

antibody PG4D2, and completed the present invention.

The present invention is as follows.

[0008] The present invention is an isolated humanized or mouse-human chimeric anti-podoplanin antibody which comprises the following amino acid sequences, or an antibody fragment containing the antigen-binding region thereof,

provided that the amino acid sequences of the heavy-chain CDRs 1 to 3 and the light-chain CDRs 1 to 3 optionally have a sequence identity of not less than 90% to the amino acid sequences represented by SEQ ID NOs: 1 to 6 below, respectively;

wherein the amino acid sequence of the heavy-chain CDR1 is the amino acid sequence represented by SEQ ID NO: 1,

the amino acid sequence of the heavy-chain CDR2 is the amino acid sequence represented by SEQ ID NO: 2,

the amino acid sequence of the heavy-chain CDR3 is the amino acid sequence represented by SEQ ID NO: 3,

the amino acid sequence of the light-chain CDR1 is the amino acid sequence represented by SEQ ID NO: 4,

the amino acid sequence of the light-chain CDR2 is the amino acid sequence represented by SEQ ID NO: 5, and

the amino acid sequence of the light-chain CDR3 is the amino acid sequence represented by SEQ ID NO: 6.

[0008a] In an aspect, the present disclosure refers

to an isolated humanized anti-podoplanin antibody, or
an antibody fragment thereof, comprising an antigen-
binding region, which comprises a variable region
comprising the amino acid sequence I or II below,

the amino acid sequences of the heavy-chain FRs 1
to 4 and the light-chain FRs 1 to 4 in the following
amino acid sequence I optionally have a sequence
identity of not less than 90% to the amino acid
sequences represented by SEQ ID NOs: 7 to 14 below,
respectively, and

the amino acid sequences of the heavy-chain FRs 1
to 4 and the light-chain FRs 1 to 4 in the following
amino acid sequence II optionally have a sequence
identity of not less than 90% to the amino acid
sequences represented by SEQ ID NOs: 7, 15, 16, 10 to
14 below, respectively;

wherein, in the amino acid sequence I,

the amino acid sequence of the heavy-chain CDR1 is
the amino acid sequence represented by SEQ ID NO: 1,

the amino acid sequence of the heavy-chain CDR2 is
the amino acid sequence represented by SEQ ID NO: 2,

the amino acid sequence of the heavy-chain CDR3 is
the amino acid sequence represented by SEQ ID NO: 3,

the amino acid sequence of the light-chain CDR1 is
the amino acid sequence represented by SEQ ID NO: 4,

the amino acid sequence of the light-chain CDR2 is
the amino acid sequence represented by SEQ ID NO: 5,

the amino acid sequence of the light-chain CDR3 is
the amino acid sequence represented by SEQ ID NO: 6,

the amino acid sequence of the heavy-chain FR1 is
the amino acid sequence represented by SEQ ID NO: 7,

the amino acid sequence of the heavy-chain FR2 is
the amino acid sequence represented by SEQ ID NO: 8,

the amino acid sequence of the heavy-chain FR3 is
the amino acid sequence represented by SEQ ID NO: 9,

the amino acid sequence of the heavy-chain FR4 is
the amino acid sequence represented by SEQ ID NO: 10,

the amino acid sequence of the light-chain FR1 is
the amino acid sequence represented by SEQ ID NO: 11,

the amino acid sequence of the light-chain FR2 is
the amino acid sequence represented by SEQ ID NO: 12,

the amino acid sequence of the light-chain FR3 is
the amino acid sequence represented by SEQ ID NO: 13,

and

the amino acid sequence of the light-chain FR4 is
the amino acid sequence represented by SEQ ID NO: 14;

and

wherein, in the amino acid sequence II,

the amino acid sequence of the heavy-chain CDR1 is
the amino acid sequence represented by SEQ ID NO: 1,

the amino acid sequence of the heavy-chain CDR2 is
the amino acid sequence represented by SEQ ID NO: 2,

the amino acid sequence of the heavy-chain CDR3 is
the amino acid sequence represented by SEQ ID NO: 3,

the amino acid sequence of the light-chain CDR1 is
the amino acid sequence represented by SEQ ID NO: 4,
the amino acid sequence of the light-chain CDR2 is
the amino acid sequence represented by SEQ ID NO: 5,
the amino acid sequence of the light-chain CDR3 is
the amino acid sequence represented by SEQ ID NO: 6,
the amino acid sequence of the heavy-chain FR1 is
the amino acid sequence represented by SEQ ID NO: 7,
the amino acid sequence of the heavy-chain FR2 is
the amino acid sequence represented by SEQ ID NO: 15,
the amino acid sequence of the heavy-chain FR3 is
the amino acid sequence represented by SEQ ID NO: 16,
the amino acid sequence of the heavy-chain FR4 is
the amino acid sequence represented by SEQ ID NO: 10,
the amino acid sequence of the light-chain FR1 is
the amino acid sequence represented by SEQ ID NO: 11,
the amino acid sequence of the light-chain FR2 is
the amino acid sequence represented by SEQ ID NO: 12,
the amino acid sequence of the light-chain FR3 is
the amino acid sequence represented by SEQ ID NO: 13,
and
the amino acid sequence of the light-chain FR4 is
the amino acid sequence represented by SEQ ID NO: 14,
wherein said anti-podoplanin antibody and antigen-
binding region thereof specifically bind to the PLAG4
domain of human podoplanin.

[0008b] In another aspect, the present disclosure refers to an isolated mouse-human chimeric anti-podoplanin antibody, or an antibody fragment thereof, comprising an antigen-binding region, which comprises variable regions comprising the following amino acid sequences,

the amino acid sequences of the heavy-chain FRs 1 to 4 and the light-chain FRs 1 to 4 optionally have a sequence identity of not less than 90% to the amino acid sequences represented by SEQ ID NO: 17 to 24 below, respectively,

wherein

the amino acid sequence of the heavy-chain CDR1 is the amino acid sequence represented by SEQ ID NO: 1,

the amino acid sequence of the heavy-chain CDR2 is the amino acid sequence represented by SEQ ID NO: 2,

the amino acid sequence of the heavy-chain CDR3 is the amino acid sequence represented by SEQ ID NO: 3,

the amino acid sequence of the light-chain CDR1 is the amino acid sequence represented by SEQ ID NO: 4,

the amino acid sequence of the light-chain CDR2 is the amino acid sequence represented by SEQ ID NO: 5,

the amino acid sequence of the light-chain CDR3 is the amino acid sequence represented by SEQ ID NO: 6,

the amino acid sequence of the heavy-chain FR1 is the amino acid sequence represented by SEQ ID NO: 17,

the amino acid sequence of the heavy-chain FR2 is

the amino acid sequence represented by SEQ ID NO: 18,
the amino acid sequence of the heavy-chain FR3 is
the amino acid sequence represented by SEQ ID NO: 19,
the amino acid sequence of the heavy-chain FR4 is
the amino acid sequence represented by SEQ ID NO: 20,
the amino acid sequence of the light-chain FR1 is
the amino acid sequence represented by SEQ ID NO: 21,
the amino acid sequence of the light-chain FR2 is
the amino acid sequence represented by SEQ ID NO: 22,
the amino acid sequence of the light-chain FR3 is
the amino acid sequence represented by SEQ ID NO: 23,
and
the amino acid sequence of the light-chain FR4 is
the amino acid sequence represented by SEQ ID NO: 24,
wherein said anti-podoplanin antibody and antigen-
binding region thereof specifically bind to the PLAG4
domain of human podoplanin.

[0008c] In another aspect, the present disclosure
refers to the use of an isolated humanized anti-
podoplanin antibody, or an antibody fragment thereof,
comprising an antigen-binding region which comprises a
variable region comprising the amino acid sequence I or
II below,

the amino acid sequences of the heavy-chain FRs 1
to 4 and the light-chain FRs 1 to 4 in the following
amino acid sequence I optionally have a sequence
identity of not less than 90% to the amino acid

sequences represented by SEQ ID NOs: 7 to 14 below,
respectively, and

the amino acid sequences of the heavy-chain FRs 1
to 4 and the light-chain FRs 1 to 4 in the following
amino acid sequence II optionally have a sequence
identity of not less than 90% to the amino acid
sequences represented by SEQ ID NOs: 7, 15, 16, 10 to
14 below, respectively;

wherein in the amino acid sequence I,

the amino acid sequence of the heavy-chain CDR1 is
the amino acid sequence represented by SEQ ID NO: 1,

the amino acid sequence of the heavy-chain CDR2 is
the amino acid sequence represented by SEQ ID NO: 2,

the amino acid sequence of the heavy-chain CDR3 is
the amino acid sequence represented by SEQ ID NO: 3,

the amino acid sequence of the light-chain CDR1 is
the amino acid sequence represented by SEQ ID NO: 4,

the amino acid sequence of the light-chain CDR2 is
the amino acid sequence represented by SEQ ID NO: 5,

the amino acid sequence of the light-chain CDR3 is
the amino acid sequence represented by SEQ ID NO: 6,

the amino acid sequence of the heavy-chain FR1 is
the amino acid sequence represented by SEQ ID NO: 7,

the amino acid sequence of the heavy-chain FR2 is
the amino acid sequence represented by SEQ ID NO: 8,

the amino acid sequence of the heavy-chain FR3 is
the amino acid sequence represented by SEQ ID NO: 9,

the amino acid sequence of the heavy-chain FR4 is
the amino acid sequence represented by SEQ ID NO: 10,
the amino acid sequence of the light-chain FR1 is
the amino acid sequence represented by SEQ ID NO: 11,
the amino acid sequence of the light-chain FR2 is
the amino acid sequence represented by SEQ ID NO: 12,
the amino acid sequence of the light-chain FR3 is
the amino acid sequence represented by SEQ ID NO: 13,
and
the amino acid sequence of the light-chain FR4 is
the amino acid sequence represented by SEQ ID NO: 14;
and
wherein, in the amino acid sequence II,
the amino acid sequence of the heavy-chain CDR1 is
the amino acid sequence represented by SEQ ID NO: 1,
the amino acid sequence of the heavy-chain CDR2 is
the amino acid sequence represented by SEQ ID NO: 2,
the amino acid sequence of the heavy-chain CDR3 is
the amino acid sequence represented by SEQ ID NO: 3,
the amino acid sequence of the light-chain CDR1 is
the amino acid sequence represented by SEQ ID NO: 4,
the amino acid sequence of the light-chain CDR2 is
the amino acid sequence represented by SEQ ID NO: 5,
the amino acid sequence of the light-chain CDR3 is
the amino acid sequence represented by SEQ ID NO: 6,
the amino acid sequence of the heavy-chain FR1 is
the amino acid sequence represented by SEQ ID NO: 7,

the amino acid sequence of the heavy-chain FR2 is
the amino acid sequence represented by SEQ ID NO: 15,
the amino acid sequence of the heavy-chain FR3 is
the amino acid sequence represented by SEQ ID NO: 16,
the amino acid sequence of the heavy-chain FR4 is
the amino acid sequence represented by SEQ ID NO: 10,
the amino acid sequence of the light-chain FRI is
the amino acid sequence represented by SEQ ID NO: 11,
the amino acid sequence of the light-chain FR2 is
the amino acid sequence represented by SEQ ID NO: 12,
the amino acid sequence of the light-chain FR3 is
the amino acid sequence represented by SEQ ID NO: 13,
and
the amino acid sequence of the light-chain FR4 is
the amino acid sequence represented by SEQ ID NO: 14,
in the manufacture of a medicament for inhibiting
the binding between podoplanin and CLEC-2 in a human
subject,
wherein said anti-podoplanin antibody and antigen-
binding region thereof specifically bind to the PLAG4
domain of human podoplanin.

[0009] In a preferred aspect of the anti-podoplanin

the amino acid sequence represented by SEQ ID NO: 20,
the amino acid sequence of the light-chain FR1 is
the amino acid sequence represented by SEQ ID NO: 21,
the amino acid sequence of the light-chain FR2 is
the amino acid sequence represented by SEQ ID NO: 22,
the amino acid sequence of the light-chain FR3 is
the amino acid sequence represented by SEQ ID NO: 23,
and

the amino acid sequence of the light-chain FR4 is
the amino acid sequence represented by SEQ ID NO: 24.

[0011] In a preferred aspect of the anti-podoplanin antibody or antibody fragment containing the antigen-binding region thereof, the anti-podoplanin antibody comprises a constant region of a human antibody of the IgG class.

[0012] Additionally, the present invention provides an agent for inhibiting the binding between podoplanin and CLEC-2 which comprises the anti-podoplanin antibody or antibody fragment containing the antigen-binding region thereof.

[0013] Additionally, the present invention provides a pharmaceutical composition which comprises the anti-podoplanin antibody or antibody fragment containing the antigen-binding region thereof.

[0013a] In another aspect, the present disclosure refers to a pharmaceutical composition, comprising, an effective amount for specifically binding to podoplanin

of an isolated humanized anti-podoplanin antibody or an antibody fragment thereof, containing an antigen-binding region which comprises a variable region comprising the amino acid sequence I or II below,

the amino acid sequences of the heavy-chain FRs 1 to 4 and the light-chain FRs 1 to 4 in the following amino acid sequence I optionally have a sequence identity of not less than 90% to the amino acid sequences represented by SEQ ID NOs: 7 to 14 below, respectively, and

the amino acid sequences of the heavy-chain FRs 1 to 4 and the light-chain FRs 1 to 4 in the following amino acid sequence II optionally have a sequence identity of not less than 90% to the amino acid sequences represented by SEQ ID NOs: 7, 15, 16, 10 to 14 below, respectively;

wherein in the amino acid sequence I,

the amino acid sequence of the heavy-chain CDR1 is the amino acid sequence represented by SEQ ID NO: 1,

the amino acid sequence of the heavy-chain CDR2 is the amino acid sequence represented by SEQ ID NO: 2,

the amino acid sequence of the heavy-chain CDR3 is the amino acid sequence represented by SEQ ID NO: 3,

the amino acid sequence of the light-chain CDR1 is the amino acid sequence represented by SEQ ID NO: 4,

the amino acid sequence of the light-chain CDR2 is the amino acid sequence represented by SEQ ID NO: 5,

the amino acid sequence of the light-chain CDR3 is
the amino acid sequence represented by SEQ ID NO: 6,

the amino acid sequence of the heavy-chain FR1 is
the amino acid sequence represented by SEQ ID NO: 7,

the amino acid sequence of the heavy-chain FR2 is
the amino acid sequence represented by SEQ ID NO: 8,

the amino acid sequence of the heavy-chain FR3 is
the amino acid sequence represented by SEQ ID NO: 9,

the amino acid sequence of the heavy-chain FR4 is
the amino acid sequence represented by SEQ ID NO: 10,

the amino acid sequence of the light-chain FR1 is
the amino acid sequence represented by SEQ ID NO: 11,

the amino acid sequence of the light-chain FR2 is
the amino acid sequence represented by SEQ ID NO: 12,

the amino acid sequence of the light-chain FR3 is
the amino acid sequence represented by SEQ ID NO: 13,

and

the amino acid sequence of the light-chain FR4 is
the amino acid sequence represented by SEQ ID NO: 14;

and

wherein, in the amino acid sequence II,

the amino acid sequence of the heavy-chain CDR1 is
the amino acid sequence represented by SEQ ID NO: 1,

the amino acid sequence of the heavy-chain CDR2 is
the amino acid sequence represented by SEQ ID NO: 2,

the amino acid sequence of the heavy-chain CDR3 is
the amino acid sequence represented by SEQ ID NO: 3,

the amino acid sequence of the light-chain CDR1 is
the amino acid sequence represented by SEQ ID NO: 4,
the amino acid sequence of the light-chain CDR2 is
the amino acid sequence represented by SEQ ID NO: 5,
the amino acid sequence of the light-chain CDR3 is
the amino acid sequence represented by SEQ ID NO: 6,
the amino acid sequence of the heavy-chain FR1 is
the amino acid sequence represented by SEQ ID NO: 7,
the amino acid sequence of the heavy-chain FR2 is
the amino acid sequence represented by SEQ ID NO: 15,
the amino acid sequence of the heavy-chain FR3 is
the amino acid sequence represented by SEQ ID NO: 16,
the amino acid sequence of the heavy-chain FR4 is
the amino acid sequence represented by SEQ ID NO: 10,
the amino acid sequence of the light-chain FRI is
the amino acid sequence represented by SEQ ID NO: 11,
the amino acid sequence of the light-chain FR2 is
the amino acid sequence represented by SEQ ID NO: 12,
the amino acid sequence of the light-chain FR3 is
the amino acid sequence represented by SEQ ID NO: 13,
and
the amino acid sequence of the light-chain FR4 is
the amino acid sequence represented by SEQ ID NO: 14;
and a pharmaceutically acceptable carrier,
wherein said anti-podoplanin antibody and antigen-
binding region thereof specifically bind to the PLAG4
domain of human podoplanin.

[0013b] In another aspect, the present disclosure refers to the use of an isolated mouse-human chimeric anti-podoplanin antibody, or an antibody fragment thereof, comprising an antigen-binding region, which comprises variable regions comprising the following amino acid sequences,

the amino acid sequences of the heavy-chain FRs 1 to 4 and the light-chain FRs 1 to 4 optionally have a sequence identity of not less than 90% to the amino acid sequences represented by SEQ ID NO: 17 to 24 below, respectively,

wherein

the amino acid sequence of the heavy-chain CDR1 is the amino acid sequence represented by SEQ ID NO: 1,

the amino acid sequence of the heavy-chain CDR2 is the amino acid sequence represented by SEQ ID NO: 2,

the amino acid sequence of the heavy-chain CDR3 is the amino acid sequence represented by SEQ ID NO: 3,

the amino acid sequence of the light-chain CDR1 is the amino acid sequence represented by SEQ ID NO: 4,

the amino acid sequence of the light-chain CDR2 is the amino acid sequence represented by SEQ ID NO: 5,

the amino acid sequence of the light-chain CDR3 is the amino acid sequence represented by SEQ ID NO: 6,

the amino acid sequence of the heavy-chain FR1 is the amino acid sequence represented by SEQ ID NO: 17,

the amino acid sequence of the heavy-chain FR2 is

the amino acid sequence represented by SEQ ID NO: 18,
the amino acid sequence of the heavy-chain FR3 is
the amino acid sequence represented by SEQ ID NO: 19,
the amino acid sequence of the heavy-chain FR4 is
the amino acid sequence represented by SEQ ID NO: 20,
the amino acid sequence of the light-chain FRI is
the amino acid sequence represented by SEQ ID NO: 21,
the amino acid sequence of the light-chain FR2 is
the amino acid sequence represented by SEQ ID NO: 22,
the amino acid sequence of the light-chain FR3 is
the amino acid sequence represented by SEQ ID NO: 23,
and
the amino acid sequence of the light-chain FR4 is
the amino acid sequence represented by SEQ ID NO: 24,
in the manufacture of a medicament for inhibiting
the binding between podoplanin and CLEC-2 in a human
subject,
wherein said anti-podoplanin antibody and antigen-
binding region thereof specifically bind to the PLAG4
domain of human podoplanin.

[0013c] In another aspect, the present disclosure
refers to a pharmaceutical composition, comprising:

an effective amount for specifically binding to
podoplanin of an isolated mouse-human chimeric anti-
podoplanin antibody, or an antibody fragment thereof,
comprising an antigen-binding region, which comprises
variable regions comprising the following amino acid

sequences,

the amino acid sequences of the heavy-chain FRs 1 to 4 and the light-chain FRs 1 to 4 optionally have a sequence identity of not less than 90% to the amino acid sequences represented by SEQ ID NO: 17 to 24 below, respectively,

wherein

the amino acid sequence of the heavy-chain CDR1 is the amino acid sequence represented by SEQ ID NO: 1,

the amino acid sequence of the heavy-chain CDR2 is the amino acid sequence represented by SEQ ID NO: 2,

the amino acid sequence of the heavy-chain CDR3 is the amino acid sequence represented by SEQ ID NO: 3,

the amino acid sequence of the light-chain CDR1 is the amino acid sequence represented by SEQ ID NO: 4,

the amino acid sequence of the light-chain CDR2 is the amino acid sequence represented by SEQ ID NO: 5,

the amino acid sequence of the light-chain CDR3 is the amino acid sequence represented by SEQ ID NO: 6,

the amino acid sequence of the heavy-chain FR1 is the amino acid sequence represented by SEQ ID NO: 17,

the amino acid sequence of the heavy-chain FR2 is the amino acid sequence represented by SEQ ID NO: 18,

the amino acid sequence of the heavy-chain FR3 is the amino acid sequence represented by SEQ ID NO: 19,

the amino acid sequence of the heavy-chain FR4 is the amino acid sequence represented by SEQ ID NO: 20,

the amino acid sequence of the light-chain FR1 is
the amino acid sequence represented by SEQ ID NO: 21,

the amino acid sequence of the light-chain FR2 is
the amino acid sequence represented by SEQ ID NO: 22,

the amino acid sequence of the light-chain FR3 is
the amino acid sequence represented by SEQ ID NO: 23,
and

the amino acid sequence of the light-chain FR4 is
the amino acid sequence represented by SEQ ID NO: 24,

and a pharmaceutically acceptable carrier,

wherein said anti-podoplanin antibody and antigen-
binding region thereof specifically bind to the PLAG4
domain of human podoplanin.

[0013d] In another aspect, the present disclosure
refers to an isolated humanized anti-podoplanin antibody,
or an antibody fragment thereof, of claim 1, or an
isolated mouse-human chimeric anti-podoplanin antibody,
or an antibody fragment thereof, of claim 3, for use in
therapy.

In a preferred aspect, the pharmaceutical
composition is for reducing the proliferation or
metastasis of a cancer cell.

antibody K~~41~~.

In this case, for example, the following nucleotide sequences can be used as nucleotide sequences encoding the above-described amino acid sequences.

The nucleotide sequences represented by SEQ ID NOs: 27 to 32 and 45 to 52 can be used as nucleotide sequences encoding the amino acid sequences represented by SEQ ID NOs: 1 to 6 and 17 to 24, respectively.

The same nucleotide sequences encoding the amino acid sequences of the constant regions as those described in Production Example 1 can be used.

[0061] The Freedom (trademark) pCHO 1.0 vector (Thermo Fisher Scientific) was used for gene expression in CHO cells (Thermo Fisher Scientific) as an expression vector.

[0062] CHO cells expressing the mouse-human chimeric antibody chPG4D2 were thawed and then eventually amplified in a 5-L stirred bioreactor in a batch mode under the following culture conditions: a stirring rate of 100 rpm, a culture temperature of 37°C, and a pH of 7.0. The culture was collected after 7 days of incubation.

The collected culture was used to purify and obtain the mouse-human chimeric antibody chPG4D2. The collected culture supernatant was used for the measurements described in Examples 2 and 3 below.

CLAIMS

~~1. An isolated humanized or mouse-human chimeric anti-podoplanin antibody which comprises the following amino acid sequences, or an antibody fragment containing the antigen-binding region thereof,~~

~~provided that the amino acid sequences of the heavy-chain CDRs 1 to 3 and the light-chain CDRs 1 to 3 optionally have a sequence identity of not less than 90% to the amino acid sequences represented by SEQ ID NOs: 1 to 6 below, respectively;~~

~~wherein the amino acid sequence of the heavy-chain CDR1 is the amino acid sequence represented by SEQ ID NO: 1,~~

~~the amino acid sequence of the heavy-chain CDR2 is the amino acid sequence represented by SEQ ID NO: 2,~~

~~the amino acid sequence of the heavy-chain CDR3 is the amino acid sequence represented by SEQ ID NO: 3,~~

~~the amino acid sequence of the light-chain CDR1 is the amino acid sequence represented by SEQ ID NO: 4,~~

~~the amino acid sequence of the light-chain CDR2 is the amino acid sequence represented by SEQ ID NO: 5, and~~

~~the amino acid sequence of the light-chain CDR3 is the amino acid sequence represented by SEQ ID NO: 6.~~

21. An isolated humanized~~The~~ anti-podoplanin antibody, or an antibody fragment thereof, comprising~~containing thean~~ antigen-binding region, thereof, according~~to claim 1, wherein the anti-podoplanin antibody is a humanized antibody~~ which comprises a variable region comprising the amino acid sequence ~~(I)~~ or ~~(II)~~ below, ~~provided that~~ the amino acid sequences of the heavy-chain FRs 1 to 4 and the light-chain FRs 1 to 4 in the following amino acid sequence ~~(I)~~ optionally have a sequence identity

of not less than 90% to the amino acid sequences represented by SEQ ID NOs: 7 to 14 below, respectively, and

the amino acid sequences of the heavy-chain FRs 1 to 4 and the light-chain FRs 1 to 4 in the following amino acid sequence ~~+II+~~ optionally have a sequence identity of not less than 90% to the amino acid sequences represented by SEQ ID NOs: 7, 15, 16, 10 to 14 below, respectively;

wherein, in the amino acid sequence ~~+I+—~~,

the amino acid sequence of the heavy-chain CDR1 is the amino acid sequence represented by SEQ ID NO: 1,

the amino acid sequence of the heavy-chain CDR2 is the amino acid sequence represented by SEQ ID NO: 2,

the amino acid sequence of the heavy-chain CDR3 is the amino acid sequence represented by SEQ ID NO: 3,

the amino acid sequence of the light-chain CDR1 is the amino acid sequence represented by SEQ ID NO: 4,

the amino acid sequence of the light-chain CDR2 is the amino acid sequence represented by SEQ ID NO: 5,

the amino acid sequence of the light-chain CDR3 is the amino acid sequence represented by SEQ ID NO: 6,

the amino acid sequence of the heavy-chain FR1 is the amino acid sequence represented by SEQ ID NO: 7,

the amino acid sequence of the heavy-chain FR2 is the amino acid sequence represented by SEQ ID NO: 8,

the amino acid sequence of the heavy-chain FR3 is the amino acid sequence represented by SEQ ID NO: 9,

the amino acid sequence of the heavy-chain FR4 is the amino acid sequence represented by SEQ ID NO: 10,

the amino acid sequence of the light-chain FR1 is the amino acid sequence represented by SEQ ID NO: 11,

the amino acid sequence of the light-chain FR2 is the amino acid sequence represented by SEQ ID NO: 12,

the amino acid sequence of the light-chain FR3 is the

amino acid sequence represented by SEQ ID NO: 13, and
the amino acid sequence of the light-chain FR4 is the
amino acid sequence represented by SEQ ID NO: 14; and
wherein, in the amino acid sequence ~~+II+~~,
the amino acid sequence of the heavy-chain CDR1 is the
amino acid sequence represented by SEQ ID NO: 1,
the amino acid sequence of the heavy-chain CDR2 is the
amino acid sequence represented by SEQ ID NO: 2,
the amino acid sequence of the heavy-chain CDR3 is the
amino acid sequence represented by SEQ ID NO: 3,
the amino acid sequence of the light-chain CDR1 is the
amino acid sequence represented by SEQ ID NO: 4,
the amino acid sequence of the light-chain CDR2 is the
amino acid sequence represented by SEQ ID NO: 5,
the amino acid sequence of the light-chain CDR3 is the
amino acid sequence represented by SEQ ID NO: 6,
the amino acid sequence of the heavy-chain FR1 is the
amino acid sequence represented by SEQ ID NO: 7,
the amino acid sequence of the heavy-chain FR2 is the
amino acid sequence represented by SEQ ID NO: 15,
the amino acid sequence of the heavy-chain FR3 is the
amino acid sequence represented by SEQ ID NO: 16,
the amino acid sequence of the heavy-chain FR4 is the
amino acid sequence represented by SEQ ID NO: 10,
the amino acid sequence of the light-chain FR1 is the
amino acid sequence represented by SEQ ID NO: 11,
the amino acid sequence of the light-chain FR2 is the
amino acid sequence represented by SEQ ID NO: 12,
the amino acid sequence of the light-chain FR3 is the
amino acid sequence represented by SEQ ID NO: 13, and
the amino acid sequence of the light-chain FR4 is the
amino acid sequence represented by SEQ ID NO: 14~~+~~.
wherein said anti-podoplanin antibody and antigen-

binding region thereof specifically bind to the PLAG4 domain of human podoplanin.

2. The isolated humanized anti-podoplanin antibody or antibody fragment thereof, containing the antigen-binding region thereof according to claim 1, wherein the anti-podoplanin antibody comprises a constant region of a human antibody of the IgG class.

3. ~~The~~An isolated mouse-human chimeric anti-podoplanin antibody, or an antibody fragment thereof, comprising an ~~containing the~~ antigen-binding region, ~~thereof according to claim 1, wherein the anti-podoplanin antibody is a mouse-human chimeric antibody~~ which comprises variable regions comprising the following amino acid sequences, ~~provided that~~ the amino acid sequences of the heavy-chain FRs 1 to 4 and the light-chain FRs 1 to 4 optionally have a sequence identity of not less than 90% to the amino acid sequences represented by SEQ ID NO: 17 to 24 below, respectively,

wherein-

the amino acid sequence of the heavy-chain CDR1 is the amino acid sequence represented by SEQ ID NO: 1,

the amino acid sequence of the heavy-chain CDR2 is the amino acid sequence represented by SEQ ID NO: 2,

the amino acid sequence of the heavy-chain CDR3 is the amino acid sequence represented by SEQ ID NO: 3,

the amino acid sequence of the light-chain CDR1 is the amino acid sequence represented by SEQ ID NO: 4,

the amino acid sequence of the light-chain CDR2 is the amino acid sequence represented by SEQ ID NO: 5,

the amino acid sequence of the light-chain CDR3 is the amino acid sequence represented by SEQ ID NO: 6,

the amino acid sequence of the heavy-chain FR1 is the amino acid sequence represented by SEQ ID NO: 17,

the amino acid sequence of the heavy-chain FR2 is the amino acid sequence represented by SEQ ID NO: 18,

the amino acid sequence of the heavy-chain FR3 is the amino acid sequence represented by SEQ ID NO: 19,

the amino acid sequence of the heavy-chain FR4 is the amino acid sequence represented by SEQ ID NO: 20,

the amino acid sequence of the light-chain FR1 is the amino acid sequence represented by SEQ ID NO: 21,

the amino acid sequence of the light-chain FR2 is the amino acid sequence represented by SEQ ID NO: 22,

the amino acid sequence of the light-chain FR3 is the amino acid sequence represented by SEQ ID NO: 23, and

the amino acid sequence of the light-chain FR4 is the amino acid sequence represented by SEQ ID NO: 24.

wherein said anti-podoplanin antibody and antigen-binding region thereof specifically bind to the PLAG4 domain of human podoplanin.

4. The isolated mouse-human chimeric anti-podoplanin antibody or antibody fragment ~~containing the~~ thereof, comprising an antigen-binding region thereof according to ~~any one of claims 1 to~~ claim 3, wherein the anti-podoplanin antibody comprises a constant region of a human antibody of the IgG class.

~~5. An agent for inhibiting the binding between podoplanin and CLEC-2, comprising the anti-podoplanin antibody or antibody fragment containing the antigen-binding region thereof according to any one of claims 1 to 4.~~

5. Use of an isolated humanized anti-podoplanin

antibody, or an antibody fragment thereof, comprising an antigen-binding region which comprises a variable region comprising the amino acid sequence I or II below,

the amino acid sequences of the heavy-chain FRs 1 to 4 and the light-chain FRs 1 to 4 in the following amino acid sequence I optionally have a sequence identity of not less than 90% to the amino acid sequences represented by SEQ ID NOs: 7 to 14 below, respectively, and

the amino acid sequences of the heavy-chain FRs 1 to 4 and the light-chain FRs 1 to 4 in the following amino acid sequence II optionally have a sequence identity of not less than 90% to the amino acid sequences represented by SEQ ID NOs: 7, 15, 16, 10 to 14 below, respectively;

wherein in the amino acid sequence I,

the amino acid sequence of the heavy-chain CDR1 is the amino acid sequence represented by SEQ ID NO: 1,

the amino acid sequence of the heavy-chain CDR2 is the amino acid sequence represented by SEQ ID NO: 2,

the amino acid sequence of the heavy-chain CDR3 is the amino acid sequence represented by SEQ ID NO: 3,

the amino acid sequence of the light-chain CDR1 is the amino acid sequence represented by SEQ ID NO: 4,

the amino acid sequence of the light-chain CDR2 is the amino acid sequence represented by SEQ ID NO: 5,

the amino acid sequence of the light-chain CDR3 is the amino acid sequence represented by SEQ ID NO: 6,

the amino acid sequence of the heavy-chain FR1 is the amino acid sequence represented by SEQ ID NO: 7,

the amino acid sequence of the heavy-chain FR2 is the amino acid sequence represented by SEQ ID NO: 8,

the amino acid sequence of the heavy-chain FR3 is the amino acid sequence represented by SEQ ID NO: 9,

the amino acid sequence of the heavy-chain FR4 is the

amino acid sequence represented by SEQ ID NO: 10,
the amino acid sequence of the light-chain FR1 is the
amino acid sequence represented by SEQ ID NO: 11,
the amino acid sequence of the light-chain FR2 is the
amino acid sequence represented by SEQ ID NO: 12,
the amino acid sequence of the light-chain FR3 is the
amino acid sequence represented by SEQ ID NO: 13, and
the amino acid sequence of the light-chain FR4 is the
amino acid sequence represented by SEQ ID NO: 14; and
wherein, in the amino acid sequence II,
the amino acid sequence of the heavy-chain CDR1 is the
amino acid sequence represented by SEQ ID NO: 1,
the amino acid sequence of the heavy-chain CDR2 is the
amino acid sequence represented by SEQ ID NO: 2,
the amino acid sequence of the heavy-chain CDR3 is the
amino acid sequence represented by SEQ ID NO: 3,
the amino acid sequence of the light-chain CDR1 is the
amino acid sequence represented by SEQ ID NO: 4,
the amino acid sequence of the light-chain CDR2 is the
amino acid sequence represented by SEQ ID NO: 5,
the amino acid sequence of the light-chain CDR3 is the
amino acid sequence represented by SEQ ID NO: 6,
the amino acid sequence of the heavy-chain FR1 is the
amino acid sequence represented by SEQ ID NO: 7,
the amino acid sequence of the heavy-chain FR2 is the
amino acid sequence represented by SEQ ID NO: 15,
the amino acid sequence of the heavy-chain FR3 is the
amino acid sequence represented by SEQ ID NO: 16,
the amino acid sequence of the heavy-chain FR4 is the
amino acid sequence represented by SEQ ID NO: 10,
the amino acid sequence of the light-chain FRI is the
amino acid sequence represented by SEQ ID NO: 11,
the amino acid sequence of the light-chain FR2 is the

amino acid sequence represented by SEQ ID NO: 12,
the amino acid sequence of the light-chain FR3 is the
amino acid sequence represented by SEQ ID NO: 13, and
the amino acid sequence of the light-chain FR4 is the
amino acid sequence represented by SEQ ID NO: 14,
in the manufacture of a medicament for inhibiting the
binding between podoplanin and CLEC-2 in a human subject,
wherein said anti-podoplanin antibody and antigen-
binding region thereof specifically bind to the PLAG4 domain
of human podoplanin.

6. A pharmaceutical composition, comprising ~~the, an~~ an
effective amount for specifically binding to podoplanin of
an isolated humanized anti-podoplanin antibody or an
antibody fragment thereof, containing ~~the-an~~ an antigen-binding
region ~~according to any one of claims 1 to 4.~~ which comprises
a variable region comprising the amino acid sequence I or II
below,

the amino acid sequences of the heavy-chain FRs 1 to 4
and the light-chain FRs 1 to 4 in the following amino acid
sequence I optionally have a sequence identity of not less
than 90% to the amino acid sequences represented by SEQ ID
NOs: 7 to 14 below, respectively, and

the amino acid sequences of the heavy-chain FRs 1 to 4
and the light-chain FRs 1 to 4 in the following amino acid
sequence II optionally have a sequence identity of not less
than 90% to the amino acid sequences represented by SEQ ID
NOs: 7, 15, 16, 10 to 14 below, respectively;

wherein in the amino acid sequence I,
the amino acid sequence of the heavy-chain CDR1 is the
amino acid sequence represented by SEQ ID NO: 1,
the amino acid sequence of the heavy-chain CDR2 is the
amino acid sequence represented by SEQ ID NO: 2,

the amino acid sequence of the heavy-chain CDR3 is the amino acid sequence represented by SEQ ID NO: 3,
the amino acid sequence of the light-chain CDR1 is the amino acid sequence represented by SEQ ID NO: 4,
the amino acid sequence of the light-chain CDR2 is the amino acid sequence represented by SEQ ID NO: 5,
the amino acid sequence of the light-chain CDR3 is the amino acid sequence represented by SEQ ID NO: 6,
the amino acid sequence of the heavy-chain FR1 is the amino acid sequence represented by SEQ ID NO: 7,
the amino acid sequence of the heavy-chain FR2 is the amino acid sequence represented by SEQ ID NO: 8,
the amino acid sequence of the heavy-chain FR3 is the amino acid sequence represented by SEQ ID NO: 9,
the amino acid sequence of the heavy-chain FR4 is the amino acid sequence represented by SEQ ID NO: 10,
the amino acid sequence of the light-chain FRI is the amino acid sequence represented by SEQ ID NO: 11,
the amino acid sequence of the light-chain FR2 is the amino acid sequence represented by SEQ ID NO: 12,
the amino acid sequence of the light-chain FR3 is the amino acid sequence represented by SEQ ID NO: 13, and
the amino acid sequence of the light-chain FR4 is the amino acid sequence represented by SEQ ID NO: 14; and
wherein, in the amino acid sequence II,
the amino acid sequence of the heavy-chain CDR1 is the amino acid sequence represented by SEQ ID NO: 1,
the amino acid sequence of the heavy-chain CDR2 is the amino acid sequence represented by SEQ ID NO: 2,
the amino acid sequence of the heavy-chain CDR3 is the amino acid sequence represented by SEQ ID NO: 3,
the amino acid sequence of the light-chain CDR1 is the amino acid sequence represented by SEQ ID NO: 4,

the amino acid sequence of the light-chain CDR2 is the amino acid sequence represented by SEQ ID NO: 5,
the amino acid sequence of the light-chain CDR3 is the amino acid sequence represented by SEQ ID NO: 6,
the amino acid sequence of the heavy-chain FR1 is the amino acid sequence represented by SEQ ID NO: 7,
the amino acid sequence of the heavy-chain FR2 is the amino acid sequence represented by SEQ ID NO: 15,
the amino acid sequence of the heavy-chain FR3 is the amino acid sequence represented by SEQ ID NO: 16,
the amino acid sequence of the heavy-chain FR4 is the amino acid sequence represented by SEQ ID NO: 10,
the amino acid sequence of the light-chain FRI is the amino acid sequence represented by SEQ ID NO: 11,
the amino acid sequence of the light-chain FR2 is the amino acid sequence represented by SEQ ID NO: 12,
the amino acid sequence of the light-chain FR3 is the amino acid sequence represented by SEQ ID NO: 13, and
the amino acid sequence of the light-chain FR4 is the amino acid sequence represented by SEQ ID NO: 14;
and a pharmaceutically acceptable carrier,
wherein said anti-podoplanin antibody and antigen-binding region thereof specifically bind to the PLAG4 domain of human podoplanin.

7. The pharmaceutical composition according to claim 6, wherein ~~the pharmaceutical composition is for reducing the proliferation or metastasis of a cancer cell~~said isolated humanized anti-podoplanin antibody, or an antibody fragment thereof is present in a therapeutically effective amount effective to inhibit the binding between podoplanin and CLEC-
2.

8. The pharmaceutical composition according to claim ~~6, wherein the pharmaceutical composition is for inhibiting~~ 7, wherein said isolated humanized anti-podoplanin antibody, or an antibody fragment thereof is present in a therapeutically effective amount effective to inhibit platelet aggregation, ~~thrombosis, or inflammation.~~

~~9. The pharmaceutical composition according to claim~~ ~~7, wherein the cancer cell is a podoplanin-positive tumor cell.~~ 9. Use of an isolated mouse-human chimeric anti-podoplanin antibody, or an antibody fragment thereof, comprising an antigen-binding region, which comprises variable regions comprising the following amino acid sequences,

the amino acid sequences of the heavy-chain FRs 1 to 4 and the light-chain FRs 1 to 4 optionally have a sequence identity of not less than 90% to the amino acid sequences represented by SEQ ID NO: 17 to 24 below, respectively,

wherein

the amino acid sequence of the heavy-chain CDR1 is the amino acid sequence represented by SEQ ID NO: 1,

the amino acid sequence of the heavy-chain CDR2 is the amino acid sequence represented by SEQ ID NO: 2,

the amino acid sequence of the heavy-chain CDR3 is the amino acid sequence represented by SEQ ID NO: 3,

the amino acid sequence of the light-chain CDR1 is the amino acid sequence represented by SEQ ID NO: 4,

the amino acid sequence of the light-chain CDR2 is the amino acid sequence represented by SEQ ID NO: 5,

the amino acid sequence of the light-chain CDR3 is the amino acid sequence represented by SEQ ID NO: 6,

the amino acid sequence of the heavy-chain FR1 is the amino acid sequence represented by SEQ ID NO: 17,

the amino acid sequence of the heavy-chain FR2 is the amino acid sequence represented by SEQ ID NO: 18,

the amino acid sequence of the heavy-chain FR3 is the amino acid sequence represented by SEQ ID NO: 19,

the amino acid sequence of the heavy-chain FR4 is the amino acid sequence represented by SEQ ID NO: 20,

the amino acid sequence of the light-chain FRI is the amino acid sequence represented by SEQ ID NO: 21,

the amino acid sequence of the light-chain FR2 is the amino acid sequence represented by SEQ ID NO: 22,

the amino acid sequence of the light-chain FR3 is the amino acid sequence represented by SEQ ID NO: 23, and

the amino acid sequence of the light-chain FR4 is the amino acid sequence represented by SEQ ID NO: 24,

in the manufacture of a medicament for inhibiting the binding between podoplanin and CLEC-2 in a human subject,

wherein said anti-podoplanin antibody and antigen-binding region thereof specifically bind to the PLAG4 domain of human podoplanin.

~~10. A DNA segment(s) encoding the protein moiety(ies) of the anti-podoplanin antibody or antibody fragment containing the antigen-binding region thereof according to any one of claims 1 to 4.~~10. A pharmaceutical composition, comprising:

an effective amount for specifically binding to podoplanin of an isolated mouse-human chimeric anti-podoplanin antibody, or an antibody fragment thereof, comprising an antigen-binding region, which comprises variable regions comprising the following amino acid sequences,

the amino acid sequences of the heavy-chain FRs 1 to 4 and the light-chain FRs 1 to 4 optionally have a sequence

identity of not less than 90% to the amino acid sequences
represented by SEQ ID NO: 17 to 24 below, respectively,
wherein
the amino acid sequence of the heavy-chain CDR1 is the
amino acid sequence represented by SEQ ID NO: 1,
the amino acid sequence of the heavy-chain CDR2 is the
amino acid sequence represented by SEQ ID NO: 2,
the amino acid sequence of the heavy-chain CDR3 is the
amino acid sequence represented by SEQ ID NO: 3,
the amino acid sequence of the light-chain CDR1 is the
amino acid sequence represented by SEQ ID NO: 4,
the amino acid sequence of the light-chain CDR2 is the
amino acid sequence represented by SEQ ID NO: 5,
the amino acid sequence of the light-chain CDR3 is the
amino acid sequence represented by SEQ ID NO: 6,
the amino acid sequence of the heavy-chain FR1 is the
amino acid sequence represented by SEQ ID NO: 17,
the amino acid sequence of the heavy-chain FR2 is the
amino acid sequence represented by SEQ ID NO: 18,
the amino acid sequence of the heavy-chain FR3 is the
amino acid sequence represented by SEQ ID NO: 19,
the amino acid sequence of the heavy-chain FR4 is the
amino acid sequence represented by SEQ ID NO: 20,
the amino acid sequence of the light-chain FR1 is the
amino acid sequence represented by SEQ ID NO: 21,
the amino acid sequence of the light-chain FR2 is the
amino acid sequence represented by SEQ ID NO: 22,
the amino acid sequence of the light-chain FR3 is the
amino acid sequence represented by SEQ ID NO: 23, and
the amino acid sequence of the light-chain FR4 is the
amino acid sequence represented by SEQ ID NO: 24,
and a pharmaceutically acceptable carrier,
wherein said anti-podoplanin antibody and antigen-

binding region thereof specifically bind to the PLAG4 domain of human podoplanin.

~~11. A recombinant vector comprising the DNA segment(s) according to claim 10.11. The pharmaceutical composition according to claim 10, wherein said isolated mouse-human chimeric anti-podoplanin antibody, or an antibody fragment thereof is present in a therapeutically effective amount effective to inhibit the binding between podoplanin and CLEC-2.~~

~~12. A host cell comprising the DNA segment(s) according to claim 10 or the recombinant vector according to claim 11.12. The pharmaceutical composition according to claim 11, wherein said isolated mouse-human chimeric anti-podoplanin antibody, or an antibody fragment thereof is present in a therapeutically effective amount effective to inhibit platelet aggregation.~~

13. An isolated humanized anti-podoplanin antibody, or an antibody fragment thereof, of claim 1, or an isolated mouse-human chimeric anti-podoplanin antibody, or an antibody fragment thereof, of claim 3, for use in therapy.