**Supplementary Fig. S1**. BiTE® molecule structure and mechanism of action to target and eliminate TAA-positive tumor cells. BiTE®, bispecific T-cell engager; mAb, monoclonal antibody; MOA, mechanism of action; TAA, tumor-associated antigen.

**Supplementary Fig. S2. Cynomolgus monkey EGFRvIII specific domain truncation within cynomolgus monkey EGFR**

The extracellular domain of cynomolgus monkey EGFRvIII was generated by deletion of 267 amino acids (aa; indicated by strike-through text) and insertion of glycine at the fusion site within the cynomolgus monkey EGFR aa sequence 1 to 665 (<https://www.ncbi.nlm.nih.gov/protein>, accession number XP\_005549616.1). For generation of an expression construct, EGFRvIII-modified cynomolgus monkey EGFR was fused to the human EGFR (<https://www.ncbi.nlm.nih.gov/protein>, accession number NP\_005219.2) coding sequence lacking aa 1–665.

**Supplementary Fig. S3. AMG 596 in vitro activity data in cytotoxicity assays with U-87 MG/EGFRvIII tumor cells**

U-87 MG/EGFRvIII tumor cells were co-cultured with human PBMC at effector to target cell ratios of 10:1 and increasing AMG 596 concentrations for 48 hours. (**A**) Redirected lysis was monitored via flow cytometric determination of PI uptake by target cells. (**B**) T‑cell activation was analyzed by flow cytometry after 48 hours. (**C**) AMG 596-induced cytokine secretion is exemplarily shown by the flow cytometric analysis of INF- in supernatants after 48 hours. EGFRvIII, epidermal growth factor receptor variant III; IFN, interferon; PBMC, peripheral blood mononuclear cells. Data points represent the mean of duplicate measurements. Error bars indicate the standard error of the mean (SEM).

**Supplementary Fig. S4. Detection of human T cells in brain sections of mice from the orthotopic tumor study**

Immunohistochemistry analysis of brain sections of mice from the orthotopic tumor study that were treated with (A) vehicle or (B) AMG 596 (0.5 mg/kg) were analyzed for the presence of human CD3 T cells at study days 44 and 19. Arrows indicate T cells. Images were recorded with a Leica LM2500 microscope (100 x magnification).

**Supplementary Figure S5. AMG 596-mediated lysis by CD3+, CD4+ and CD8+ T cells**

U-87 MG/EGFRvIII tumor cells were co-cultured with human (**A**) CD3+, (**B**) CD4+ and (**C**) CD8+ T cells at effector to target cell ratios of 10:1 and increasing AMG 596 concentrations for 48 hours. Redirected lysis was monitored via flow cytometric determination of PI uptake by target cells. T cells were untouched isolated (T cell isolation kits from Miltenyi) from indicated donor PBMC. EGFRvIII, epidermal growth factor receptor variant III; Data points represent the mean of duplicate measurements. Error bars indicate SEM.

**Supplementary Fig S6. AMG 596 TDCC assays with U-251 MG cells previously co-cultured in transwells with U-251 MG/EGFRvIII cells**

In transwell experiments, U-251 MG (bottom) and U-251 MG/EGFRvIII cells (cell impermeable membrane, BD Bioscience) were co-cultured for 72 hours. Thereafter, U-251 MG cells harvested from the bottom wells, U-251 MG cells (negative control) and U-251 MG/EGFRvIII cells (positive control) were co-cultured with PBMC at effector to target ratios of 10:1 with 1 µg/mL AMG 596 for 72 hours. (**A**) AMG 596‑mediated cytotoxicity was analyzed by flow cytometry as loss of target cell membrane integrity, reflected by the nuclear uptake of propidium iodide. (**B**, **C**) Upregulation of T-cell activation markers CD69 and CD25 on T cells was analyzed by flow cytometry using fluorescent antibodies against CD4, CD8 (identification of T cells) and CD25, CD69 (all antibodies from BD Bioscience). Cytotoxicity and T-cell activation were analyzed on a FACSCanto II flow cytometer (BD Bioscience) and data were evaluated using FACS Diva software (BD) and GraphPad Prism (GraphPad). EGFRvIII, epidermal growth factor receptor variant III.