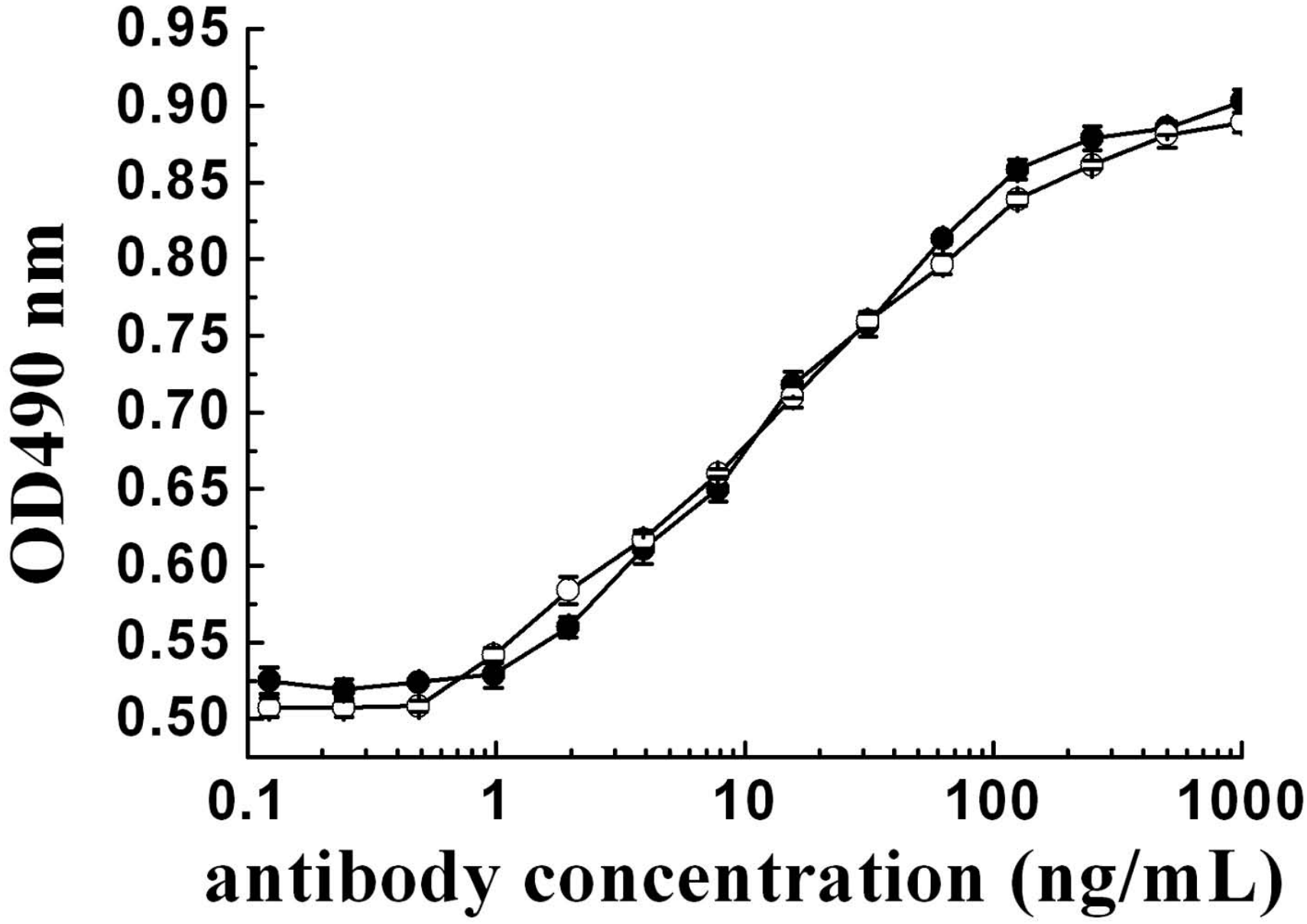

OD490 nm
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.1
1
10
100
1000
antibody concentration (ng/mL)

Supplemental Data

Supplemental Materials and Methods

Cell lines, antibodies, and animals

A431 cells, an EGFR-overexpressing epidermal cancer cell line, were obtained from the American Type Culture Collection (ATCC). Erbitux was purchased from ImClone Systems Incorporated. CMAB009, a recombinant human/mouse chimeric anti-EGFR monoclonal antibody, was produced using CHO cells by National Engineering Research Center of Antibody Medicine. Eight-week-old female nude Balb/c mice were housed in pathogen-free conditions and were treated in accordance with guideline of the Committee on Animals of the Second Military Medical University.

Competitive binding assay

The relative binding affinity of Erbitux and CMAB009 was determined using flow cytometry as described in serum level assay. A431 cells at 1×10^6/mL were incubated with a sub-saturating concentration of the FITC-cetuximab and different concentrations of competing antibodies for 45min at 4°C. The cells were then washed and analyzed by FCM. The EC50 values of competitors were calculated using a four-variable algorithm.

In vitro inhibition of cell growth

The in vitro inhibitory activity of Erbitux and CMAB009 was determined and compared. Breifly, A431 cells (8×10^3 cells/well) were plated in 96-well microtiter plates in complete growth medium. Serial dilutions of Erbitux and CMAB009 were added. Cells were allowed to proliferate for 4 days at 37°C in a humidified 5% CO_2

atmosphere, and then the cell number was determined by means of the MTS/PMS method (Promega). The absorbance was measured at 490 nm, reference 630 nm using an ELISA plate reader.

Antibody-dependent cellular cytotoxicity (ADCC)

Antibody-dependent cellular cytotoxicity (ADCC) activities were measured by lactate dehydrogenase (LDH)-releasing assay using the CytoTox 96 non-Radioactive Cytotoxicity Assay kit (Promega) according to the manufacturer's instructions. Briefly, the cells were incubated for 1 h in phenol red-free DMEM culture medium in a 5% CO2 incubator at 37°C, followed by serial dilutions of Erbitux and CMAB009, then exposed to peripheral blood mononuclear cells (PBMCs) at effector/target ratios (E/T) of 20:1 for 7 h. The cell lysis was determined by measuring the amount of LDH released into the culture supernatant. The optical density (OD) was measured at 490 nm with ELISA plate reader.

Mouse tumor xenograft models

For the animal studies, athymic nude mice (Balb/c; 6-8 week) were housed under clean conditions. Animals were inoculated subcutaneous with 5×10^6 A431 cells. Tumors grew uniformly and could be easily measured by a caliper every other day, and tumor volume was calculated using the following formula: tumor volume (mm^3) = length × $(width)^2$ / 2. One week after inoculation, when tumors reached an average volume of 50 mm^3, the mice were randomly allocated to six treatment groups (n = 10 mice per group). Then the mice were injected i.v. with CMAB009, Erbitux, cis-platinum, cis-DDP/CMAB009, cis-DDP/Erbitux, or the PBS control. Cis-DDP

was given at 6 μg/g body weight once weekly for 2 weeks and antibodies were administered at 1 mg per mouse once weekly for 4 weeks. Control animals received injections of PBS. The mice were observed daily and euthanized at the onset of hind leg paralysis.

Supplemental Table 1. The composition of CMAB009 and Erbitux

CMAB009		Erbitux	
components	Amount per vial (10 mL)	components	Amount per vial (50 mL)
chimeric antibody	100 mg	chimeric antibody	100 mg
Sodium chloride	80 mg	Sodium chloride	424 mg
Sodium dihydrogen phosphate dihydrate	4.1 mg	Sodium dihydrogen phosphate dihydrate	20 mg
Disodium phosphate dihydrate	25.1 mg	Disodium phosphate dihydrate	66 mg
saccharobiose	100 mg		
polysorbate 80	0.7 mg		

Supplemental Figure Legends

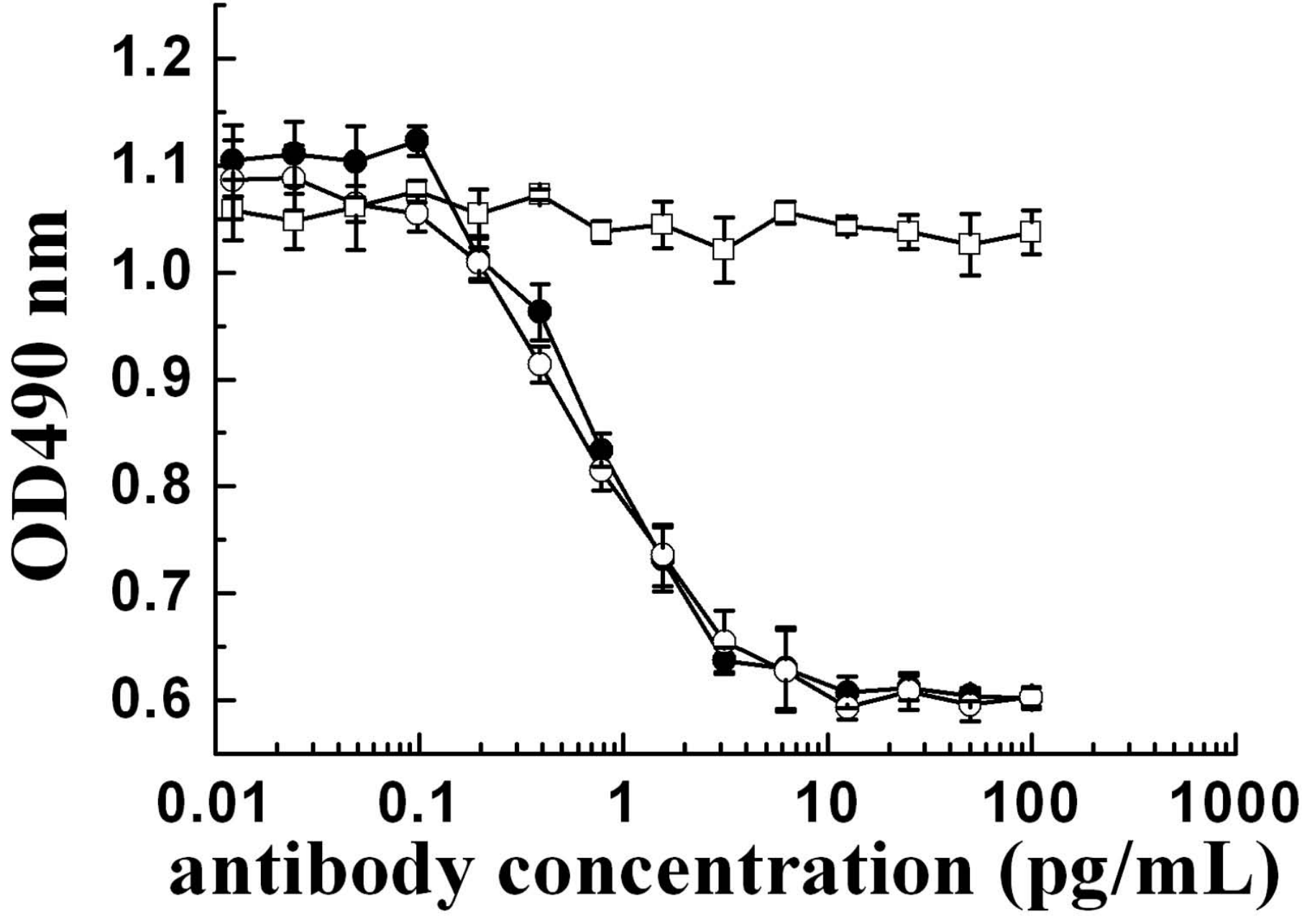
Supplemental Figure 1 CMAB009 induced ADCC as measured by LDH-releasing assay. The A431 cells were preincubated in phenol red-free DMEM culture medium with serial dilutions of the CMAB009 (●) and Erbitux (○). Thereafter human PBMC were added to the wells (E/T = 20:1) .The cell lysis was determined by measuring the amount of LDH released into the culture supernatant. The absorbance was determined with a microplate reader using a 490 nm filter with a 630 nm reference filter. Data from multiple wells in one experiment are presented and expressed as mean ± SD, n = 3.

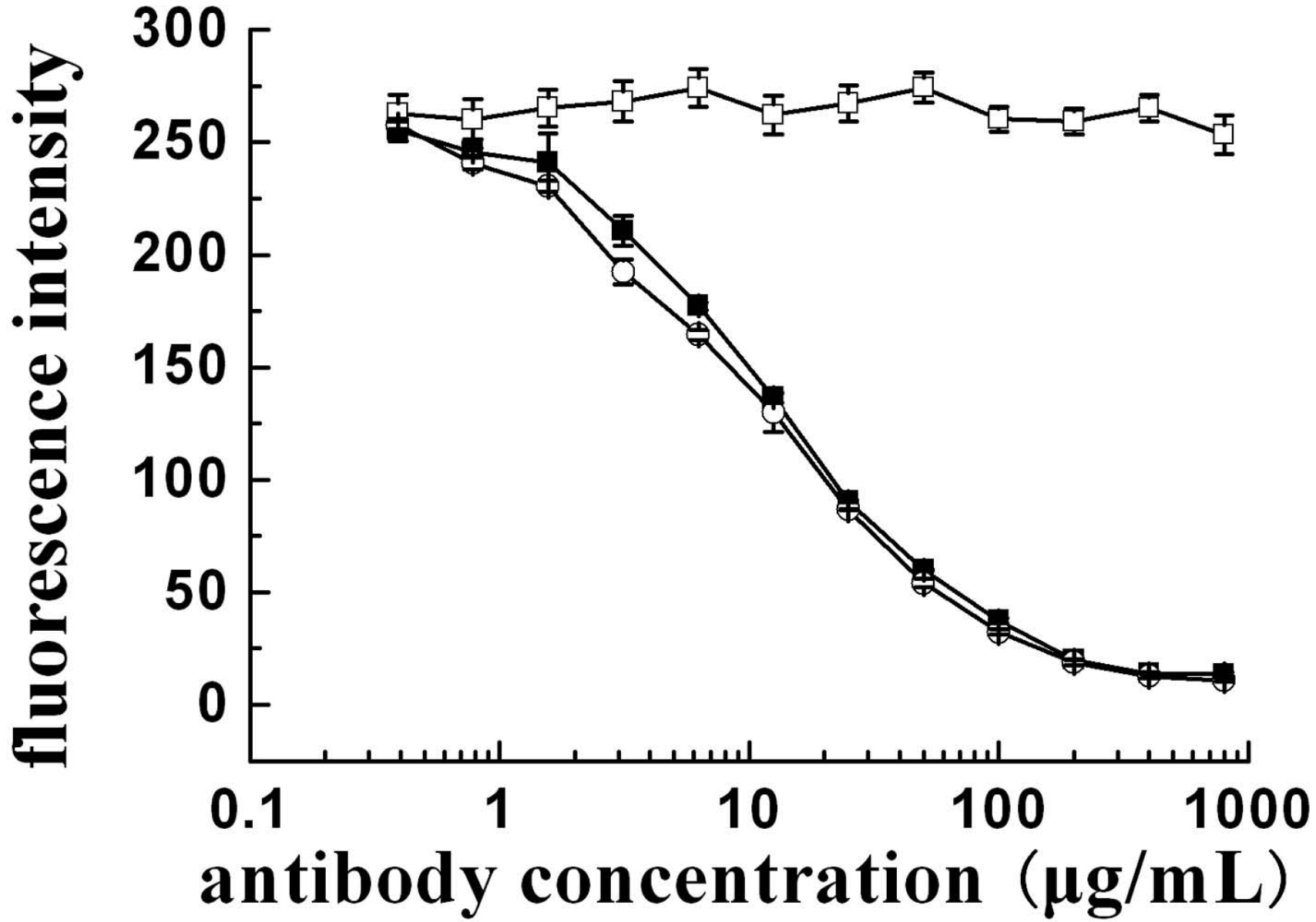
Supplemental Figure 2 Growth inhibition of cultured A431 cells by CMAB009. The A431 cells were incubated with various concentrations of CMAB009 (●), positive control antibody Erbitux (○), and human IgG as negative control (□) for 4 days. Thereafter, the cell number was determined by means of the MTS/PMS method. The absorbance was determined with a microplate reader using a 490 nm filter with a 630 nm reference filter. Data from multiple wells in one experiment are presented and expressed as mean ± SD, n = 3.

Supplemental Figure 3 Antigen-binding activity of CMAB009. The A431 cells were incubated with a subsaturating concentration of FITC-labeled anti-EGFR antibody and serial dilutions concentrations of competing antibodies CMAB009 (●), positive control antibody Erbitux (○), and human IgG as negative control (□) for 45min at 4°C. The cells were then washed and analyzed by FCM. Data from multiple tubes in one experiment are presented and expressed as mean ± SD, n = 3.

Supplemental Figure 4 Efficacy of CMAB009 in tumor xenograft models using BALB/c mice. Groups of 10 mice were inoculated subcutaneous with 5×10^6 A431 cells. 7 days after tumor cell inoculation, the mice then received various treatments. A, treatment with PBS, Erbitux, and CMAB009; B, treatment with PBS, cis-DDP, cis-DDP/Erbitux, and cis-DDP/CMAB009. Data are presented as mean ± SD, n = 10.

Supplemental Figure 5 Concentration-time curve of CMAB009 after a single intravenous infusion of 100 mg/m^2 in individual subjects.


OD490 nm
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.01
0.1
1
10
100
1000
antibody concentration (pg/mL)


fluorescence intensity
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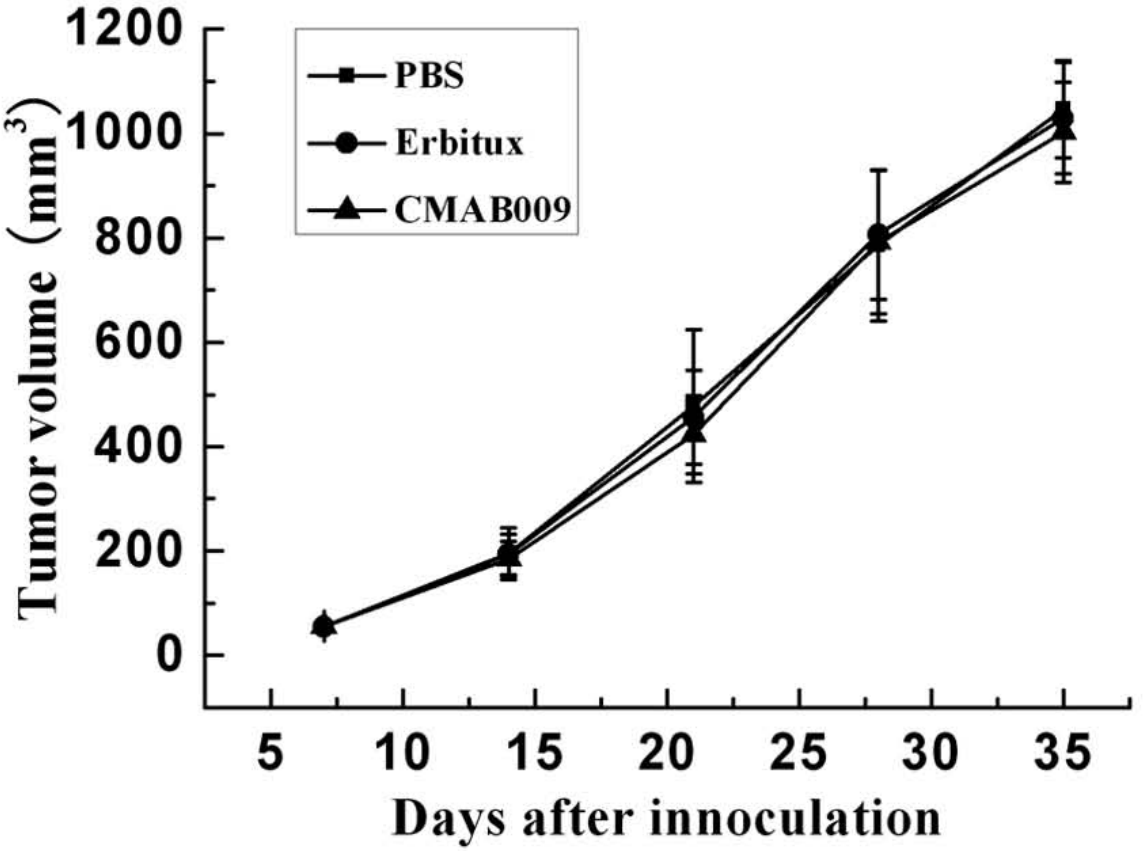
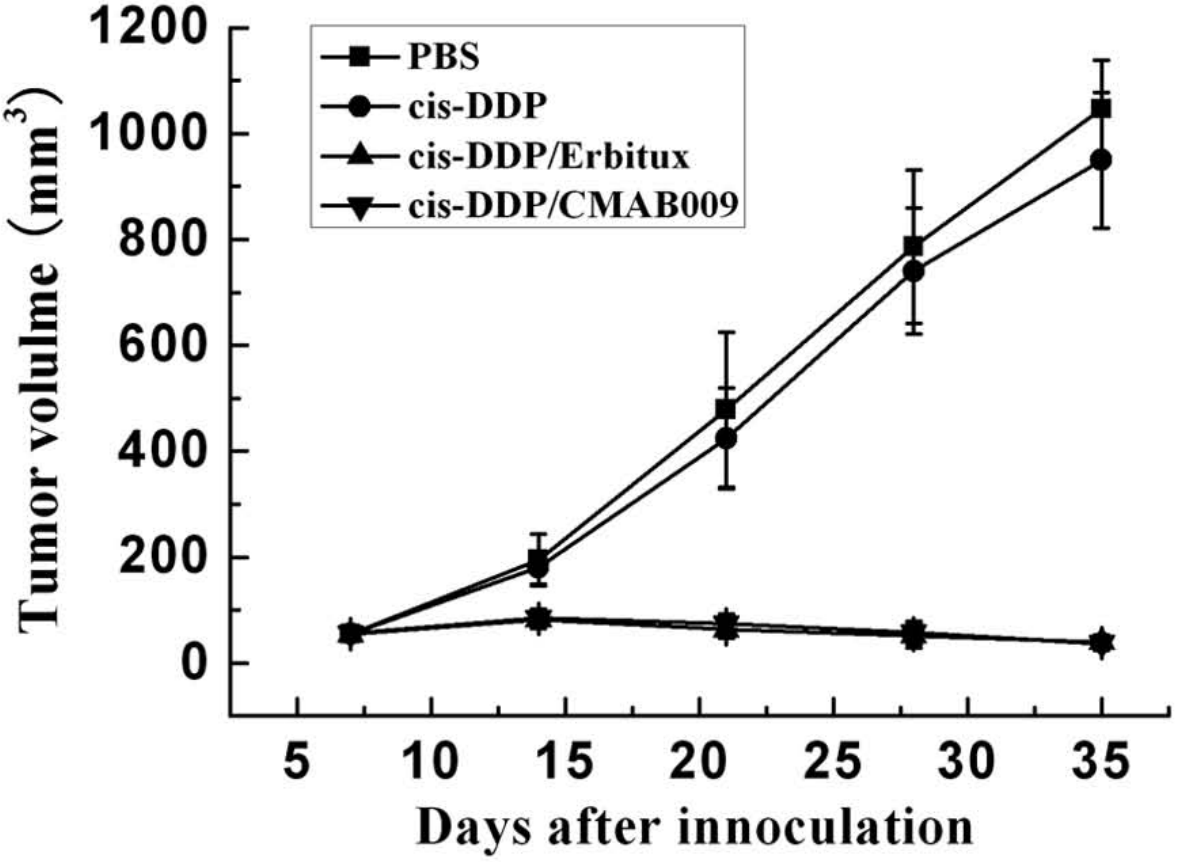
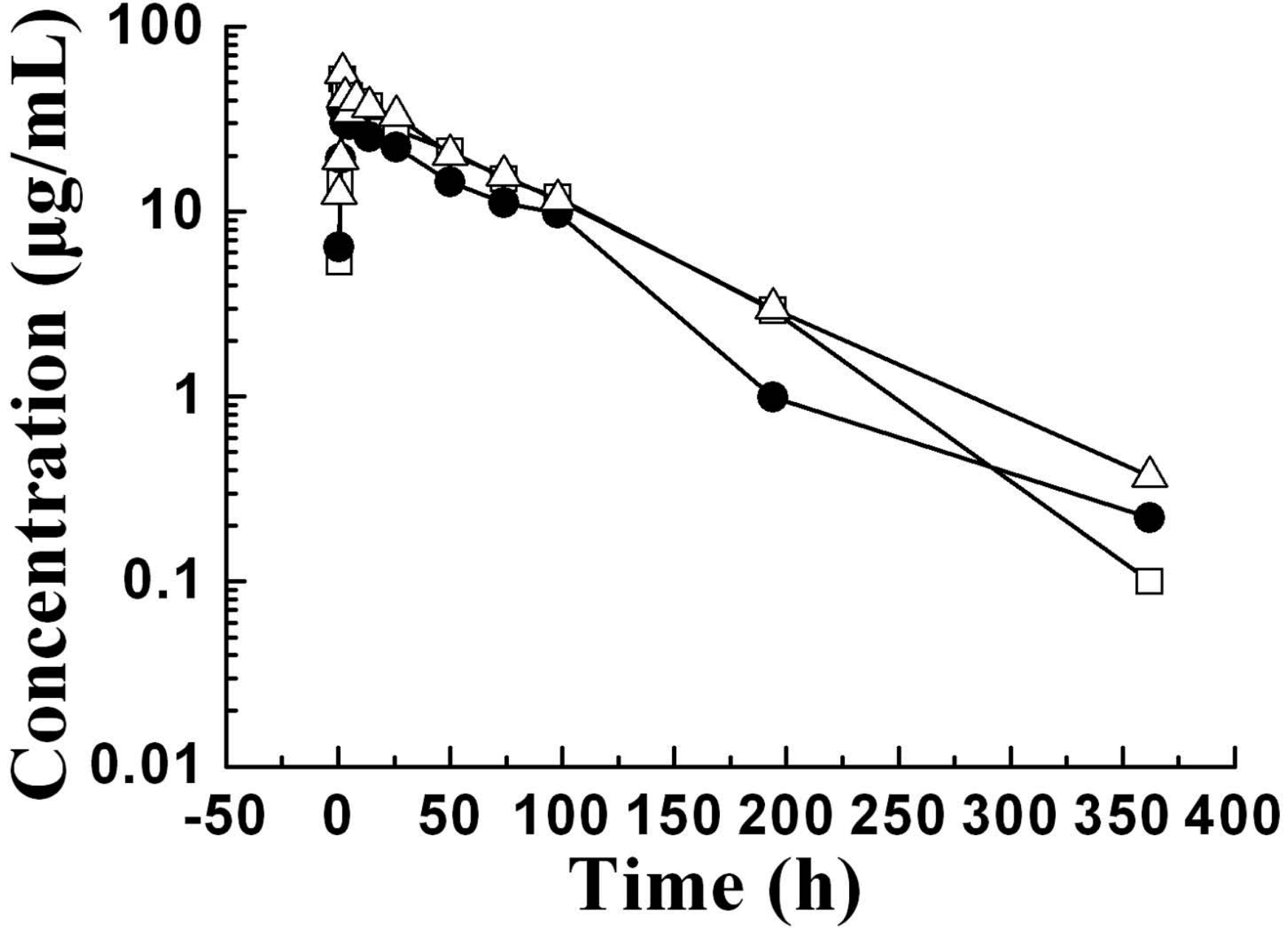
250
200
150
100
50
0
0.1
1
10
100
1000
antibody concentration (μg/mL)

A


PBS
Erbitux
CMAB009
Tumor volume (mm3)
1200
1000
800
600
400
200
0
5
10
15
20
25
30
35
Days after innoculation

B


PBS
cis-DDP
cis-DDP/Erbitux
cis-DDP/CMAB009
Tumor volulme (mm3)
1200
1000
800
600
400
200
0
5
10
15
20
25
30
35
Days after innoculation


Concentration (μg/mL)
100
10
1
0.1
0.01
-50
0
50
100
150
200
250
300
350
400
Time (h)