Moore et al (2018, Future Oncol. 14(17) 1669-1678) report the results of a phase III study with the ADC mirvetuximab soravtansine, comprising mirvetuximab, a humanized anti-FRα antibody, conjugated with an average of 3 to 4 maytansinoid molecules as the payload, for the treatment of ovarian cancer. The mechanism of action of the maytansin payload of this ADC is microtubule inhibition.

Hence, there is still a need for an antibody-drug conjugate comprising an anti-FRα antibody which is efficient, has high specificity, low toxicity (improved therapeutic index) and differentiated mechanism of action of the payload.

As shown in the Examples, the present disclosure provides topoisomerase I inhibitor-based anti-FRα antibody-drug conjugates with excellent in vivo efficacy in solid tumor cancer models and low toxicity, in particular when compared with reference prior art ADCs targeting FRα–expressing antibody with other payload and drug linkers, such as mirvetuximab soravtansine.

Summary

In a first aspect of the present disclosure, there is provided an antibody-drug conjugate of the formula (I):

Ab-[L-D]*p* (I),

wherein :

- Ab is an anti-folate receptor alpha (FRα) antibody which binds specifically to SEQ ID NO:12,
- L is a cleavable linker moiety bonded to said antibody, preferably via a thiol residue,
- D is a cytotoxic drug moiety bonded to L,
- *p* is from 1 to 8, preferably from 6 to 8, and more preferably *p* is 8.

In another aspect of the present disclosure, there is provided a use of an antibody-drug conjugate as disclosed herein, in the manufacture of a medicament, for the treatment of a tumor in a subject in need thereof, for example a solid tumor, more specifically selected from the group consisting of ovarian cancer, breast cancer, lung cancer, or mesothelioma.

In yet another aspect of the present disclosure, there is provided a use of an antibody-drug conjugate as disclosed herein, in the manufacture of a medicament for the treatment of a cancer, in a subject in need thereof, selected from the group consisting of ovarian cancer, triple negative breast cancer, and non-small cell lung cancer.

In a further aspect of the present disclosure, there is provided a pharmaceutical composition comprising an antibody-drug conjugate as disclosed herein, in combination with one or more pharmaceutical acceptable excipient, diluent or carrier, optionally comprising other active ingredients.

In another aspect of the present disclosure, there is provided an antibody-drug conjugate as disclosed herein for use as a medicament.

Accordingly, a first object of the disclosure relates to an antibody-drug conjugate (ADC) of the formula (I):

Ab-[L-D]*p* (I),

wherein :

Ab is an anti-folate receptor alpha (FRα) antibody which binds specifically to SEQ ID NO:12,

L is a cleavable linker moiety bonded to said anti-folate receptor alpha (FRα) antibody preferably via a thiol residue,

D is a cytotoxic drug moiety bonded to L,

p is from 1 to 8, preferably from 6 to 8, and more preferably *p* is 8.

In specific examples, Ab is an antibody comprising a human IgG1 isotype constant region.

In a preferred example, Ab is an antibody comprising a mutant or chemically modified constant region of human IgG1 isotype, wherein said mutant or chemically modified constant region confers no or decreased ADCC activity to said antibody when compared to a corresponding antibody with wild type human IgG1 isotype constant region.

In other specific examples, Ab is an antibody which comprises a human IgG4 isotype constant region, or a mutant or chemically modified IgG4 constant region, wherein said mutant or chemically modified constant region confers no or decreased ADCC activity to said antibody when compared to a corresponding antibody with wild type IgG4 isotype constant region.

In preferred examples, Ab is an anti-FRα antibody comprising either:

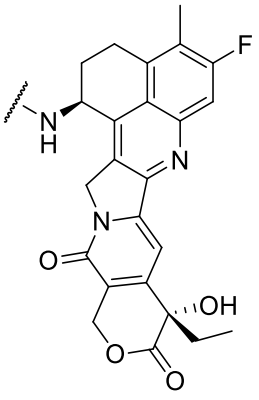
(a) a variable heavy chain polypeptide comprising HCDR1 of SEQ ID NO:1, HCDR2 of SEQ ID NO:2, HCDR3 of SEQ ID NO:3 and a variable light chain polypeptide comprising LCDR1 of SEQ ID NO:4, LCDR2 of SEQ ID NO:5 and LCDR3 of SEQ ID NO:6; or,

(b) a variable heavy chain polypeptide comprising VH of SEQ ID NO:7 and a variable light chain polypeptide comprising VL of SEQ ID NO:8.

In specific examples, said Ab comprises or essentially consists of a heavy chain of SEQ ID NO:9 and a light chain of SEQ ID NO:10;

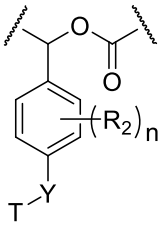
In specific examples, said Ab comprises or essentially consists of a heavy chain of SEQ ID NO:11 and a light chain of SEQ ID NO:10.

In specific examples, D is an inhibitor of topoisomerase I, preferably selected from the group consisting of camptothecine analogues, and more preferably D is the drug moiety of Exatecan of formula (II) below



(II)

In specific examples, L is a cleavable linker moiety of formula -A-W-, wherein A is an optional stretcher unit linked to Ab, and W is a cleavable moiety linked to D. In a more specific example, L is a lysosomal protease-sensitive linker, and W comprises a cleavable peptide moiety, for example selected from the group consisting of valine-citrulline (Val-Cit), alanine-alanine-asparagine (Ala-Ala-Asn), valine-alanine (Val-Ala) and phenylalanine-lysine (Phe-Lys). In another specific example, L is a protease sensitive cleavable linker and W comprises a sugar cleavable unit, preferably selected from a β-glucuronide or a β-galactoside moiety. In another specific example, L is a glutathione-sensitive linker and W comprises a disulfide moiety. In a specific example, W is of the following formula (III)



(III)

wherein

each R_2 is independently selected from the group consisting of electron-withdrawing groups and C_1-C_4 alkyl;

n is 0, 1 or 2 ;

T is a sugar cleavable unit or a polypeptide cleavable unit;

Y is O when T is a sugar cleavable unit, or NR_3 when T is a polypeptide cleavable unit; and

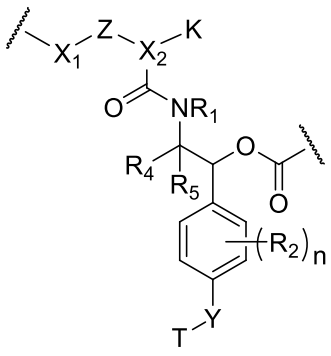
R_3 is selected from the group consisting of H, C_1-C_{24} alkyl, C_2-C_6 alkenyl; optionally substituted polyether, aryl having 6 to 10 ring atoms, C_3-C_8 cycloalkyl, heterocycloalkyl having 3 to 10 ring atoms, heteroaryl having 5 to 10 ring atoms, and any combination thereof,

said alkyl and alkenyl being optionally interrupted by one or more heteroatom or chemical groups selected from -O-, -S-, -C(O)-, -NR''-, -C(O)NR''-, -NR''-C(O)-, -NR''-C(O)-NR'''-, -NR''-C(O)-O-, -O-C(O)NR''- and triazole,

R'' and R''' being independently selected from H and C_1-C_6 alkyl,

and pharmaceutically acceptable salts thereof

In a preferred example, L corresponds to a linker -A-W- of formula (IV)



(IV)

wherein

X_1 is a connector unit;

Z is an optional spacer;

X_2 is a connector unit;

K is an optional hydrophobicity masking entity, preferably selected from polysarcosine and polyethylene glycol;

R_1 is selected from the group consisting of H, C_1-C_{24} alkyl, C_2-C_6 alkenyl; optionally substituted polyether, aryl having 6 to 10 ring atoms, C_3-C_8 cycloalkyl, heterocycloalkyl having 3 to 10 ring atoms, heteroaryl having 5 to 10 ring atoms, and any combination thereof,

said alkyl and alkenyl being optionally interrupted by one or more heteroatom or chemical groups selected from -O-, -S-, -C(O)-, -NR''-, -C(O)NR''-, -NR''-C(O)-, -NR''-C(O)-NR'''-, -NR''-C(O)-O-, -O-C(O)NR''- and triazole;

R_4 is selected from the group consisting of H, C_1-C_6 alkyl and C_2-C_6 alkenyl, said alkyl and alkenyl being optionally interrupted by one or more heteroatom or chemical groups selected from -O-, -S-, -C(O)-, and -NR''-;

R_5 is selected from the group consisting of H, C_1-C_6 alkyl and C_2-C_6 alkenyl, said alkyl and alkenyl being optionally interrupted by one or more heteroatom or chemical groups selected from -O-, -S-, -C(O)-, and -NR''-.

In a more specific example, X_1 and X_2 are independently selected from the group consisting of one or more amino acid(s), one or more N-substituted amino acid, optionally substituted polyether, C_1-C_{12} alkylene, arylene having 6 to 10 ring atoms, C_3-C_8 cycloalkylene, heterocycloalkylene having 5 to 10 ring atoms, heteroarylene having 5 to 10 ring atoms, C_2-C_{10} alkenylene, and any combination thereof,

said alkylene and alkenylene being optionally interrupted by one or more heteroatom or chemical groups selected from -O-, -S-, -C(O)-, -NR''-, -C(O)NR''-, -NR''-C(O)-, -NR''-C(O)-NR'''-, -NR''-C(O)-O-, -O-C(O)NR''- and triazole,

and said alkylene, arylene, cycloalkylene, heterocycloalkylene, heteroarylene, and alkenylene being optionally substituted with one or more of the substituents selected from : halogen, oxo, -OH, -NO_2, –CN, C_1-C_6 alkyl, C_3-C_6 cycloalkyl, heterocyclyl having 5 to 10 ring atoms, aryl having 6 to 10 ring atoms, heteroaryl having 5 to 10 ring atoms, C_1-C_6 alkoxy, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, -(CO)-R', -O-(CO)-R', -(CO)-O-R', -(CO)-NR''R''', -NR''-(CO)-R', and -NR''R''';

R', R'' and R''' being independently selected from H and C_1-C_6 alkyl.

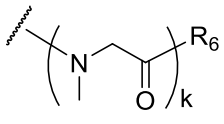
In a more specific example, Z is independently selected from the group consisting of one or more amino acid(s), one or more N-substituted amino acid, optionally substituted polyether, C_1-C_{12} alkylene, arylene having 6 to 10 ring atoms, C_3-C_8 cycloalkylene, heterocycloalkylene having 5 to 10 ring atoms, heteroarylene having 5 to 10 ring atoms, C_2-C_{10} alkenylene, and any combination thereof,

said alkylene and alkenylene being optionally interrupted by one or more heteroatom or chemical groups selected from -O-, -S-, -C(O)-, -NR''-, -C(O)NR''-, -NR''-C(O)-, -NR''-C(O)-NR'''-, -NR''-C(O)-O-, -O-C(O)NR''- and triazole,

and said alkylene, arylene, cycloalkylene, heterocycloalkylene, heteroarylene, and alkenylene being optionally substituted with one or more of the substituents selected from : halogen, oxo, -OH, $-NO_2$, –CN, C1-C6 alkyl, C3-C6 cycloalkyl, heterocyclyl having 5 to 10 ring atoms, aryl having 6 to 10 ring atoms, heteroaryl having 5 to 10 ring atoms, C_1-C_6 alkoxy, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, -(CO)-R', -O-(CO)-R', -(CO)-O-R', -(CO)-NR''R''', -NR''-(CO)-R', and -NR''R''';

R', R'' and R''' being independently selected from H and C_1-C_6 alkyl.

In specific examples, K is a polysarcosine, preferably of the following formula (V)



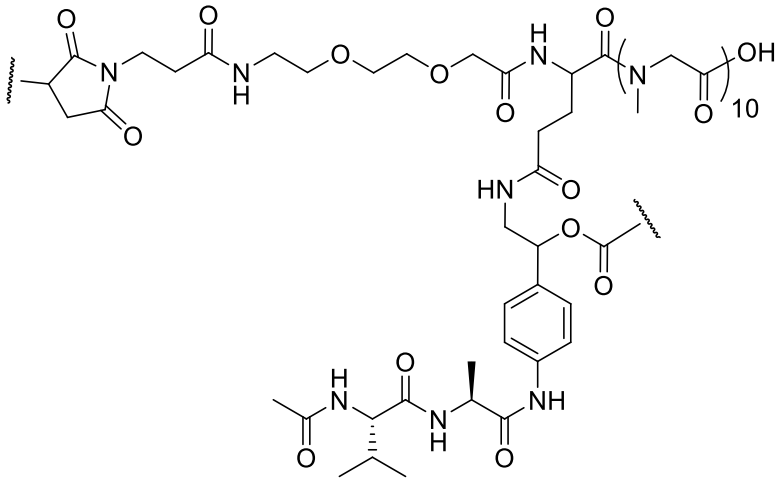
(V),

wherein k is an integer between 2 and 50, preferably between 4 and 30,
and R_6 corresponds to OH or NH_2.

In specific examples, T is a sugar cleavable unit which is a glucuronide or a galactoside.

In other specific examples, T is a dipeptide, preferably selected from Val-Cit, Val-Ala and Phe-Lys.

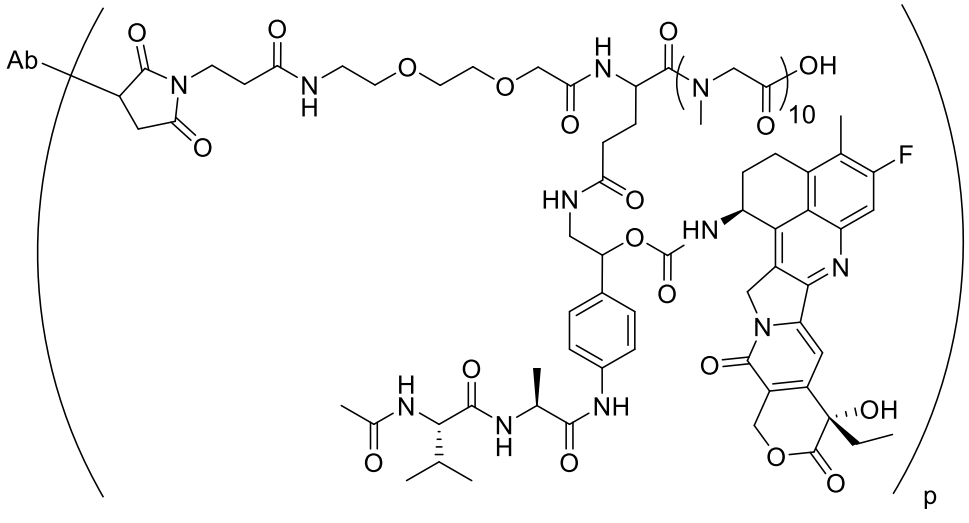
In specific examples, L is covalently bonded to one or more thiol residues of said antibody, preferably, said L corresponding to a linker of formula (VI):



(VI).

In specific examples, Ab includes full-length antibodies or antibodies fragments containing antigen binding portions.

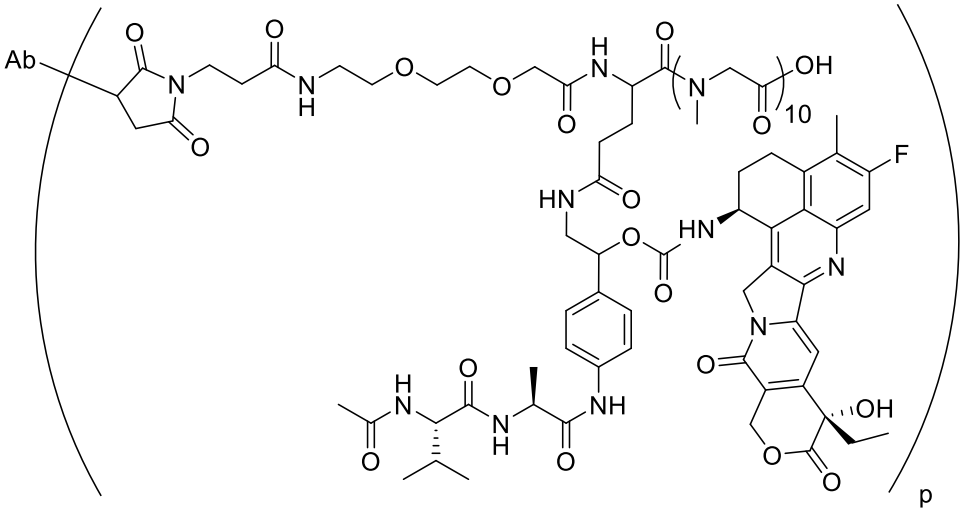
In specific examples, the antibody drug conjugate corresponds to the following formula (VII)



(VII)

wherein Ab is an anti-FRα antibody, such as farletuzumab, or its silent IgG1 variants, typically comprising alanine substitutions in Leucine 234 and Leucine 235 of IgG1 Fc constant region, and p is from 4 to 8.

In a preferred example, the antibody drug conjugate corresponds to the following formula (VII)



(VII)

(VII)

wherein Ab is an anti-FRα antibody, such as farletuzumab, or its silent IgG1 variants, typically a mutant variant of human IgG1 comprising alanine substitutions in Leucine 234 and Leucine 235 of IgG1 Fc constant region, also commonly called as LALA mutations, and p is 8.

In a more preferred example of the ADC of formula (I),

Ab is an anti-folate receptor alpha antibody or an antigen-binding fragment thereof, comprising

(i) a variable heavy chain polypeptide comprising HCDR1 of SEQ ID NO:1, HCDR2 of SEQ ID NO:2, HCDR3 of SEQ ID NO:3 and

(ii) a variable light chain polypeptide comprising LCDR1 of SEQ ID NO:4, LCDR2 of SEQ ID NO:5 and LCDR3 of SEQ ID NO:6;

L is a cleavable linker of formula -A-W-, wherein A is an optional stretcher unit linked to the Ab, and W is a cleavable moiety linked to D

D is exatecan;

p is from 1 to 8, for example between 4 and 8, preferably between 6 and 8, and for example p is between 7 and 8.

Another object of the disclosure relates to the above ADCs, for use as a medicament, preferably for use in the treatment of a tumor, for example a solid tumor, more specifically selected from the group consisting of ovarian cancer, breast cancer, lung cancer, or mesothelioma.

Another object of the disclosure relates to the use of above ADCs, in the preparation of a medicament or pharmaceutical composition for the treatment of a tumor, for example a solid tumor, more specifically selected from the group consisting of ovarian cancer, breast cancer, lung cancer, or mesothelioma.

In specific examples, the ADCs may be preferably used in the treatment of a cancer selected from the group consisting of ovarian cancer, triple negative breast cancer, and non-small cell lung cancer.

The disclosure further relates to a pharmaceutical composition comprising an antibody-drug conjugate as disclosed herein, in combination with one or more pharmaceutical acceptable excipient, diluent or carrier, optionally comprising other active ingredients, for example anti-cancer drugs or immunotherapeutic drugs such as immune checkpoint inhibitors.

The disclosure also relates to the process for obtaining an ADC of the disclosure, wherein the method comprises:

(a) culturing a host cell under conditions suitable for production of the anti-FRα antibody as defined herein,
(b) isolating said anti-FRα antibody,
(c) synthesis of exatecan bonded to the linker L of formula (VIII):

CLAIMS

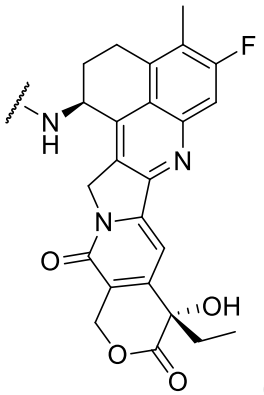
1. An antibody-drug conjugate of the formula (I):

Ab-[L-D]*p* (I),

wherein :

- Ab is an anti-folate receptor alpha (FRα) antibody which binds specifically to SEQ ID NO:12,
- L is a cleavable linker moiety bonded to said antibody, preferably via a thiol residue,
- D is a cytotoxic drug moiety bonded to L,
- *p* is from 1 to 8, preferably from 6 to 8, and more preferably *p* is 8.

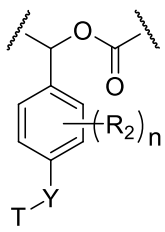
2. The antibody-drug conjugate according to claim 1, wherein D is an inhibitor of topoisomerase I, preferably selected from the group consisting of camptothecine analogues, and more preferably D is the drug moiety of Exatecan of formula (II) below



(II).

3. The antibody-drug conjugate according to any one of claims 1-2, wherein L is a cleavable linker moiety of formula -A-W-, wherein A is an optional stretcher unit linked to Ab, and W is a cleavable moiety linked to D.

4. The antibody-drug conjugate according to claim 3, wherein W is of the following formula (III)



(III)

wherein

each R_2 is independently selected from the group consisting electron-withdrawing groups and C1-C4 alkyl;

n is 0, 1 or 2 ;

T is a sugar cleavable unit or a polypeptide cleavable unit;

Y is O when T is a sugar cleavable unit, or NR_3 when T is a polypeptide cleavable unit; and

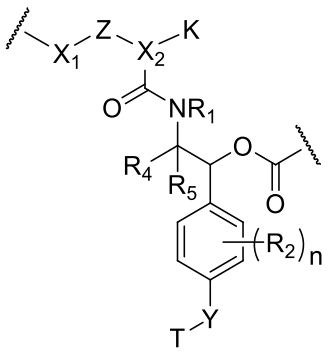
R3 is selected from the group consisting of H, C1-C24 alkyl, C2-C6 alkenyl; optionally substituted polyether, aryl having 6 to 10 ring atoms, C3-C8 cycloalkyl, heterocycloalkyl having 3 to 10 ring atoms, heteroaryl having 5 to 10 ring atoms, and any combination thereof,

said alkyl and alkenyl being optionally interrupted by one or more heteroatom or chemical groups selected from -O-, -S-, -C(O)-, -NR''-, -C(O)NR''-, -NR''-C(O)-, -NR''-C(O)-NR'''-, -NR''-C(O)-O-, -O-C(O)NR''- and triazole,

R'' and R''' being independently selected from H and C1-C6 alkyl,

and pharmaceutically acceptable salts thereof.

5. The antibody-drug conjugate according claim 4, wherein L corresponds to a linker -A-W- of formula (IV)



(IV)

wherein

X_1 is a connector unit;

Z is an optional spacer;

X_2 is a connector unit;

K is an optional hydrophobicity masking entity, preferably selected from polysarcosine and polyethylene glycol;

R_1 is selected from the group consisting of H, C1-C24 alkyl, C_2-C_6 alkenyl; optionally substituted polyether, aryl having 6 to 10 ring atoms, C3-C8 cycloalkyl,

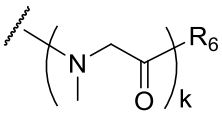
heterocycloalkyl having 3 to 10 ring atoms, heteroaryl having 5 to 10 ring atoms, and any combination thereof,

said alkyl and alkenyl being optionally interrupted by one or more heteroatom or chemical groups selected from -O-, -S-, -C(O)-, -NR''-, -C(O)NR''-, -NR''-C(O)-, -NR''-C(O)-NR'''-, -NR''-C(O)-O-, -O-C(O)NR''- and triazole;

R_4 is selected from the group consisting H, C1-C6 alkyl and C2-C6 alkenyl, said alkyl and alkenyl being optionally interrupted by one or more heteroatom or chemical groups selected from -O-, -S-, -C(O)-, and -NR''-; and

R_5 is selected from the group consisting H, C_1-C_6 alkyl and C_2-C_6 alkenyl, said alkyl and alkenyl being optionally interrupted by one or more heteroatom or chemical groups selected from -O-, -S-, -C(O)-, and -NR''-.

6. The antibody-drug conjugate according to claim 5, wherein K is a polysarcosine, preferably of the following formula (V)



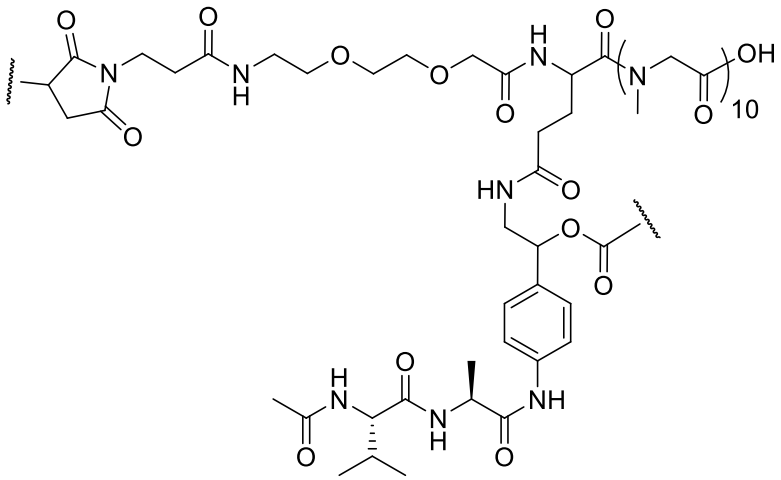
(V),

wherein k is an integer between 2 and 50, preferably between 4 and 30,
and R_6 corresponds to OH or NH_2.

7. The antibody-drug conjugate according to any one of claims 5-6, wherein T is a sugar cleavable unit which is a glucuronide or a galactoside.

8. The antibody-drug conjugate according to any of claims 5-7, wherein T is a dipeptide, preferably selected from Val-Cit, Val-Ala and Phe-Lys.

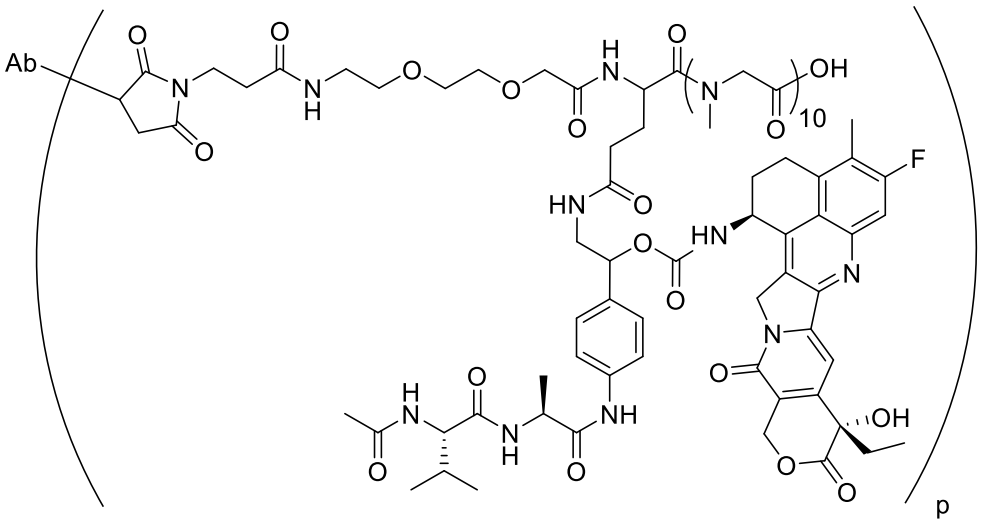
9. The antibody-drug conjugate according to any one of claims 1-3, wherein L corresponds to a linker of formula (VI):



(VI),

and L is covalently bonded to one or more thiol residues of said antibody.

10. The antibody-drug conjugate according to any one of claims 1-9, which corresponds to the following formula (VII)



(VII)

wherein Ab is an anti-FRα antibody, such as farletuzumab, or its silent IgG1 variants, typically comprising alanine substitutions in Leucine 234 and Leucine 235 of IgG1 Fc constant region, and p is from 4 to 8.

11. The antibody-drug conjugate according to any one of claims 1-10, wherein Ab is an anti-FRα antibody which comprises either:

(i) a variable heavy chain polypeptide comprising HCDR1 of SEQ ID NO:1, HCDR2 of SEQ ID NO:2, HCDR3 of SEQ ID NO:3 and a variable light chain polypeptide

comprising LCDR1 of SEQ ID NO:4, LCDR2 of SEQ ID NO:5 and LCDR3 of SEQ ID NO:6; or

(ii) a variable heavy chain polypeptide comprising VH of SEQ ID NO:7 and a variable light chain polypeptide comprising VL of SEQ ID NO:8.

12. The antibody-drug conjugate according to any one of Claims 1-11, wherein

(i) Ab is an anti-folate receptor alpha antibody or an antigen-binding fragment thereof, comprising

- a variable heavy chain polypeptide comprising HCDR1 of SEQ ID NO:1, HCDR2 of SEQ ID NO:2, HCDR3 of SEQ ID NO:3 and
- a variable light chain polypeptide comprising LCDR1 of SEQ ID NO:4, LCDR2 of SEQ ID NO:5 and LCDR3 of SEQ ID NO:6;

(ii) L is a cleavable linker of formula -A-W-, wherein A is an optional stretcher unit linked to the Ab, and W is a cleavable moiety linked to D;

(iii) D is an inhibitor of topoisomerase I, e.g., exatecan ; and,

(iv) *p* is from 1 to 8, preferably from 4 to 8, more preferably about 8.

13. Use of an antibody-drug conjugate according to any one of claims 1-12, in the manufacture of a medicament, for the treatment of a tumor in a subject in need thereof, for example a solid tumor, more specifically selected from the group consisting of ovarian cancer, breast cancer, lung cancer, or mesothelioma.

14. Use of an antibody-drug conjugate according to any one of claims 1-12, in the manufacture of a medicament for the treatment of a cancer, in a subject in need thereof, selected from the group consisting of ovarian cancer, triple negative breast cancer, and non-small cell lung cancer.

15. A pharmaceutical composition comprising an antibody-drug conjugate according to any one of claims 1-12, in combination with one or more pharmaceutical acceptable excipient, diluent or carrier, optionally comprising other active ingredients.

16. An antibody-drug conjugate according to any one of claims 1-12 for use as a medicament.