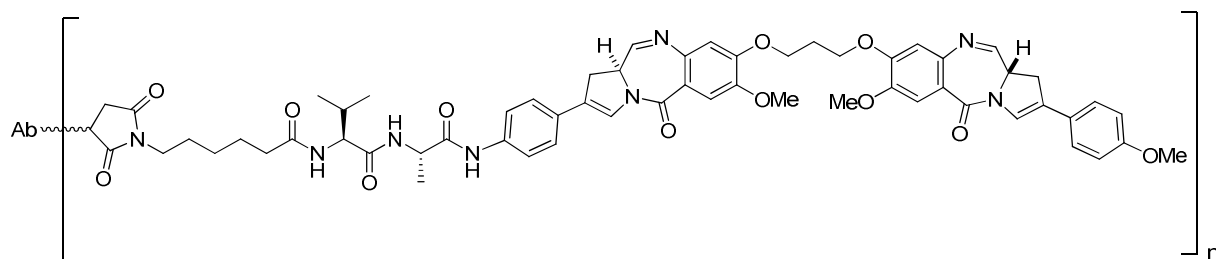


SUMMARY

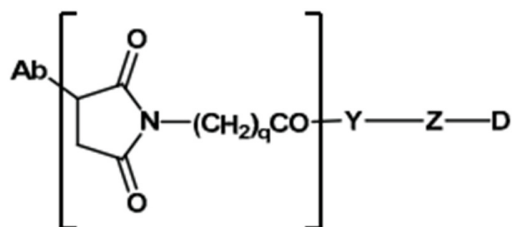
[0011] The present disclosure provides antibody-drug conjugates (ADCs) comprising a cytotoxic or cytostatic agent linked to an anti-EGFR antibody by way of a linker, compositions comprising the ADCs, methods of making the ADCs, and methods of treating a cancer comprising administering the ADCs to a subject having cancer. As described in more detail in the Examples, and while not intending to be bound by any particular theory of operation, the data included herein demonstrate that anti-EGFR ADCs comprising specific linkers and specific cytotoxic and/or cytostatic agents (i.e., a pyrrolobenzodiazepine (PBD) dimer), exert potent anti-tumor activities. Moreover, the anti-EGFR ADCs of the present disclosure are characterized by a fixed low drug loading, which surprisingly provides a highly efficacious ADC.

[0011a] The present disclosure provides an antibody-drug conjugate (ADC) comprising the structure of Formula (X), or a salt thereof:



wherein Formula (X) comprises an anti-EGFR antibody (Ab) conjugated to a cytotoxic warhead, wherein the anti-EGFR antibody comprises: a heavy chain variable region comprising a CDRH1 sequence comprising SEQ ID NO: 3, a CDRH2 sequence comprising SEQ ID NO: 4, and a CDRH3 sequence comprising SEQ ID NO: 5; a light chain variable region comprising a CDRL1 sequence comprising SEQ ID NO: 8, a CDRL2 sequence comprising SEQ ID NO: 9, and a CDRL3 sequence comprising SEQ ID NO: 10; and a mutation comprising S239C in a heavy chain constant region, wherein the numbering is in accordance with Kabat; wherein the anti-EGFR antibody is conjugated to the cytotoxic warhead through the mutation comprising S239C, and wherein n is 2.

[0011b] The present disclosure provides an antibody-drug conjugate (ADC) comprising the structure of formula (IX), or a salt thereof,



(IX),

wherein D comprises a pyrrolobenzodiazepine (PBD) dimer; Ab is an anti-EGFR antibody, Y is Val, Z is Ala, and q is 1, 2, 3, 4, 5, 6, 7, or 8, and wherein the anti-EGFR antibody comprises a heavy chain variable region comprising a CDRH1 sequence comprising SEQ ID NO: 3, a CDRH2 sequence comprising SEQ ID NO: 4, and a CDRH3 sequence comprising SEQ ID NO: 5; a light chain variable region comprising a CDRL1 sequence comprising SEQ ID NO: 8, a CDRL2 sequence comprising SEQ ID NO: 9, and a CDRL3 sequence comprising SEQ ID NO: 10; a mutation comprising S239C in a heavy chain constant region, wherein the numbering is in accordance with Kabat; wherein the anti-EGFR antibody Ab is conjugated to the structure of Formula (IX) through the mutation comprising S239C, and n is 2.

[0011c] The present disclosure provides a pharmaceutical composition comprising the ADC as described herein in combination with at least one pharmaceutically acceptable excipient, carrier, or diluent.

[0012] Accordingly, in examples, the present disclosure provides ADCs that specifically bind EGFR, and in particular human EGFR (hEGFR).

[0013] In examples, the present disclosure provides an antibody drug conjugate (ADC) comprising a cytotoxic and/or cytostatic agent linked to an antibody by way of a linker, wherein the ADC is a compound according to the structural formula (I):



(I),

or a salt thereof, where D comprises a pyrrolobenzodiazepine (PBD) dimer, L is a linker, and Ab is an anti-human epidermal growth factor receptor antibody. In examples, the anti-EGFR Ab comprises (i) a heavy chain CDRH1 domain comprising the amino acid sequence set forth in

SEQ ID NO: 3; a heavy chain CDRH2 domain comprising the amino acid sequence set forth in SEQ ID NO: 4, and a heavy chain CDRH3 domain comprising the amino acid sequence set forth in SEQ ID NO: 5; (ii) a light chain CDRL1 domain comprising the amino acid sequence set forth in SEQ ID NO: 8; a light chain CDRL2 domain comprising the amino acid sequence set forth in SEQ ID NO: 9; a light chain CDRL3 domain comprising the amino acid sequence set forth in SEQ ID NO: 10; and (iii) a mutation comprising S239C in a heavy chain constant region, wherein the numbering is in accordance with Kabat. XY represents a covalent linkage linking linker L to antibody Ab through the S239C mutation. In examples, n is any integer. In examples, n is 2. In examples, the antibody Ab has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 2, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 7. In examples, the antibody Ab has a heavy chain comprising the amino acid sequence of SEQ ID NO: 1, and a light chain comprising the amino acid sequence of SEQ ID NO: 6. In examples, XY is a maleimide-sulfhydryl linkage. In examples, L comprises the linker as described in Formula III, IV, V, VI, VII, VIII, or IX. For example, in examples, L comprises the linker as described in Formula IX. In examples, the linker is a maleimidocaproyl-Valine-Alanine (mc-Val-Ala) linker. In examples, the anti-EGFR antibody comprises an IgG1 isotype. In certain examples, the heavy chain constant region of the anti-EGFR antibody either lacks a C-terminal lysine or comprises an amino acid other than lysine at a C-terminus of the heavy chain constant region. In examples, the anti-EGFR antibody is a humanized antibody.

[0014] In examples, the present disclosure provides an antibody-drug conjugate (ADC) comprising a cytotoxic and/or cytostatic agent linked to an antibody by way of a linker, wherein the antibody drug conjugate is a compound according to structural Formula (I)



(I),

or a salt thereof, where D comprises a pyrrolobenzodiazepine (PBD) dimer; L is a linker; Ab is an anti-EGFR antibody comprising (i) a heavy chain variable region comprising SEQ ID NO:2, (ii) a light chain variable region comprising SEQ ID NO: 7; and (iii) a mutation comprising S239C in a heavy chain constant region, wherein the numbering is in accordance with Kabat; XY represents a covalent linkage linking linker L to antibody Ab; and n is any integer. In examples,

n is 2 or 4. In examples, n is 2. In examples, XY is a linkage formed with a sulfhydryl group on antibody Ab. In examples, XY is a maleimide-sulfhydryl linkage. In examples, L comprises the linker as described in Formula III, IV, V, VI, VII, VIII, or IX. In examples, L comprises the linker as described in Formula IX. In examples, the anti-EGFR antibody comprises an IgG1 isotype. In examples, the heavy chain constant region of the anti-EGFR antibody either lacks a C-terminal lysine or comprises an amino acid other than lysine at a C-terminus of the heavy chain constant region. In examples, the anti-EGFR antibody is a humanized antibody.

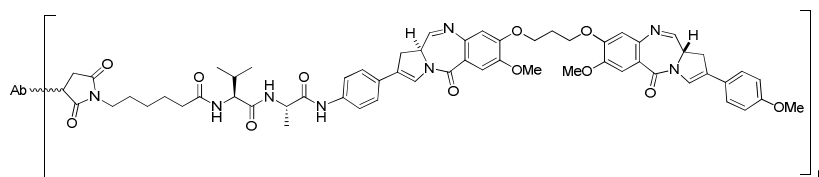
[0015] In examples, the present disclosure provides an antibody drug conjugate comprising a cytotoxic and/or cytostatic agent linked to an antibody by way of a linker, wherein the antibody drug conjugate is a compound according to structural formula (I):



(I),

or a salt thereof, where D comprises a pyrrolobenzodiazepine (PBD) dimer; L is a linker; Ab is an anti-EGFR antibody comprising (i) a heavy chain comprising the amino acid sequence as set forth in SEQ ID NO: 1, (ii) a light chain comprising the amino acid sequence set forth in SEQ ID NO: 6; XY represents a covalent linkage linking linker L to antibody Ab; and n is any integer. In examples, n is 2 or 4. In examples, n is 2. In examples, XY is a linkage formed with a sulfhydryl group on antibody Ab. In examples, XY is a maleimide-sulfhydryl linkage. In examples, L comprises the linker as described in Formula III, IV, V, VI, VII, VIII, or IX. In examples, L comprises the linker as described in Formula IX. In examples, the anti-EGFR antibody comprises an IgG1 isotype. In examples, the heavy chain constant region of the anti-EGFR antibody either lacks a C-terminal lysine or comprises an amino acid other than lysine at a C-terminus of the heavy chain constant region. In examples, the anti-EGFR antibody is a humanized antibody.

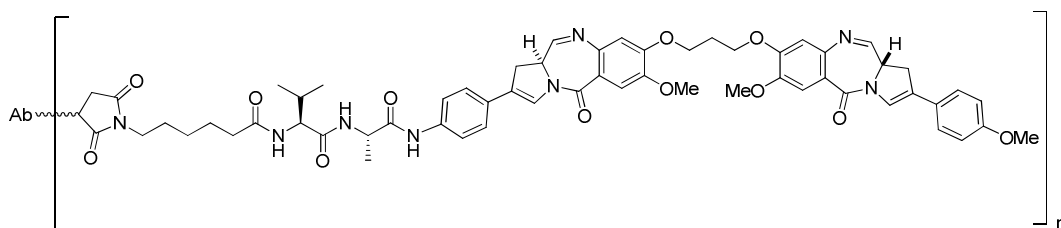
[0016] In examples, the present disclosure features an ADC comprising the structure of Formula (X):



(X),

or a salt thereof, wherein Ab comprises an anti-EGFR antibody comprising (i) a heavy chain variable region comprising a CDRH1 sequence comprising SEQ ID NO: 3, a CDRH2 sequence comprising SEQ ID NO: 4, and a CDRH3 sequence comprising SEQ ID NO: 5; (ii) a light chain variable region comprising a CDRL1 sequence comprising SEQ ID NO: 8, a CDRL2 sequence comprising SEQ ID NO: 9, and a CDRL3 sequence comprising SEQ ID NO: 10; (iii) a mutation comprising S239C in a heavy chain constant region, wherein the numbering is in accordance with Kabat; wherein n is 2. In examples, the heavy chain variable region comprises SEQ ID NO: 2, and the light chain variable region comprises SEQ ID NO: 7. In examples, the ADC comprises a full heavy chain comprising SEQ ID NO: 1, and a full light chain comprising SEQ ID NO: 6. In examples, the anti-EGFR antibody comprises an IgG1 isotype. In examples, the heavy chain constant region of the anti-EGFR antibody either lacks a C-terminal lysine or comprises an amino acid other than lysine at a C-terminus of the heavy chain constant region. In examples, the anti-EGFR antibody is a humanized antibody.

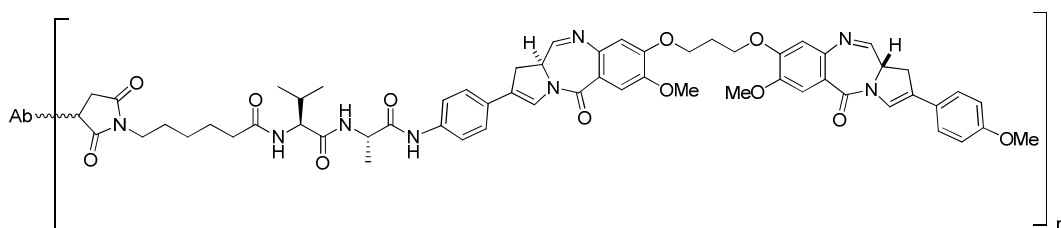
[0017] In examples, the present disclosure features an ADC comprising the structure of Formula (X):



(X),

or a salt thereof, wherein Ab comprises an anti-EGFR antibody comprising (i) a heavy chain variable region comprising SEQ ID NO: 2; (ii) a light chain variable region comprising SEQ ID NO: 7; (iii) a mutation comprising S239C in a heavy chain constant region, wherein the numbering is in accordance with Kabat; wherein n is 2. In examples, the heavy chain variable region comprises SEQ ID NO: 2, and the light chain variable region comprises SEQ ID NO: 7. In examples, the ADC comprises a full heavy chain comprising SEQ ID NO: 1, and a full light chain comprising SEQ ID NO: 6. In examples, the anti-EGFR antibody comprises an IgG1 isotype. In examples, the heavy chain constant region of the anti-EGFR antibody either lacks a C-terminal lysine or comprises an amino acid other than lysine at a C-terminus of the heavy chain constant region. In examples, the anti-EGFR antibody is a humanized antibody.

[0018] In examples, the present disclosure features an ADC comprising the structure of Formula (X):



(X),

or a salt thereof, wherein Ab comprises an anti-EGFR antibody comprising (i) a heavy chain comprising SEQ ID NO: 1; (ii) a light chain comprising SEQ ID NO: 6; wherein n is 2.

[0019] In examples, the present disclosure provides a composition comprising an ADC described herein. In examples, the composition further comprises at least one excipient, a carrier, and/or a diluent. In examples, the composition of the present disclosure is formulated for pharmaceutical use in humans.

[0020] In examples, the present disclosure provides a method of making an ADC, comprising contacting an anti-EGFR antibody with a synthon according to structural Formula (Ia) D-L-R^x, wherein D is a cytotoxic and/or cytostatic agent capable of crossing a cell membrane, L is a linker capable of being cleaved by a lysosomal enzyme, and R^x comprises a functional group capable of covalently linking the synthon to the antibody, under conditions in which the synthon covalently links the synthon to the antibody, wherein D is a PBD dimer, and wherein the antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 1, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 6.

[0021] In examples, the present disclosure provides a method of making an ADC, comprising contacting an anti-EGFR antibody with a synthon according to structural Formula (Ia) D-L-R^x, wherein D is a cytotoxic and/or cytostatic agent capable of crossing a cell membrane, L is a linker capable of being cleaved by a lysosomal enzyme, and R^x comprises a functional group capable of covalently linking the synthon to the antibody, under conditions in which the synthon covalently links the synthon to the antibody, wherein D is a PBD dimer, and wherein the antibody comprises (i) a heavy chain variable region comprising a CDRH1 sequence comprising SEQ ID NO: 3, a CDRH2 sequence comprising SEQ ID NO: 4, and a CDRH3 sequence comprising SEQ ID NO: 5; (ii) a light chain variable region comprising a CDRL1 sequence comprising SEQ ID NO: 8, a CDRL2 sequence comprising SEQ ID NO: 9, and a CDRL3 sequence comprising SEQ ID NO: 10; and (iii) a mutation comprising S239C in a heavy chain constant region, wherein the numbering is in accordance with Kabat. In examples, the anti-EGFR antibody comprises an IgG1 isotype. In examples, the heavy chain constant region of the anti-EGFR antibody either lacks a C-terminal lysine or comprises an amino acid other than lysine at a C-terminus of the heavy chain constant region. In examples, the anti-EGFR antibody is a humanized antibody.

[0022] In examples, the present disclosure provides a method of making an ADC, comprising contacting an anti-EGFR antibody with a synthon according to structural Formula (Ia) D-L-R^x, wherein D is a cytotoxic and/or cytostatic agent capable of crossing a cell membrane, L is a linker capable of being cleaved by a lysosomal enzyme, and R^x comprises a functional group capable of covalently linking the synthon to the antibody, under conditions in which the synthon covalently links the synthon to the antibody, wherein D is a PBD dimer, and wherein the antibody comprises (i) a heavy chain variable region comprising a CDRH1 sequence comprising SEQ ID NO: 3, a CDRH2 sequence comprising SEQ ID NO: 4, and a CDRH3 sequence comprising SEQ ID NO: 5; (ii) a light chain variable region comprising a CDRL1 sequence comprising SEQ ID NO: 8, a CDRL2 sequence comprising SEQ ID NO: 9, and a CDRL3 sequence comprising SEQ ID NO: 10; and (iii) a mutation comprising S239C in a heavy chain constant region, wherein the numbering is in accordance with Kabat; and wherein R^x is a sulfhydryl group or a maleimide-sulfhydryl group. In examples, the anti-EGFR antibody comprises an IgG1 isotype. In examples, the heavy chain constant region of the anti-EGFR antibody either lacks a C-terminal lysine or comprises an amino acid other than lysine at a C-terminus of the heavy chain constant region. In examples, the anti-EGFR antibody is a humanized antibody.

[0023] In examples, the present disclosure provides a method of making an ADC, comprising contacting an anti-EGFR antibody with a synthon according to structural Formula (Ia) D-L-R^x, wherein D is a cytotoxic and/or cytostatic agent capable of crossing a cell membrane, L is a linker capable of being cleaved by a lysosomal enzyme, and R^x comprises a functional group capable of linking the synthon to the antibody, wherein D is a PBD dimer; wherein L comprises the linker as described in Formula III, IV, V, VI, VII, VIII, or IX; and wherein the antibody comprises (i) a heavy chain variable region comprising a CDRH1 sequence comprising SEQ ID NO: 3, a CDRH2 sequence comprising SEQ ID NO: 4, and a CDRH3 sequence comprising SEQ ID NO: 5; (ii) a light chain variable region comprising a CDRL1 sequence comprising SEQ ID NO: 8, a CDRL2 sequence comprising SEQ ID NO: 9, and a CDRL3 sequence comprising SEQ ID NO: 10; and (iii) a mutation comprising S239C in a heavy chain constant region, wherein the numbering is in accordance with Kabat; and wherein R^x is a

sulfhydryl group or a maleimide-sulfhydryl group. In examples, the anti-EGFR antibody comprises an IgG1 isotype. In examples, the heavy chain constant region of the anti-EGFR antibody either lacks a C-terminal lysine or comprises an amino acid other than lysine at a C-terminus of the heavy chain constant region. In examples, the anti-EGFR Antibody is a humanized antibody.

[0024] In examples, the present disclosure provides a method of making an ADC, comprising contacting an anti-EGFR antibody with a synthon according to structural Formula (Ia) D-L-R^x, wherein D is a cytotoxic and/or cytostatic agent capable of crossing a cell membrane, L is a linker capable of being cleaved by a lysosomal enzyme, and R^x comprises a functional group capable of linking the synthon to the antibody, wherein D is a PBD dimer; wherein L comprises the linker as described in Formula IX; and wherein the antibody comprises (i) a heavy chain variable region comprising a CDRH1 sequence comprising SEQ ID NO: 3, a CDRH2 sequence comprising SEQ ID NO: 4, and a CDRH3 sequence comprising SEQ ID NO: 5; (ii) a light chain variable region comprising a CDRL1 sequence comprising SEQ ID NO: 8, a CDRL2 sequence comprising SEQ ID NO: 9, and a CDRL3 sequence comprising SEQ ID NO: 10; and (iii) a mutation comprising S239C in a heavy chain constant region, wherein the numbering is in accordance with Kabat; and wherein R^x is a sulfhydryl group or a maleimide-sulfhydryl group. In examples, the anti-EGFR antibody comprises an IgG1 isotype. In examples, the heavy chain constant region of the anti-EGFR antibody either lacks a C-terminal lysine or comprises an amino acid other than lysine at a C-terminus of the heavy chain constant region. In examples, the anti-EGFR antibody is a humanized antibody.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] Figure 1 shows a schematic of EGFR and the regions bound by Ab1 and Ab2 (an antibody having the same six CDR amino acid sequences of cetuximab).

[0026] Figure 2 shows a preparation of AbA (S239C)-PBD. The conjugation process consists of reduction of the interchain disulfides, quantitative oxidation, and conjugation with excess PBD drug linker, as described in Example 2.

[0027] **Figure 3** provides the variable heavy (VH) and variable light (VL) chain region amino acid sequences of Ab1 and AbA. CDR sequences within the VH and VL regions are boxed, and differences between the Ab1 VH sequence and the AbA VH sequence are shaded.

[0028] **Figure 4** describes the full length light and heavy chains for Ab1 and AbA. Differences between the Ab1 sequence and the AbA sequence in the heavy chain are highlighted.

[0029] **Figure 5** shows the flow cytometry analysis of Ab1 and AbA, the S239C mutant forms Ab1(S239C) and AbA(S239C), and the PBD conjugates Ab1(S239C)-PBD and AbA(S239C)-PBD to human cells. Increasing concentrations of antibodies were added to wild-type EGFR-overexpressing (**Figure 5A**) and EGFR CA mutant-overexpressing (**Figure 5B**) NR6 cells in which the EGFR epitope recognized by Ab1 and AbA is exposed. As shown and described in Example 3, the conjugation of Cys-engineered AbA(S239C) to PBD does not alter the binding properties compared to the parental antibody AbA(S239C) or AbA.

[0030] **Figure 6** shows the EGFR number for SW-48 (a colorectal adenocarcinoma cell line that expresses EGFR, >200,000 receptors per cell, IHC H-score 228), NCI-H441 (a lung adenoma xenograft model with moderate to low EGFR expression, ~100,000 receptors per cell; IHC H-score 150) and LoVo (a *KRAS* mutant colorectal adenocarcinoma with lower EGFR expression, <100,000 receptors per cell, IHC H-score 140), in comparison to a number of other EGFR-