

Figure S1: AGS67E But Not AGS67C Exhibits Anti-Tumor Activity In MOLM-13 Xenografts. Molm-13 xenografts were treated with AGS67E or the naked antibody of AGS67E (AGS67C) at 1 mg/Kg, QW for 4 doses. Complete tumor regression was observed with the AGS67E. AGS67C did not demonstrate anti-tumor activity and was comparable to isotype controls.

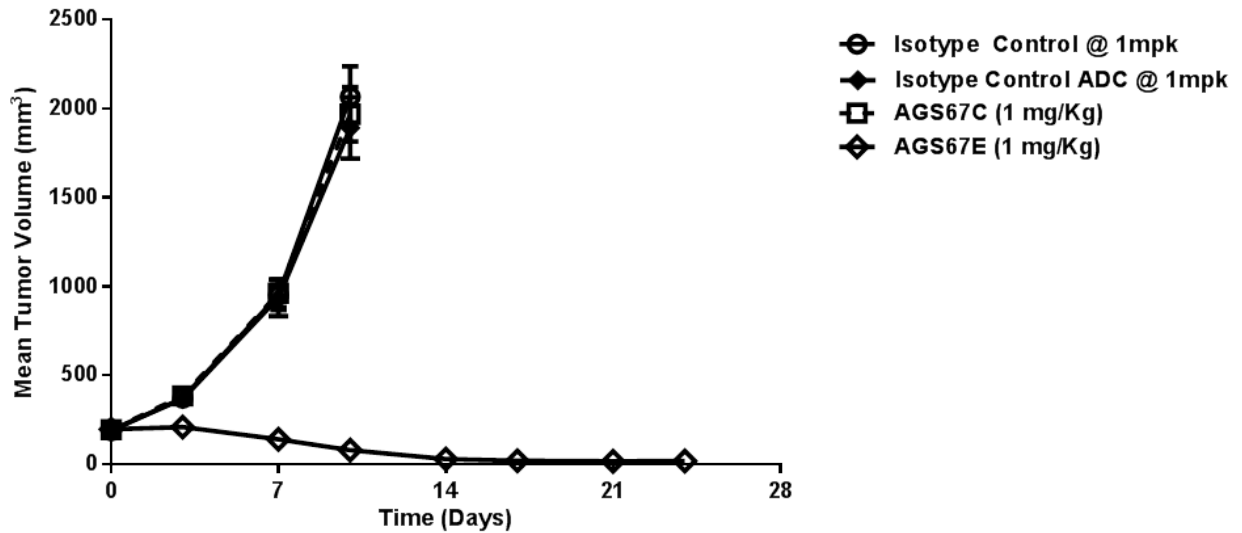
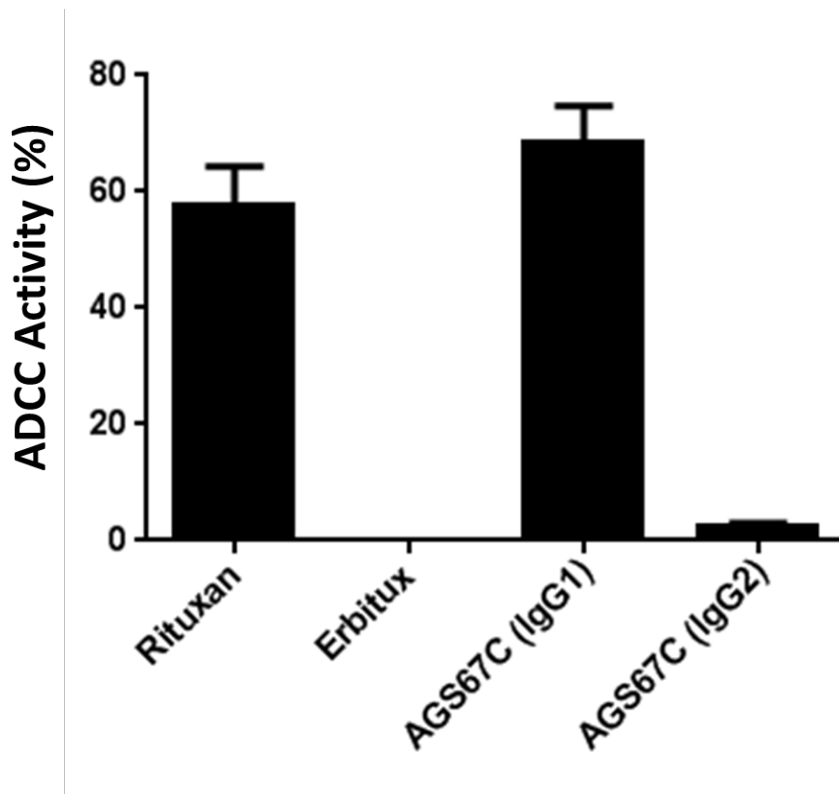


Figure S2: AGS67C IgG1 But Not IgG2 Induces ADCC Activity. Daudi cells were labeled with Chromium-51 and served as the target for effector cells in the presence of AGS67C comprised of a human IgG1 or a human IgG2 backbone. Effector PBMCs were prepared from normal human whole blood and incubated with Daudi cells at an effector:target ratio of 100:1 for 4 hours at 37C. Rituxan and Erbitux were used as positive and negative controls respectively. Percent lysis was plotted as ADCC activity.



METHODS:

Figure S1: Five week-old female CB17/SCID mice (Charles River) were maintained and used at Agensys' animal facility using IACUC approved protocols. 1×10^6 Molm-13 AML cells were injected into the right flanks of individual SCID mice and tumor volumes were allowed to reach 100-300 mm³. Animals and their tumors were size matched and randomized into treatment and control groups. AGS67C and an isotype control were dosed by intravenous bolus injection at 1.0 mg/Kg, QW for 4 and 2 doses respectively. Tumor growth was monitored using caliper measurements every 3 to 4 days until the end of study. Tumor volume was calculated as $\text{width}^2 \times \text{length} / 2$, where width is the smallest dimension and length is the largest. Animals were euthanized when tumors reached 2000 mm³. Mean tumor volume data for each group was plotted over time with standard error bars. A statistical analysis of the tumor volume data for the last day before animal sacrifice was performed using the Kruskal-Wallis test. Pairwise comparisons were made using Tukey's test procedures (2-sided) to protect the experiment-wise error rate. This implementation of Tukey's test was performed on the ranks of the data. The percent tumor growth inhibition in each treated group versus a control group was calculated as $[(\text{Control} - \text{Control baseline}) - (\text{Treated} - \text{Treated baseline})] / (\text{Control} - \text{Control baseline}) \times 100\%$.

Figure S2: The B lymphoma target cell line, Daudi, was harvested and labeled using chromium-51 radioisotope at a concentration of 20 uCi per 1×10^6 cells for 45 minutes at 37C. The labeled cells were washed 3x with assay buffer (RPMI-1640+ 1% FBS). After the last wash, cells were re-suspended in assay buffer at a concentration of 2×10^5 cells/mL. Effector PBMCs were isolated from normal whole blood by density centrifugation with Ficoll-Hypaque (GE Healthcare) and concentrated to 2×10^6 cells/mL. Test samples were diluted in assay buffer to a final concentration of 2.5 ug/mL. Equal volumes of target cells, test sample and effector cells (at a 100:1 effector:target ratio) were added to 96-well plates in triplicate and incubated for 4 hours in a humidified 37C incubator. After incubation, the plates were centrifuged (2100 RPM for 1

minute) and a 25 uL aliquot of each supernatant was transferred to 96-well flexi plates (Perkin Elmer) along with 150 uL of OptiPhase Supermix Cocktail (Perkin Elmer). Following a 15 minute incubation, counts per minute (CPM) were measured using a 1450 MicroBeta TriLux Microplate Scintillation Counter (Perkin Elmer). Cytotoxicity was reported as a percentage and calculated as 100 multiplied by (experimental release-spontaneous release) divided by (maximum release-spontaneous release).