

FIG. 1 shows the killing rate of the anti-Her2 bispecific antibodies against BT474 Her2+++ tumor cells. The ADCC-enhanced anti-Her2 bispecific antibodies (represented as 23C2 HER2-1 and 23C2 HER2-2 in FIG. 1) had better killing effects on BT474 tumor cells than the combination of trastuzumab and pertuzumab and than the anti-Her2 bispecific antibodies (Expi HER2-1 and Expi HER2-2) expressed by CHO-S cells; wherein the EC₅₀ of the combination of trastuzumab and pertuzumab (1:1) is 8.627 ng/mL, the EC₅₀ of Expi HER2-1 is 38.05 ng/mL, and the EC₅₀ of Expi HER2-2 is 35.17 ng/mL, so that the Expi HER2-2 is superior to Expi HER2-1; the EC₅₀ of the ADCC-enhanced 23C2 HER2-1 is 4.728 ng/mL, and the EC₅₀ of ADCC-enhanced 23C2 HER2-2 is 3.658 ng/mL, so that the ADCC-enhanced 23C2 HER2-2 is superior to 23C2 HER2-1.

Example 9. Killing of Her2 Positive Target Cell NCI-N87 by Anti-Her2 Bispecific Antibodies

The killing effects of the anti-Her2 bispecific antibodies on target cells (NCI-N87 Her2++, source: the Cell Bank of Type Culture Collection Committee of the Chinese Academy of Sciences) were studied using NK cells provided by human PBMCs (peripheral blood mononuclear cells), and the *in vitro* activity of the anti-Her2 bispecific antibodies was assessed by EC₅₀ value.

The specific procedures are as follows: NCI-N87 cells were adjusted to a cell density of 3×10^5 cells/mL using 1640 medium containing 2% FBS (fetal bovine serum) and seeded in a 96-well cell culture plate (eppendorf, Cat. No. 0030730199) at 50 μ L per well. Different concentrations of the anti-Her2 bispecific antibodies or control drugs (8.1 nM, 2.7 nM, 0.9 nM, 0.3 nM, 0.1 nM, 0.03 nM, 0.01 nM, 0.003 nM, 0.001 nM and 0.0004 nM) were prepared using medium for experiment and added to the above 96-well cell culture plate at 50 μ L per well. Human PBMCs were adjusted to a cell density of 1.5×10^6 cells/mL using medium for experiment and added at 100 μ L per well. An administration group (target cell + effector cell + antibody or control drug), a target cell group (NCI-N87 cell), an effector cell group (human PBMC), a target cell + effector cell group, a blank control group (medium) and a lysis solution control group, and a target cell maximum release group (target cell + lysis solution) were set, with the effector-to-target cell ratio being 10:1. 45 min prior to the assay, 20 μ L/well of lysis solution (Promega, Cat. No. G182A) was added to the target cell maximum release group and the lysis solution control group. After 45 min, the cell lysis rates were measured using a CytoTox96® nonradioactive cytotoxicity assay (Promega, G1780). Trastuzumab, T-DM1 (trastuzumab-emtansine conjugate, under trade name Kadcyla®), the trastuzumab and pertuzumab (1:1), and Expi HER2-1 were used as the control drugs.

$$\text{Rate of lysis (\%)} = (\text{OD}_{\text{administration group}} - \text{OD}_{\text{target cell + effector cell group}}) / (\text{OD}_{\text{target cell maximum release group}} - \text{OD}_{\text{target cell group}}) \times 100\%$$

FIG. 2 shows the killing rate of anti-Her2 bispecific antibodies against NCI-N87 tumor cells. The ADCC-enhanced anti-Her2 bispecific antibody 23C2 HER2-2 had a better killing effect on NCI-N87 tumor cells than the combination of trastuzumab and pertuzumab, trastuzumab, T-DM1, and Expi HER2-1; wherein the EC₅₀ of the ADCC-enhanced 23C2 HER2-2 is 0.02447 nM, the EC₅₀ of the combination of trastuzumab and pertuzumab is 0.08267 nM, the EC₅₀ of Expi HER2-1 is 0.1048 nM, the EC₅₀ of T-DM1 is 0.07392 nM, and the EC₅₀ of trastuzumab is 0.07468 nM.

Example 10. Killing of Trastuzumab-Resistant Cell JIMT-1 by Anti-Her2 Bispecific Antibodies

The killing effects of the anti-Her2 bispecific antibodies on target cells (JIMT-1, source: AddexBio, Cat. No. C0006005) were studied using NK cells provided by human PBMCs (peripheral blood mononuclear cells), and the *in vitro* activity of the anti-Her2 bispecific antibodies was assessed by EC₅₀ value.

The specific procedures are as follows: JIMT-1 cells were adjusted to a cell density of 3×10^5 cells/mL using 1640 medium containing 2% FBS (fetal bovine serum) and seeded in a 96-well cell culture plate (eppendorf, Cat. No. 0030730199) at 50 μ L per well. Different concentrations of the anti-Her2 bispecific antibodies or control drugs (8.1 nM, 2.7 nM, 0.9 nM, 0.3 nM, 0.1 nM, 0.03 nM, 0.01 nM, 0.003 nM, 0.001 nM and 0.0004 nM) were prepared using medium for experiment and added to the above 96-well cell culture plate at 50 μ L per well. Human PBMCs were adjusted to a cell density of 1.5×10^6 cells/mL using medium for experiment and added at 100 μ L per well. An administration group (target cell + effector cell + antibody or control drug), a target cell group (~~BT474~~ JIMT-1 cell), an effector cell group (human PBMC), a target cell + effector cell group, a blank control group (medium) and a lysis solution control group, and a target cell maximum release group (target cell + lysis solution) were set, with the effector-to-target cell ratio being 20:1. 45 min prior to the assay, 20 μ L/well of lysis solution (Promega, Cat. No. G182A) was added to the target cell maximum release group and the lysis solution control group. After 45 min, the cell lysis rates were measured using a CytoTox96® nonradioactive cytotoxicity assay (Promega, G1780). Trastuzumab, T-DM1, the combination of trastuzumab and pertuzumab (1:1), and Expi HER2-1 were used as the control drugs.

$$\text{Rate of lysis (\%)} = (\text{OD}_{\text{administration group}} - \text{OD}_{\text{target cell + effector cell group}}) / (\text{OD}_{\text{target cell maximum release group}} - \text{OD}_{\text{target cell group}}) \times 100\%$$