEQ ID NOS: 20, 21, and 22.

2. The formulation of claim 1, wherein the auristatin is Monomethylauristatin F (MMAF).

3. The formulation of claim 2, wherein the MMAF is conjugated to the antibody with a maleimidocaproyl linker.

4. The formulation of any one of claims 1-3, wherein the surfactant is a polysorbate.

5. The formulation of any one of claims 1-4, wherein the formulation comprises about 1-120 mg of the anti-EGFR ADC.

6. The formulation of any one of claims 1-5, wherein the sugar is selected from the group consisting of mannitol, sorbitol, sucrose, and trehalose.

7. The formulation of any one of claims 1-5, wherein the sugar is sucrose and the surfactant is a polysorbate.

8. The formulation of claim 4 or claim 7, wherein the polysorbate is polysorbate 80.

9. The formulation of any one of claims 1-3 , wherein said formulation is reconstituted and comprises about 1 - 100 mg/ml of the anti-EGFR ADC, about 1 -10 mg/mL histidine, about 50 -90 mg/ml of the sugar, and about 0.01 - 0.2 mg/ml of the surfactant.

10. The formulation of claim 9, wherein the sugar is sucrose, optionally wherein the surfactant is polysorbate 80, and/or the formulation has a pH of about 5.5 to 6.5.

11. The formulation of claim 9 or claim 10, comprising 10-100 mg/ml of the anti-EGFR ADC.

12. The formulation of claim 9 or claim 10, comprising 1-40 mg/ml of the anti-EGFR ADC.

13. The formulation of any one of claims 1-12, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 13, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 18.

14. The formulation of any one of claims 1-13, wherein the formulation comprises an ADC mixture having an average DAR of about 3 or an ADC mixture having a DAR of about 2-4.

15. A lyophilized formulation comprising

an anti-EGFR ADC comprising an anti-EGFR antibody conjugated to monomethylauristatin F (MMAF), wherein said ADC comprises a heavy chain variable region comprising complementarity domain regions (CDRs) comprising the amino acid sequences set forth in SEQ ID NOS: 15, 16, and 17, and comprises a light chain variable region comprising CDRs comprising the amino acid sequences set forth in SEQ ID NOS: 20, 21, and 22,

sucrose,

histidine, and

polysorbate 80,

wherein the formulation comprises an ADC mixture having an average DAR of about 3 or an ADC mixture having a DAR of about 2-4.

16. The formulation of claim 15, wherein the anti-EGFR antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 13, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 18.

17. The formulation of any one of claims 1-16, which is a pharmaceutical formulation.

18. A method of preparing the formulation of any one of claims 1-3 or 15, said method comprising lyophilizing an aqueous formulation having a pH ranging from about 5 to 7 and comprising 1-20 mg of histidine, about 320-410 mg of the sugar, about 0.1 to 0.9 mg of the surfactant, and about 1-150 mg of the anti-EGFR ADC.

19. Use of the formulation of any one of claims 1-17 in the manufacture of a medicament for treating a disorder requiring treatment with the anti-EGFR ADC.

20. The use according to claim 19, wherein the disorder requiring treatment with the anti-EGFR ADC is cancer.

21. The use according to claim 20, wherein the cancer is selected from the group consisting of glioblastoma, non-small cell lung cancer, lung cancer, colon cancer, head and neck cancer, breast cancer, squamous cell tumors, anal cancer, skin cancer, vulvar cancer, squamous tumors and glioma.

22. The use according to claim 21, wherein the squamous tumor is selected from the group consisting of squamous lung tumor, a squamous tumor of the head or neck, and a squamous cervical tumor.

23. The use according to claim 21, wherein the cancer is glioblastoma or glioblastoma multiforme.

24. The use according to claim 20, wherein the cancer is a solid tumor having overexpression of EGFR.

25. The use according to any one of claims 19-24, wherein the formulation is to be administered intravenously.

26. A kit comprising the formulation of any one of claims 1-17.

27. The formulation of any one of claims 1-17 for use in therapy.