

CLAIMS

1. Use of an anti-ALK2 antibody or an antigen-binding fragment thereof comprising a property of binding to the ALK2, a property of cross-linking the ALK2, and a property of inhibiting BMP signal transduction in the manufacture of a medicament for treating and/or preventing a disease in a patient wherein the disease is ectopic ossification, ~~wherein:~~
~~the patient has an active mutation in Activin like kinase 2 (ALK2) protein which is responsible for ectopic ossification; and~~

~~an amino acid residue at position 330 of the ALK2 is proline,—~~

wherein prior to administration of the medicament to a patient the following steps to select the patient, have been performed:

(a) detecting the presence or absence in patients of an active mutation in ALK2 protein which is responsible for ectopic ossification and is at least one selected from L196P, delP197_F198insL, R202I, R206H, Q207E, R258S, R258G, G325A, G328E, G328R, G328W, G356D, and R375P, preferably R206H.

(b) selecting a patient having the active mutation in ALK2; and

(c) confirming that the patient to be administered the medicament has proline at position 330 of ALK2.

~~2. The use according to claim 1, wherein the ALK2 has no G328V mutation.~~

~~3. The use according to claim 1, wherein prior to administration of the medicament to a patient the following steps to select the patient, have been performed:~~

~~(a) detecting the presence or absence of an active mutation in ALK2 in patients;—~~

~~(b) selecting a patient having the active mutation in ALK2; and~~

~~(c) confirming that the patient to be administered the medicament has no mutation of an amino acid residue at position 330 of ALK2.—~~

~~42. The use according to claim 31, wherein the step (c) further comprises the step of confirming that the ALK2 of the patient has no G328V mutation.~~

~~53. The use according to any one of claims 1 to 4 claim 1 or 2, wherein the anti-ALK2 antibody or the antigen-binding fragment thereof specifically binds to a polypeptide consisting of amino acid residues from position 21 to position 123 in the amino acid sequence of SEQ ID NO: 1.~~

64. The use according to any one of claims 1 to 53, wherein the anti-ALK2 antibody or the antigen-binding fragment thereof binds to:

(i) an epitope comprising each residue of glutamic acid at position 38, glycine at position 39, isoleucine at position 59, asparagine at position 60, aspartic acid at position 61, glycine at position 62, phenylalanine at position 63, histidine at position 64, valine at position 65, tyrosine at position 66, asparagine at position 102, threonine at position 104, glutamine at position 106, and leucine at position 107 in the amino acid sequence of SEQ ID NO: 1; or

(ii) an epitope comprising each residue of glutamic acid at position 38, glycine at position 39, leucine at position 40, isoleucine at position 59, asparagine at position 60, aspartic acid at position 61, glycine at position 62, phenylalanine at position 63, histidine at position 64, valine at position 65, tyrosine at position 66, and threonine at position 104 in the amino acid sequence of SEQ ID NO: 1.

75. The use according to any one of claims 1 to 53, wherein the anti-ALK2 antibody or the antigen-binding fragment thereof competes, for binding to ALK2, with the anti-ALK2 antibody or the antigen-binding fragment thereof according to claim 64.

86. The use according to any one of claims 1 to 75, wherein the anti-ALK2 antibody or the antigen-binding fragment thereof is a monoclonal antibody, a polyclonal antibody, a chimeric antibody, a humanized antibody, a human antibody, a diabody, a multispecific antibody, or F(ab')₂.

97. The use according to any one of claims 1 to 86, wherein
(p-1) a heavy chain sequence of the anti-ALK2 antibody or the antigen-binding fragment thereof comprises a variable region having CDRH1, CDRH2, and CDRH3, wherein the CDRH1, the CDRH2, and the CDRH3 consist of the amino acid sequences of:

SEQ ID NO: 5, SEQ ID NO: 6, and SEQ ID NO: 7, respectively; and

a light chain sequence thereof comprises a variable region having CDRL1, CDRL2, and CDRL3, wherein the CDRL1, the CDRL2, and the CDRL3 consist of the amino acid sequences of:

SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10, respectively;

(p-2) a heavy chain sequence of the anti-ALK2 antibody or the antigen-binding fragment thereof comprises a variable region having CDRH1, CDRH2, and CDRH3, wherein the CDRH1, the CDRH2, and the CDRH3 consist of the amino acid sequences of:

SEQ ID NO: 5, SEQ ID NO: 6, and SEQ ID NO: 7, respectively; and

a light chain sequence thereof comprises a variable region having CDRL1, CDRL2, and CDRL3, wherein the CDRL1, the CDRL2, and the CDRL3 consist of the amino acid sequences of:

SEQ ID NO: 8, SEQ ID NO: 17, and SEQ ID NO: 10, respectively;

(p-3) a heavy chain sequence of the anti-ALK2 antibody or the antigen-binding fragment thereof comprises a variable region having CDRH1, CDRH2, and CDRH3, wherein the CDRH1, the CDRH2, and the CDRH3 consist of the amino acid sequences of:

SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13, respectively; and

a light chain sequence thereof comprises a variable region having CDRL1, CDRL2, and CDRL3, wherein the CDRL1, the CDRL2, and the CDRL3 consist of the amino acid sequences of:

SEQ ID NO: 14, SEQ ID NO: 15, and SEQ ID NO: 16, respectively;

(p-4) a heavy chain sequence of the anti-ALK2 antibody or the antigen-binding fragment thereof comprises a variable region having CDRH1, CDRH2, and CDRH3, wherein the CDRH1, the CDRH2, and the CDRH3 consist of the amino acid sequences of:

SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20, respectively; and

a light chain sequence thereof comprises a variable region having CDRL1, CDRL2, and CDRL3, wherein the CDRL1, the CDRL2, and the CDRL3 consist of the amino acid sequences of:

SEQ ID NO: 21, SEQ ID NO: 22, and SEQ ID NO: 23, respectively; or

(p-5) a heavy chain sequence of the anti-ALK2 antibody or the antigen-binding fragment thereof comprises a variable region having CDRH1, CDRH2, and CDRH3, wherein the CDRH1, the CDRH2, and the CDRH3 consist of the amino acid sequences of:

SEQ ID NO: 24, SEQ ID NO: 25, and SEQ ID NO: 26, respectively, and

a light chain sequence thereof comprises a variable region having CDRL1, CDRL2, and CDRL3, wherein the CDRL1, the CDRL2, and the CDRL3 consist of the amino acid sequences of:

SEQ ID NO: 27, SEQ ID NO: 28, and SEQ ID NO: 29, respectively.

~~a heavy chain sequence of the anti-ALK2 antibody or the antigen-binding fragment thereof comprises a variable region having CDRH1, CDRH2, and CDRH3, wherein the CDRH1, the CDRH2, and the CDRH3 consist of the amino acid sequences of:~~

~~SEQ ID NO: 5, SEQ ID NO: 6, and SEQ ID NO: 7, respectively;~~

~~SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13, respectively;~~

~~SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20, respectively; or~~

~~SEQ ID NO: 24, SEQ ID NO: 25, and SEQ ID NO: 26, respectively, and~~

~~a light chain sequence thereof comprises a variable region having CDRL1, CDRL2, and CDRL3, wherein the CDRL1, the CDRL2, and the CDRL3 consist of the amino acid sequences of:~~

~~SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10, respectively;
SEQ ID NO: 8, SEQ ID NO: 17, and SEQ ID NO: 10, respectively;
SEQ ID NO: 14, SEQ ID NO: 15, and SEQ ID NO: 16, respectively;
SEQ ID NO: 21, SEQ ID NO: 22, and SEQ ID NO: 23, respectively; or
SEQ ID NO: 27, SEQ ID NO: 28, and SEQ ID NO: 29, respectively.~~

408. The use according to any one of claims 1 to 97, wherein

(p-6) the heavy chain variable region sequence of the anti-ALK2 antibody or the antigen-binding fragment thereof is:

a1) an amino acid sequence consisting of amino acid residues from position 20 to position 142 of the amino acid sequence of SEQ ID NO: 31; and the light chain variable region sequence is:

b1) an amino acid sequence consisting of amino acid residues from position 21 to position 133 of the amino acid sequence of SEQ ID NO: 32;

(p-7) the heavy chain variable region sequence of the anti-ALK2 antibody or the antigen-binding fragment thereof is:

a2) an amino acid sequence consisting of amino acid residues from position 20 to position 142 of the amino acid sequence of SEQ ID NO: 33; and the light chain variable region sequence is:

b1) an amino acid sequence consisting of amino acid residues from position 21 to position 133 of the amino acid sequence of SEQ ID NO: 32;

(p-8) the heavy chain variable region sequence of the anti-ALK2 antibody or the antigen-binding fragment thereof is:

a3) an amino acid sequence consisting of amino acid residues from position 20 to position 140 of the amino acid sequence of SEQ ID NO: 34; and the light chain variable region sequence is:

b2) an amino acid sequence consisting of amino acid residues from position 21 to position 129 of the amino acid sequence of SEQ ID NO: 35;

(p-9) the heavy chain variable region sequence of the anti-ALK2 antibody or the antigen-binding fragment thereof is:

a4) an amino acid sequence consisting of amino acid residues from position 20 to position 140 of the amino acid sequence of SEQ ID NO: 36; and the light chain variable region sequence is:

b3) an amino acid sequence consisting of amino acid residues from position 21 to position 129 of the amino acid sequence of SEQ ID NO: 37;

(p-10) the heavy chain variable region sequence of the anti-ALK2 antibody or the antigen-binding fragment thereof is:

a5) an amino acid sequence consisting of amino acid residues from position 20 to position 140 of the amino acid sequence of SEQ ID NO: 38; and the light chain variable region sequence is:

b2) an amino acid sequence consisting of amino acid residues from position 21 to position 129 of the amino acid sequence of SEQ ID NO: 35;

(p-11) the heavy chain variable region sequence of the anti-ALK2 antibody or the antigen-binding fragment thereof is:

a6) an amino acid sequence consisting of amino acid residues from position 20 to position 140 of the amino acid sequence of SEQ ID NO: 39; and the light chain variable region sequence is:

b3) an amino acid sequence consisting of amino acid residues from position 21 to position 129 of the amino acid sequence of SEQ ID NO: 37;

(p-12) the heavy chain variable region sequence of the anti-ALK2 antibody or the antigen-binding fragment thereof is:

a7) an amino acid sequence having at least 95% identity to any one amino acid sequence selected from the amino acid sequences a1) to a6); and the light chain variable region sequence is:

b4) an amino acid sequence having at least 95% identity to any one amino acid sequence selected from the amino acid sequences b1) to b3);

(p-13) the heavy chain variable region sequence of the anti-ALK2 antibody or the antigen-binding fragment thereof is:

a8) an amino acid sequence having at least 99% identity to any one amino acid sequence selected from the amino acid sequences a1) to a6); and the light chain variable region sequence is:

b5) an amino acid sequence having at least 99% identity to any one amino acid sequence selected from the amino acid sequences b1) to b3); or

(p-14) the heavy chain variable region sequence of the anti-ALK2 antibody or the antigen-binding fragment thereof is:

a9) an amino acid sequence comprising a substitution(s), a deletion(s), or an addition(s) of one or several amino acid residues in any one amino acid sequence selected from the amino acid sequences a1) to a6); and the light chain variable region sequence is:

b6) an amino acid sequence comprising a substitution(s), a deletion(s), or an addition(s) of one or several amino acid residues in any one amino acid sequence selected from the amino acid sequences b1) to b3).

~~a1) an amino acid sequence consisting of amino acid residues from position 20 to position 142 of the amino acid sequence of SEQ ID NO: 31;~~

~~a2) an amino acid sequence consisting of amino acid residues from position 20 to position 142 of the amino acid sequence of SEQ ID NO: 33;~~

~~a3) an amino acid sequence consisting of amino acid residues from position 20 to position 140 of the amino acid sequence of SEQ ID NO: 34;~~

~~a4) an amino acid sequence consisting of amino acid residues from position 20 to position 140 of the amino acid sequence of SEQ ID NO: 36;~~

~~a5) an amino acid sequence consisting of amino acid residues from position 20 to position 140 of the amino acid sequence of SEQ ID NO: 38;~~

~~a6) an amino acid sequence consisting of amino acid residues from position 20 to position 140 of the amino acid sequence of SEQ ID NO: 39;~~

~~a7) an amino acid sequence having at least 95% identity to any one amino acid sequence selected from the amino acid sequences a1) to a6);~~

~~a8) an amino acid sequence having at least 99% identity to any one amino acid sequence selected from the amino acid sequences a1) to a6); or~~

~~a9) an amino acid sequence comprising a substitution(s), a deletion(s), or an addition(s) of one or several amino acid residues in any one amino acid sequence selected from the amino acid sequences a1) to a6), and~~

~~the light chain variable region sequence is~~

~~b1) an amino acid sequence consisting of amino acid residues from position 21 to position 133 of the amino acid sequence of SEQ ID NO: 32;~~

~~b2) an amino acid sequence consisting of amino acid residues from position 21 to position 129 of the amino acid sequence of SEQ ID NO: 35;~~

~~b3) an amino acid sequence consisting of amino acid residues from position 21 to position 129 of the amino acid sequence of SEQ ID NO: 37;~~

~~b4) an amino acid sequence having at least 95% identity to any one amino acid sequence selected from the amino acid sequences b1) to b3);~~

~~b5) an amino acid sequence having at least 99% identity to any one amino acid sequence selected from the amino acid sequences b1) to b3); or~~

~~b6) an amino acid sequence comprising a substitution(s), a deletion(s), or an addition(s) of one or several amino acid residues in any one amino acid sequence selected from the amino acid sequences b1) to b3).~~

~~149.~~ The use according to claim ~~108~~, wherein the anti-ALK2 antibody is:

an antibody consisting of a heavy chain comprising a heavy chain variable region consisting of amino acid residues from position 20 to position 142 of the amino acid sequence of SEQ ID NO: 31 and a light chain comprising a light chain variable region consisting of amino acid residues from position 21 to position 133 of the amino acid sequence of SEQ ID NO: 32;

an antibody consisting of a heavy chain comprising a heavy chain variable region consisting of amino acid residues from position 20 to position 142 of the amino acid sequence of SEQ ID NO: 33 and a light chain comprising a light chain variable region consisting of amino acid residues from position 21 to position 133 of the amino acid sequence of SEQ ID NO: 32;

an antibody consisting of a heavy chain comprising a heavy chain variable region consisting of amino acid residues from position 20 to position 140 of the amino acid sequence of SEQ ID NO: 34 and a light chain comprising a light chain variable region consisting of amino acid residues from position 21 to position 129 of the amino acid sequence of SEQ ID NO: 35;

an antibody consisting of a heavy chain comprising a heavy chain variable region consisting of amino acid residues from position 20 to position 140 of the amino acid sequence of SEQ ID NO: 36 and a light chain comprising a light chain variable region consisting of amino acid residues from position 21 to position 129 of the amino acid sequence of SEQ ID NO: 37;

an antibody consisting of a heavy chain comprising a heavy chain variable region consisting of amino acid residues from position 20 to position 140 of the amino acid sequence of SEQ ID NO: 38 and a light chain comprising a light chain variable region consisting of amino acid residues from position 21 to position 129 of the amino acid sequence of SEQ ID NO: 35; or

an antibody consisting of a heavy chain comprising a heavy chain variable region consisting of amino acid residues from position 20 to position 140 of the amino acid sequence of SEQ ID NO: 39 and a light chain comprising a light chain variable region consisting of amino acid residues from position 21 to position 129 of the amino acid sequence of SEQ ID NO: 37.

~~12. The use according to any one of claims 1 to 11, wherein the active mutation in ALK2 is at least one selected from L196P, delP197_F198insL, R202I, R206H, Q207E, R258S, R258G, G325A, G328E, G328R, G328W, G356D, and R375P.~~

~~13. The use according to any one of claims 1 to 11 wherein the active mutation in ALK2 is R206H mutation.~~

~~1410.~~ The use according to any one of claims 1 to ~~139~~, wherein the ectopic ossification is fibrodysplasia ossificans progressiva (FOP).

~~1511.~~ Use of an anti-ALK2 antibody or an antigen-binding fragment thereof comprising a property of binding to the ALK2, a property of cross-linking the ALK2, and a property of inhibiting BMP signal transduction in the manufacture of a medicament for treating and/or preventing a patient having brain tumor, ~~wherein the patient has an active mutation in Activin like kinase 2 (ALK2) protein which is responsible for brain tumor.~~

wherein prior to administration of the medicament to a patient the following steps to select the patient, have been performed:

(a) detecting the presence or absence in patients of an active mutation in ALK2 protein which is responsible for brain tumor and is at least one selected from R206H, R258G, G328E, G328W, and G356D;

(b) selecting a patient having the active mutation in ALK2; and

(c) confirming that the patient to be administered the medicament has proline at position 330 of ALK2.

~~16.~~ The use according to claim 15, ~~wherein an amino acid residue at position 330 of ALK2 in the patient is proline.~~

~~17.~~ The use according to claim 15 or 16, ~~wherein the active mutation in ALK2 is at least one selected from R206H, R258G, G328E, G328W, and G356D.~~

~~1812.~~ The use according to ~~any one of claims 15 to 17~~claim 11, wherein the anti-ALK2 antibody or the antigen-binding fragment thereof is an anti-ALK2 antibody or an antigen-binding fragment thereof defined in any one of claims ~~5 to 113~~ to 9.

~~1913.~~ The use according to ~~any one of claims 15 to 18~~claim 11 or 12, wherein the brain tumor is diffuse intrinsic pontine glioma (DIPG).

~~2014.~~ A method for predicting a risk of developing an adverse reaction ascribable to administration of an anti-ALK2 antibody or an antigen-binding fragment thereof, comprising the following steps of:

(a) detecting the presence or absence of an active mutation in ALK2 and a mutation of an amino acid residue at position 330 of ALK2 of a patient; and

(b) determining that when the patient has the active mutation in ALK2 and has no mutation of an amino acid residue at position 330 of ALK2, the patient has a low risk of

developing an adverse reaction ascribable to the administration of an anti-ALK2 antibody or an antigen-binding fragment thereof.

2415. A method for predicting responsiveness to treatment and/or prevention by administration of an anti-ALK2 antibody or an antigen-binding fragment thereof, comprising the following steps of:

(a) detecting the presence or absence of an active mutation in ALK2 and a mutation of an amino acid residue at position 330 of ALK2 of a patient; and

(b) determining that when the patient has the active mutation in ALK2 and has no mutation of an amino acid residue at position 330 of ALK2, the patient has responsiveness to treatment and/or prevention by the administration of an anti-ALK2 antibody or an antigen-binding fragment thereof.

~~2216.~~ A method for selecting a patient to be treated and/or ~~to be prevented~~ a patient requiring circumvention or suppression of onset of a disease by administration of an anti-ALK2 antibody or an antigen-binding fragment thereof, comprising the following steps of:

(a) detecting the presence or absence of an active mutation in ALK2 and a mutation of an amino acid residue at position 330 of ALK2 of a patient; and

(b) selecting the patient as the patient to be treated and/or prevented by the administration of an anti-ALK2 antibody or an antigen-binding fragment thereof when the patient has the active mutation in ALK2 and has no mutation of an amino acid residue at position 330 of ALK2.

~~2317.~~ The use according to claim ~~31~~, wherein when the medicament is to be administered, when steps (a) to (c) have been carried out, one of the following steps is carried out;

a) determining that the patient has a low risk of developing an adverse reaction ascribable to the administration of the medicament; or

b) determining that the patient has responsiveness to the medicament.

~~24~~18. The method according to any one of claims ~~20 to 22~~14 to 16, or the use according claim 17, wherein the administration of the anti-ALK2 antibody or the antigen-binding fragment thereof is ~~according to any one of claims 1 to 19~~ defined in any one of claims 1 to 13 is in the form of a pharmaceutical composition.

~~25~~19. The method according to any one of claims ~~20 to 22 and 24~~14 to 16 and 18, or the use according to claim ~~23~~17, wherein the step (b) in claims ~~20 to 22 and 24~~14 to 16 and 18 and step a) and step b) of claim ~~23~~17 further comprises confirming that the active mutation in ALK2 is not G328V mutation.

~~26~~20. The method according to any one of claims ~~20 to 22, 24 and 25~~14 to 16, 18 and 19, or the use according to claim ~~23~~17, wherein the active mutation in ALK2 is at least one selected from L196P, delP197_F198insL, R202I, R206H, Q207E, R258S, R258G, G325A, G328E, G328R, G328W, G356D, and R375P.

~~27~~21. The method according to any one of claims ~~20 to 22, 24 and 25~~14 to 16, 18 and 19, or the use according to claim ~~23~~17, wherein the active mutation in ALK2 is at least one selected from R206H, R258G, G328E, G328W, and G356D.

~~28~~22. The method according to any one of claims ~~24 to 27~~18 to 21, wherein the patient has ectopic ossification or a brain tumor.

~~29~~23. The method according to claim ~~28~~22, wherein the patient has fibrodysplasia ossificans progressiva (FOP) or diffuse intrinsic pontine glioma (DIPG).

~~30~~24. A pharmaceutical composition for use as a medicament in a patient with ectopic ossification, wherein the compodesition comprises, as an active ingredient, an anti-ALK2 antibody or an antigen-binding fragment thereof as defined in any one of claims ~~1 to 19 and 23, 1 to 13 and 17~~, comprising a property of binding to the ALK2, a property of cross-linking the ALK2, and a property of inhibiting BMP signal transduction, and the patient has an active mutation in

ALK2 protein which is responsible for ectopic ossification and, in the ALK2 protein, an amino acid residue at position 330 is proline.