

The results show that, except for no binding of nivolumab, VP101(hG4WT) and VP101(hG1DM) to C1q, other antibodies bound to C1q, and VP101(hG1WT) had a similar affinity to bevacizumab.

Example 8: ADCC Activity Assay of VP101(hG1WT) and VP101(hG1DM) on CHO-K1-PD1 Cells Expressing PD-1 Antigen

In order to detect the ADCC effect of the antibodies VP101(hG1WT) and VP101(hG1DM) at the cellular level, the inventors constructed CHO-K1-PD1 cells expressing PD-1 antigen, and established a system of co-culture of normal human PBMC and target cells for detecting the ADCC activity of the antibodies at the cytological level.

The method for detecting the ADCC activity of VP101(hG1WT) and VP101(hG1DM) on CHO-K1-PD1 cells expressing PD-1 antigen is specified as follows:

In this experiment, a human PD-1 overexpression vector pCDH-CMV-PD1FL-Puro (pCDH-CMV-Puro purchased from Youbio) was firstly constructed, the expression vector was packaged by virus and then infected CHO-K1 cells, and a CHO-K1-PD1 stable cell line of the drug-resistant stable expression membrane PD-1 protein was obtained after dosing and screening with Puromycin (2 µg/mL). Normal human PBMCs were isolated according to the Ficoll peripheral blood mononuclear cell isolation instruction. The isolated PBMCs were resuspended in a 1640 complete medium and stained with trypan blue, cells were counted and the viability of cells was determined. The cells were incubated overnight in a saturated humidity incubator at 37 °C and 5% CO₂. The next day, CHO-K1-PD1 cells and PBMC were collected and centrifuged to remove the supernatant, and then the cell