Supplementary materials

A computationally designed Rituximab/CD3 T cell engager targeting CD20+ cancers with multiple mechanisms of action

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Supplementary methods

Bispecific antibody purification

GB261 was produced by co-transfecting plasmids encoding the CD20 heavy chain, CD3 heavy chain and their common light chain into Expi293 cells using ExpiFectamine 293 Transfection Kit (Thermo Fisher) according to manufacturer's instructions. At 72 h after transfection, the cell culture supernatant was centrifuged at 3000 g for 10 min. The supernatant was filtered with a 0.45 μm membrane, and BsAb concentration was measured using a Protein A probe on gator (Probe Life). The BsAb was purified using a Protein A column on an AKTA Explorer 100 purification system (buffer A: PBS, pH=7.4; buffer B: 0.1 M glycine, pH=2.5), dialyzed in PBS (pH=7.4) twice, and then further purified using cation exchange chromatography (POROS GoPure HS Pre-packed Column, Thermo Fisher), with a salt gradient (Buffer A: 20 mM phosphate buffer, pH=7.4; Buffer B: 20 mM phosphate buffer, 1 M NaCl, pH=7.4). The purified BsAb was dialyzed in PBS twice, filtered with a 0.22 μM filter and tested by a T cell activation assay. Then, the Endotoxin content was quantified using the Pierce LAL Chromogenic Endotoxin Quantitation Kit and removed using the Pierce high capacity endotoxin removal columns, according to manufacturer's protocols. The BsAb was then filtered again with a 0.22 μm filter before using for the experiments.

Rituximab resistant Raji cells

Rituximab resistant Raji cells (RRCL) were developed as follows: Raji or Raji-GFP-Luc cells were treated with 102 µg/ml Rituximab and 10% pooled normal human serum (Innovative Research, Inc., IRLA-SER-23970); Initially, the antibody-containing old media was replaced with fresh antibody-containing media every 3 days up to 2 weeks to allow the cells to become healthy. Then, the serum concentration was increased, and the rituximab concentration was maintained at 102 µg/ml in the media. After 52 days, RRCLs were maintained in media with 10% serum.

Antibody-mediated cell bridging assay

Jurkat cells were labelled with the CellVue Claret Far Red dye and washed as described previously [23]. Raji-GFP cells only or Raji-GFP cells mixed with labeled Jurkat cells at 1:1 ratio in RPMI-1640 containing 10% FCS, treated with antibodies (20 µg/ml), and incubated overnight at 37 °C, 5% CO2. The cells were washed once with FACS buffer and analyzed using FACS. For microscopic assays, the cells were fixed with 4% paraformaldehyde and washed with PBS. Then, the cells were blocked with 10% normal goat serum for 30 min. The cells were stained with DyLight 594-conjugated goat anti-human IgG Fc cross-absorbed antibody for 1 h at RT. The cells were mounted on glass slides, covered with coverslips, and imaged with an Olympus microscope.

Supplementary figure legends

Figure S1. GB261- and BM-induced cancer cell killing at different E:T ratios. The RRCL-GFP-Luc cells or Raji-GFP-Luc cells were mixed with human PBMC at E:T ratios of 1:1 (A), 1:3 (B), and 1:9 (C) and incubated overnight at 37 °C with the indicated concentrations of antibodies in the presence of 10% human complement serum. Then, the percentage of cell death relative to the isotype control was determined by FACS based on the GFP+ live cells.

Figure S2. GB261 has high manufacturability with favorable physico-chemical characteristics. A) GB261 was purified by Protein A via AEX. B) The GB261 sample was prepared in a reducing and non-reducing labeling buffer and analyzed by capillary electrophoresis

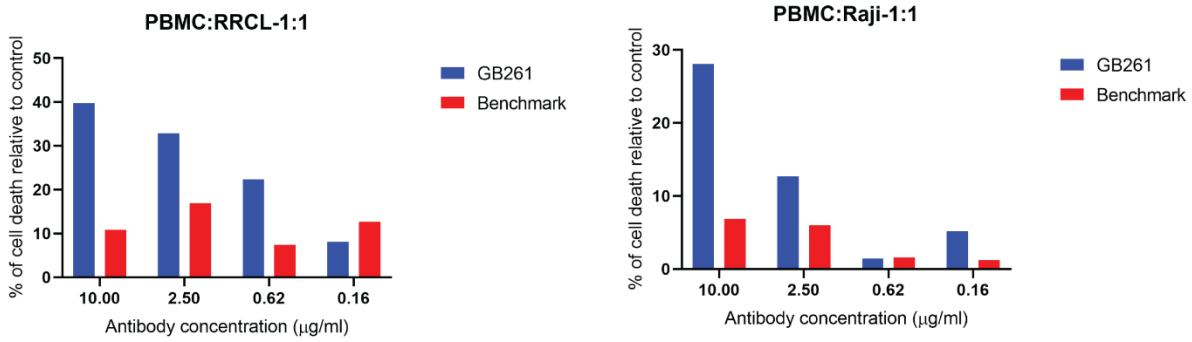
(CE). B) Reducing CE-SDS assay of the AEX-purified GB261 is shown here. C) Non-reducing CE-SDS assay of the AEX-purified GB261. D) thermostability (DSF, SLS) and aggregation potential of the AEX-purified GB261.

Figure S3. In-vivo dosing study of GB261. The pre-mixed RRCL-GFP-Luc cells and hPBMC at ~1:1 ratio was mixed with either PBS (Control) or different concentrations (per mouse) of GB261 as indicated, and injected into mice by i.v. A total of 3 mice were used per each group. Each mouse received 5 x 10^5 of Raji-GFP-Luc cells and PBMC. They were imaged for the tumor luminescence at days 3, 7, 10, 14, 17, and 21 post i.v. The tumor volumes were quantified (upper panel) and the luminescent images of the mice are shown (lower panel).

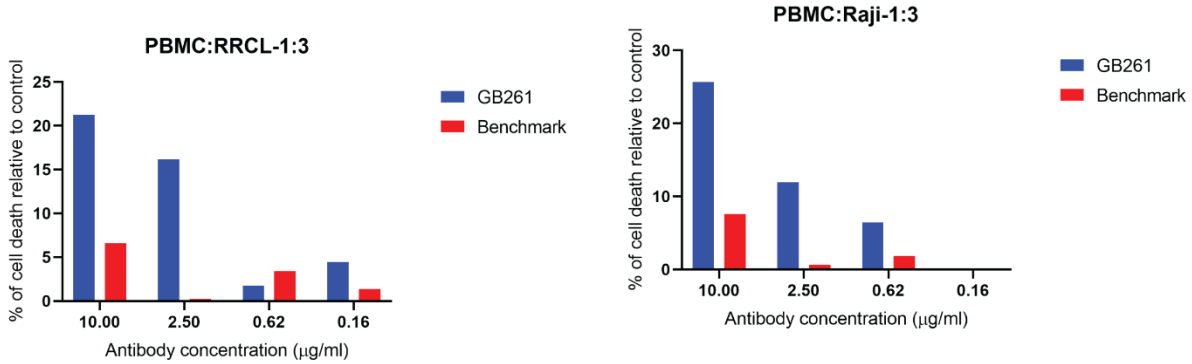
Supplementary figures

Figure 1

A



B



C

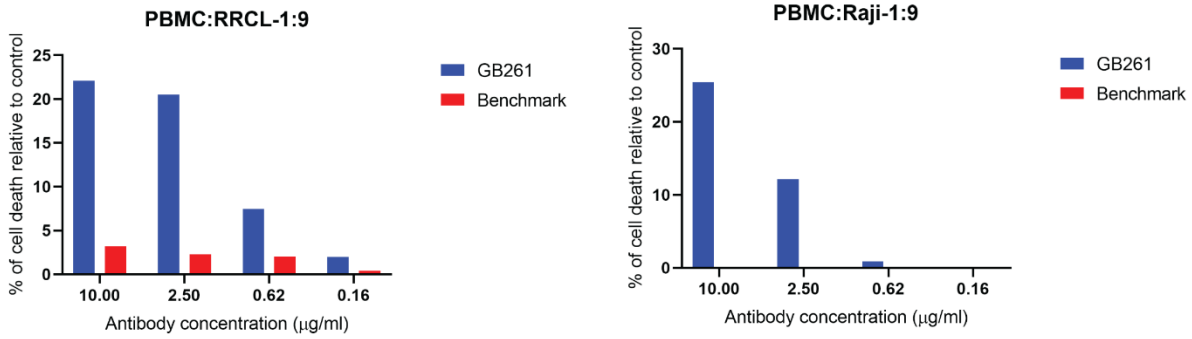


Figure 2

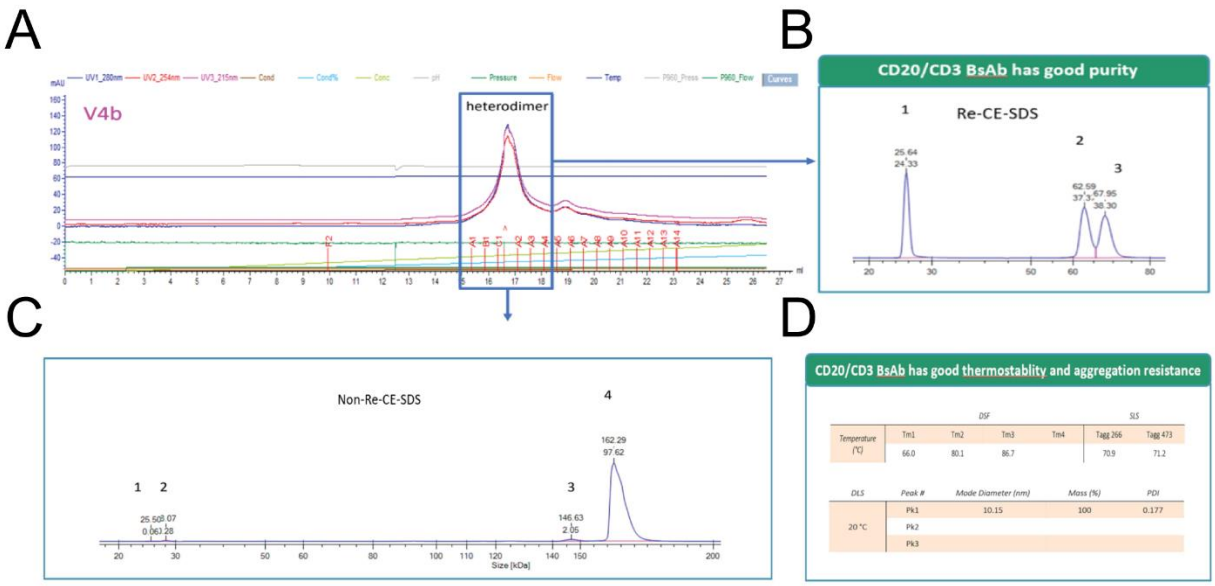
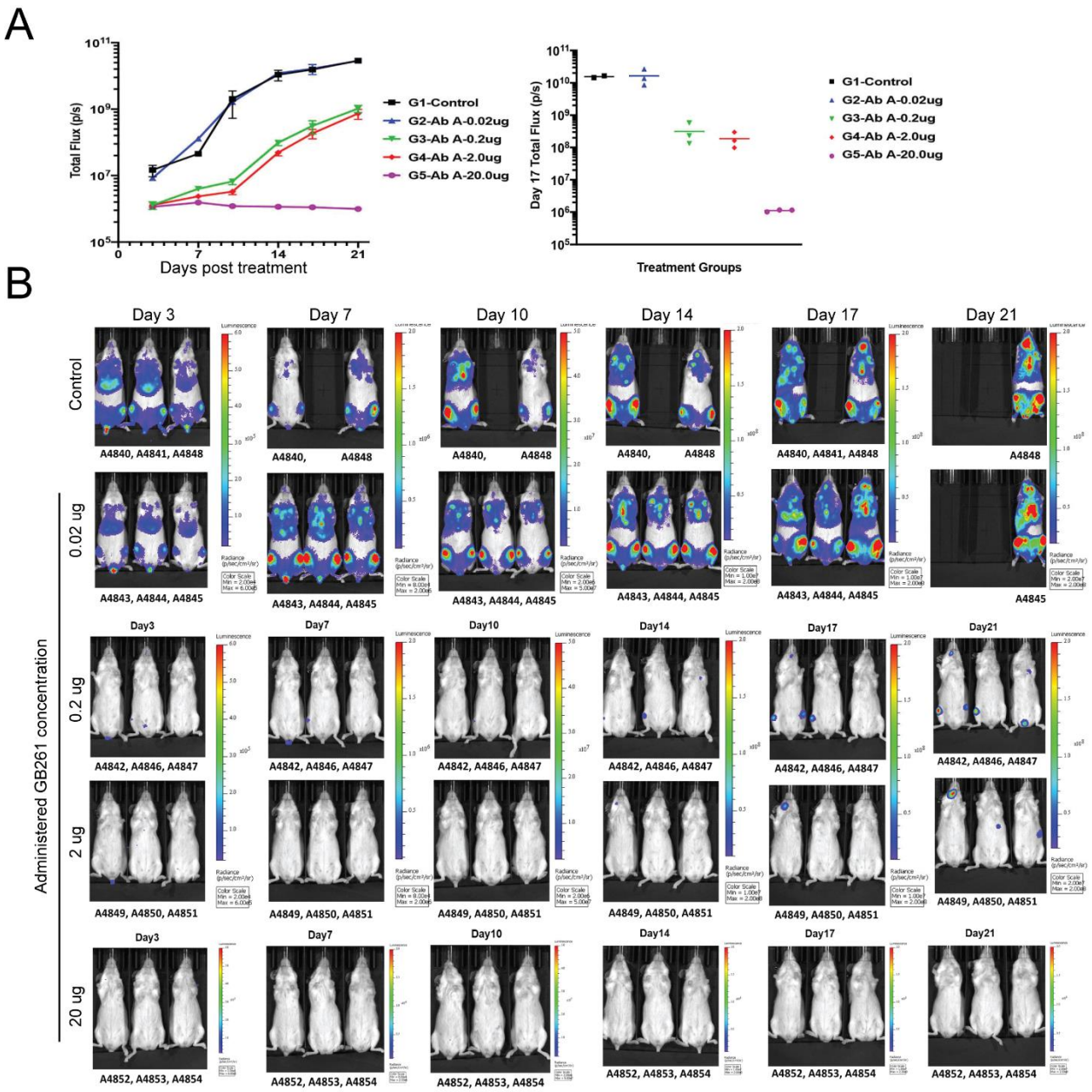

A
B
V4b
heterodimer
CD20/CD3 BsAb has good purity
Re-CE-SDS
1
2
3
25.64
24.33
62.59
37.52
67.95
38.30
C
D
Non-Re-CE-SDS
4
1
2
3
162.29
97.62
146.63
2.06
25.50
8.07
0.06
3.28
Size [kDa]
CD20/CD3 BsAb has good thermostablity and aggregation resistance
DSF
SLS
Temperature (°C)
Tm1
Tm2
Tm3
Tm4
Tagg 266
Tagg 473
66.0
80.1
86.7
70.9
71.2
DLS
Peak #
Mode Diameter (nm)
Mass (%)
PDI
20 °C
Pk1
10.15
100
0.177
Pk2
Pk3

Figure 3


A
Total Flux (p/s)
Days post treatment
G1-Control
G2-Ab A-0.02ug
G3-Ab A-0.2ug
G4-Ab A-2.0ug
G5-Ab A-20.0ug
Day 17 Total Flux (p/s)
Treatment Groups
B
Day 3
Day 7
Day 10
Day 14
Day 17
Day 21
Control
0.02 ug
0.2 ug
2 ug
20 ug
Administered GB261 concentration
A4840, A4841, A4848
A4840, A4848
A4843, A4844, A4845
A4842, A4846, A4847
A4849, A4850, A4851
A4852, A4853, A4854
A4848
A4845