

Claims

1. A method for screening an antibody or an antigen-binding fragment thereof, which binds to NRP1 and is
5 internalized into cells of the tumor spheroids, the method comprising the steps of:

(i) treating patient-derived tumor spheroids, which express NRP1, with a library comprising antibodies or antigen-binding fragments thereof, and then firstly screening
10 antibodies or antigen-binding fragments thereof which bind to NRP1 and are internalized into cells of the tumor spheroids by performing phage display;

(ii) treating patient-derived tumor spheroids, which do not express the NRP1, with the first screened antibodies or
15 antigen-binding fragments thereof which bind to NRP1 and are internalized into cells of the tumor spheroids, to perform negative selection on antibodies or antigen-binding fragments thereof binding to the patient-derived tumor spheroids which do not express the NRP1;

20 (iii) administering antibodies or antigen-binding fragments thereof, obtained by separating/removing antibodies or antigen-binding fragments thereof that binds to the patient-derived tumor spheroids of step (ii) from the antibodies or antigen-binding fragments thereof screened in
25 step (i), to animal models transplanted with the patient-

derived tumor spheroids which overexpress the NRPl by intra-tumoral injection, and dissociating cancer tissue in animal models into single cells, to secondly screen antibodies or antigen-binding fragments which bind to NRPl; and

5 (iv) separating or removing antibodies or antigen-binding fragments thereof, which bind to an antigen other than the NRPl, from the second screened antibodies or antigen-binding fragments thereof.

10 2. The method of claim 1, wherein the patient-derived tumor spheroids overexpress the NRPl are obtained by performing the following steps:

15 (a) dissociating isolated cancer patient-derived cancer tissue, and collecting a cell fraction from the dissociated tissue; and

 (b) treating the collected cell fraction with protease, followed by filtration, centrifugation and suspension, thereby obtaining single cells.

20 3. The method of claim 1 or claim 2, wherein the animal models transplanted with the patient-derived tumor spheroids overexpressing the NRPl are immunodeficient mice.

25 4. The method of claim 3, wherein the immunodeficient mice are nude mice, NOD (non-obese diabetic) mice, SCID

(Severe combined immunodeficiency) mice, NOD-SCID mice, or
NOG (NOD/SCID Il2rg^{-/-}) mice.

5 5. The method of any one of claims 1 to 4, further
comprising a step of converting the antibody or antigen-
binding fragment thereof screened by the method to an IgG
format.

10 ~~6. An antibody or an antigen-binding fragment thereof,~~
~~which is screened by the method of any one of claims 1 to 5~~
~~and binds specifically to NRP1.~~

15 ~~7. Use of the antibody or antigen binding fragment~~
~~thereof of claim 6 in the manufacture of a medicament for the~~
~~treatment or prevention of a cancer, wherein the cancer over~~
~~expresses NRP1.~~