**SUPPLEMENTARY MATERIALS AND METHODS**

**Derivation of Cetux-CAR and Nimo-CAR.** The sequence of Cetux-CAR is as follows: GMCSF signal peptide (NP\_758452.1, aa 1-22), cetuximab light chain (PDB:1YY9\_C, aa 1-213), Whitlow linker (AAE377080.1, aa 1-18), cetuximab heavy chain (PDB:1YY9\_D, aa 1-221), IgG4 (AAG00912.1, aa 161-389), CD28 (transmembrane and cytosolic, NP\_006130, aa 153-220), and CD3-ζ (cytosolic, NP\_932170.1, aa 52-164). Nimo-CAR sequence is identical to Cetux-CAR sequence, with the exception of the light chain (nimotuzumab light chain, PDB:3GKWL, aa 1-219) and heavy chain (nimotuzumab heavy chain, PDB:3GKWL, aa 1-222).

**Cell lines and propagation.** All cell lines in this study were maintained in complete media defined as Dulbecco’s modified eagle media (DMEM, Life Technologies, Grand Island, NY), supplemented with 10% heat inactivated fetal bovine serum (FBS) (Hyclone, ThermoScientific) and 2 mM Glutamax-100 (Gibco, Life Technologies) in a tissue culture incubator maintained at 5%CO2, 95% humidity and 37ºC. Adherent cell lines were cultured to 80-90% confluence and then passaged 1:10 after dissociation with 0.05% Trypsin-EDTA (Gibco) (cat #CRL-9854, ATCC). Primary human renal cortical epithelial cells (HRCE) (cat #CC-3190, Lonza) were cultured in complete Renal Growth Media (cat #CC-3190, Lonza), supplemented with recombinant human epidermal growth factor (hEGF), epinephrine, insulin, triiodothyronine, hydrocortisone, transferring, 10% heat-inactivated FBS (HyClone), and 2 mM Glutamax-100 (Gibco).

**Genetic Modification of Cell Lines.** K562 clone 27 was derived from K562 clone 9 by limiting dilution after genetic modified by SB system to stably express a membrane-bound IL15-IL15Rα fusion protein ([1](#_ENREF_1)). Clone 27 was further modified to express truncated EGFR by SB transfection of tErbB1-F2A-Neo/pSBSO (**Supplementary Fig. S1C**). EL4 were genetically modified to express truncated CD19 (from DNA plasmid CD19-F2A-Neo), truncated EGFR (from tEGFR-F2A-Neo) or CAR-L (from CAR-L-F2A-Neo) by electro-transfer of SB-derived DNA plasmids as previously described ([2](#_ENREF_2)). Twenty-four hours after electroporation 1 mg/mL G418 (Invivogen, San Diego, CA) was added to select for EL4 cells stably expressing transgenes. U87MG, also designated U87 (cat # HTB-14, ATCC) were genetically modified to generate U87low that expressed a low level of tEGFR as achieved by electro-transfer (106 cells/cuvette) of 3 µg/cuvette tErbB1-F2A-Neo/pSBSO and 2 µg/cuvette pCMV-SB11([3](#_ENREF_3))([3](#_ENREF_3))([3](#_ENREF_3))([3](#_ENREF_3))([3](#_ENREF_3)) (to express SB11 transposase) using Nucleofector (model AAB-1001, Lonza) and Cell Line Nucleofector kit T (cat# VACA-/mL1002, Lonza). The following day, 0.35 mg/mL G418 (Invivogen) was added to culture to select for tEGFR expression. To generate U87med cells, 4 µg tErbB1-F2A-Neo and 2 µg SB11 were transferred with Lipofectamine 2000 (Invitrogen) according to manufacturer’s instructions. The following day, 0.35 mg/mL G418 was added to culture to select for tEGFR expression.

**Propagation of Genetically Modified T cells**. T cells were modified by Sleeping Beauty transposition and expanded ex vivo as previously described ([3](#_ENREF_3)). Twenty-four hours after electroporation with CAR, T cells were stimulated with 100 Gy-irradiated tEGFR+ AaPC at a ratio of 2 CAR+ T cells to 1 AaPC and restimulated every 7 to 9 days. Throughout the 21 to 28 day culture period, T cells received soluble 30 ng/mL IL-21 (cat # AF-200-21, Peprotech) added to culture every 2 to 3 days beginning the day of addition of aAPC. IL-2 (Aldesleukin) was added to culture at 50 U/mL every 2-3 days beginning the day of the second and subsequent additions of AaPC. At day 14, if contaminating CD3negCD56+ cells NK cells represented >10% of cell population they were depleted using CD56+ magnetic beads (cat # 130-050-401, Miltenyi Biotec) on LS column (cat # 130-042-401, Miltenyi Biotec). Inferred cell count was calculated by multiplying the fold expansion by the total number of CD3+CAR+ T cells from previous stimulation.

**Phospho-flow Cytometry.** T cells were lysed and fixed by addition of 20 volumes of 1x pre-warmed to 37ºC PhosFlow Lyse/Fix buffer (cat # 558049, BD Biosciences), and incubated at 37ºC for 10 minutes. After centrifugation, T cells were permeabilized by addition while vortexing of ice-cold PhosFlow Perm III Buffer (cat # 558050, BD Biosciences) and incubated on ice in the dark for 20 minutes. Cells were washed with FACS Buffer and resuspended for 20 minutes in the dark at room temperature in 100 µL staining solution composed of 1:25 dilution of mAbs against CD4 (clone SK3, BD Bioscience), CD8 (clone SK1, BD Bioscience), pErk1/2 (clone 20A, BD Bioscience), pp38 (clone 36/p38, BD Bioscience) in FACS buffer. Cells were fixed with 0.5% paraformaldehyde and analyzed by flow cytometry within 24 hours.

**Long-term growth inhibition assay.** U87 and U87high cells were plated in 6-well plate in complete media and incubated overnight, following which CAR+ T cells were added at a 1:5 (T cell to target cell) ratio. At each time point, T cells were removed and adherent cells were dissociated by 0.05% Trypsin-EDTA (Gibco) and counted by trypan blue exclusion. Percent surviving target cells was calculated as [*cell number after T cell co-culture*]/[*cell number with no T cell co-culture*] x 100.

**Implantation of guide-screw and intracranial tumor.** Implantation of the guide-screw and establishment of an intracranial tumor was performed as previously described ([5](#_ENREF_5)). Once unresponsive to stimuli, a surgical area was prepared by shaving fur and treating skin with povidone-iodine antiseptic solution. A 1 cm incision was made down the middle of the cranium, and an opening was made 1 mm anterior to the coronal suture, and 2.5 mm from the sagittal suture in the right frontal lobe using a 1 mm drill bit extended 1 mm from drill. A guide-screw was inserted into the opening. Incision sites were sutured and mice were given 0.01 mg/mL buprenorphine dosed at 0.1 mL/10 g as a post-surgical analgesic. Mice recovered from surgery on low-power heat source until full mobility was regained. Two to three weeks following surgical implantation of guide-screw, U87 or U87med glioma cells (genetically modified to co-express firefly luciferase (ffLuc) fused to mKate fluorescent reporter gene ([6](#_ENREF_6)) by lentiviral transduction according to previously described protocol ([7](#_ENREF_7)) were implanted, designated day 0. Guide-screw was exposed by small incision and 250,000 ffLuc+mKate+ U87 parental cells or ffLuc+mKate+ U87med cells suspended in 5 µL sterile PBS were injected to a depth of 2.5 mm. The incision site was closed by suture. T-cell treatment began on day 5 and continued every 7 days for a total of 3 injections using 3x106 T cells with >85% CAR expression suspended in 5 µL of PBS and injected 2.5 mm into intracranial space through guide-screw.

**SUPPLEMENTARY REFERENCES**

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