Supplementary figure 1. Evaluation of m971-scFv CAR. Second generation (m971-CD28/CD3-zeta, triangle), third generation (m971-CD28/4-1BB/CD3-zeta, inverted gray triangle), and mock (circle) transduced T cells were compared in 4 hour $^{51}$Cr-release assays using Raji, NALM-6, and K-562 line as targets at the E:T ratios listed on the x-axis. Average and SEM of triplicate wells is shown. Significant differences are noted at discrete E:T ratios, *= p < 0.05.

Supplementary figure 2. Re-stimulation and expansion of CAR-transduced T cells. T cells were activated with plate-bound OKT3, transduced with vector s/n (mock, CD19, HASH and m971 in 2nd and 3rd generation formats) and cultured in 300 u/mlIL-2 in 6-well plates or flasks. 10 days post activation, CAR-transduced T cells were co-cultured with irradiated A) Raji or B) NALM6-GL cell lines at a 1:1 ratio and fed with fresh IL-2 containing media every 2-3 days. Representative cell counts from one of three separate expansions are shown.

Supplementary figure 3. In vivo analysis of HA22-CAR-treated animals. A) T cells were transduced with HA22SH-BBz, HA22SH-28z, and HA22SH-28BBz vectors and analyzed for CAR surface expression by flow cytometry with CD22-Fc (red = CD22 CAR transduced, blue = mock transduced). Percent transduction is listed in each panel. B) Mice were i.v. injected with $0.5 \times 10^6$ NALM-6GL, imaged on day 3, at which time 1 $\times 10^7$ CAR-transduced T cells were injected, and subsequently imaged on days 7 and 17. C) Kaplan-Meier survival curves for each group, analyzed as in figure 6. p values are listed in the figure.
Supplemental Figure 1

RAJI
CD22^{Hi}

NALM6-GL
CD22^{Low}

K562
CD22^{Live}

% Lysis

E:T

20:1 10:1 5:1 2.5:1 1.25:1 0.625:1 0.3125:1 0.15:1

Mock
m971-28z
m971-28BBz
Supplemental Figure 2

A
NALM6-GL

B
RAJI

Total Cell Number (x 10^6)

Days

- mock
- CD19-28z
- HA22SH 28z
- HA22SH 28BBz
- HA22SH BBz
- m971-28z
- m971-28BBz