**Supplemental Digital Content 1**

Chimeric antigen receptor (CAR) T-cell engineering process and CAR construct.(**a**)Schematic overview of CAR T construct. (**b**) To produce a defined composition of CAR-expressing T cells, CD4+ and CD8+ T-cell populations were isolated from apheresis samples obtained from consenting normal donors or patients with diffuse large B-cell lymphoma. CD4-enriched and CD8-enriched populations were separately activated, transduced with a lentiviral vector encoding both the CD19-specific CAR and a truncated epidermal growth factor receptor (EGFRt, a surrogate marker for transduction), and expanded. CAR-engineered cells were cryopreserved, stored at temperatures below −130°C, and thawed prior to use. CAR-positive cells were used at a target CD4+:CD8+ ratio of ≈ 1:1. To account for any variability in transduction efficiency, numbers of EGFRt-positive cells were normalized across experiments. CD28tm, CD28 transmembrane domain; huEGFRt, human EGFRt; LTR, long terminal repeat; PBMC, peripheral blood mononuclear cell; EGFR, epidermal growth factor receptor; scFv, single-chain variable fragment.



**Supplemental Digital Content 2**

CD19 expression and proliferation profile of Nalm-6 cells.(**a**) Expression level of CD19 on Nalm-6 compared with K562.CD19. (**b**) Growth of Nalm-6 cells measured by percentage of confluence in the presence of ibrutinib or acalabrutinib.



**Supplemental Digital Content 3**

RNA expression of kinases from serially stimulated chimeric antigen receptor (CAR) T cells (day 18).Box and whisker plot of kinase expressions of CAR T cells from 3 donors in transcripts per million (TPM). Aca, acalabrutinib; BLK, B lymphocyte kinase; BMX, bone marrow tyrosine kinase; BTK, Bruton tyrosine kinase; ctrl, control; EGFR, epidermal growth factor receptor; ERBB2, erb-b2 receptor tyrosine kinase 2; ibr, ibrutinib; ITK, interleukin 2–inducible T-cell kinase; JAK3, Janus kinase 3; TEC, Tec protein tyrosine kinase; TXK, tyrosine protein kinase TXK.



**Supplemental Digital Content 4**

Secreted cytokine concentrations from chimeric antigen receptor (CAR) T cells after in vitro treatment with ibrutinib or acalabrutinib.(**a** and **b**) Cytokine concentrations of interferon gamma (IFN-γ), interleukin 2 (IL-2) and tumor necrosis factor-alpha (TNF-α) 2 days after stimulation with 30 µg/mL anti-idiotypic antibody of CAR T cells treated with (**a**) ibrutinib or (**b**) acalabrutinib.
(**c** and **d**) Cytokine concentrations 2 days after stimulation with K562.CD19 of CAR T cells treated with (**c**) ibrutinib or (**d**) acalabrutinib. (**e**) Comparison of cytokine concentrations between short-term stimulated CAR T cells (day 2) and chronic stimulated CAR T cells (day 6) following restimulation, representative data from 1 donor. (**f** and **g**) Prior to restimulation, CAR T cells were stimulated with 30 µg anti-idiotypic antibody for 6 days with (**f**) ibrutinib or (**g**) acalabrutinib. Day 6 CAR T cells were then restimulated for 24 hours with 30 µg/mL anti-idiotypic antibody in the absence of Bruton tyrosine kinase inhibitor, and cytokine concentrations were measured. Representative data from 2 independent experiments and 3 CAR T cell donors (mean ± SEM).

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**Supplemental Digital Content 5**

Chimeric antigen receptor (CAR) T phenotypic marker expression in the presence of Bruton tyrosine kinase inhibitor in the 6-day anti-idiotypic antibody stimulation assay. (**a**) Expression of CD8, CD25, CD69, CD38, PD-1, CD107a, CD62L, CD45RO, and CCR7 on population 1 gated from multivariate T-distributed stochastic neighbor embedding (t-SNE) analysis of 3 different donors after 6 days of 30 µg/mL anti-idiotypic antibody stimulation. (**b**) Percentage of total cells that are in population 1 based on t-SNE analysis after 6 days of stimulation and treatment with ibrutinib or acalabrutinib. PD-1, programmed cell death 1.

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**Supplemental Digital Content 6**

Activation markers during initial stimulation. (**a** and **b**) Expression of CD25, CD38, CD39, and CD95 in the first 4 days after initial stimulation in the presence of (**a**) ibrutinib or (**b**) acalabrutinib. Representative result shown from cells derived from donor 2.

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**Supplemental Digital Content 7**

Changes in chimeric antigen receptor (CAR) T cell memory subsets following culture in the presence of Bruton tyrosine kinase inhibitor. (**a** and **b**) Percentage of central memory T cells (TCMs; CCR7+CD45RA−) and effector memory T cells (TEMs; CCR7−CD45RA−) over 4 days after initial stimulation in the presence of (**a**) ibrutinib or (**b**) acalabrutinib. Representative result shown from donor 2.

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**Supplemental Digital Content 8**

Surface activation markers modulated in the presence ofBruton tyrosine kinase inhibitor*.* (**a** and **b**) Expression of CD69, CD107a, and PD-1 in the first 4 days after initial stimulation in the presence of (**a**) ibrutinib or (**b**) acalabrutinib. Data from 2 independent experiments with cells from 3 donors. Plots were analyzed by two-way analysis of variance. Statistically significant differences from control are indicated as *P* < 0.05 (\*).

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**Supplemental Digital Content 9**

Chimeric antigen receptor (CAR) T cell phenotypic marker expression in the presence of Bruton tyrosine kinase inhibitor in the serial stimulation assay. (**a** and **b**) Expression of CD25, CD38, CD39, CD45RO, CD62L, CD69, CD107a, and PD-1 at days 0, 11, 18, and 21 of serial stimulation in the presence of (**a**) ibrutinib or (**b**) acalabrutinib. Representative result shown from donor 2.



**Supplemental Digital Content 10**

Chimeric antigen receptor (CAR) T cells in combination with ibrutinib or acalabrutinib decreases tumor burden in mice. (**a** and **b**)Tumor growth over time as indicated by measuring average radiance by bioluminescence from mice treated with ibrutinib or acalabrutinib in drinking water and CAR T cells from (**a**) donor 2 and (**b**) donor 3. Analysis was performed with the two-way analysis of variance. Statistically significant differences are indicated as *P* < 0.05 (\*) and
*P* < 0.01 (\*\*). p/s/cm2/sr, photons per second per centimeter squared per steradian.

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**Supplemental Digital Content 11**

Number of chimeric antigen receptor (CAR) T cells in mice treated with oral (PO) ibrutinib.(**a** and **b**) CAR T cells from mice injected with donor 1 cells and treated with vehicle or PO ibrutinib were sacrificed on day 13, 19, and 26. Cells were quantified from the (**a**) blood or (**b**) tumor site (bone marrow). Analysis was performed with the one-way analysis of variance, and *P* values are shown.

