Science Translational Medicine
AAAS

stm.sciencemag.org/cgi/content/full/12/559/eaaz1863/DC1

Supplementary Materials for

Effective combination immunotherapy using oncolytic viruses to deliver CAR targets to solid tumors

Anthony K. Park, Yuman Fong, Sang-In Kim, Jason Yang, John P. Murad, Jianming Lu, Brook Jeang, Wen-Chung Chang, Nanhai G. Chen, Sandra H. Thomas, Stephen J. Forman, Saul J. Priceman*

*Corresponding author. Email: spriceman@coh.org

Published 2 September 2020, *Sci. Transl. Med.* **12**, eaaz1863 (2020)
DOI: 10.1126/scitranslmed.aaz1863

The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at stm.sciencemag.org/cgi/content/full/12/559/eaaz1863/DC1)

Data file S1 (Microsoft Excel format). Primary data.

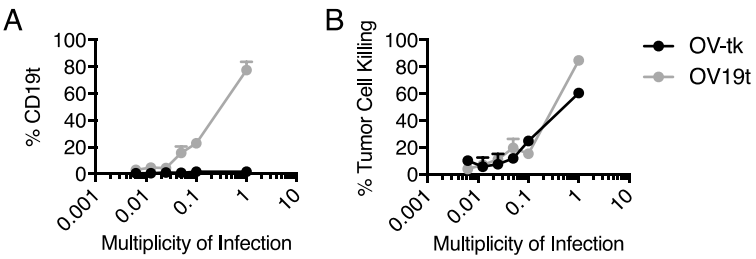

Fig. S1. Replacing *tk* gene with hCD19t in OV does not significantly affect the infection efficiency or cell killing of MDA-MB-468. A) Quantification of cell surface CD19t expression following 24 h co-culture with indicated MOIs of OV-*tk* (control) or OV19t. B) Tumor killing assessed by flow cytometry comparing MDA-MB-468 treated with indicated MOIs of OV-*tk* (control) or OV19t. Data presented are from duplicate wells and shown as mean + SD.

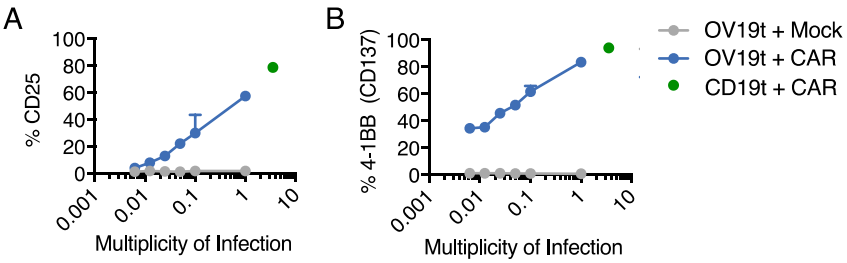

Fig. S2. Activation of CD19-CAR T cells against OV19t-infected tumor cells expressing CD19t. Quantification of A) CD25 and B) CD137 expression on untransduced T cells (Mock) or CD19-CAR T cells following a 24 h co-culture with tumor cells at an E:T ratio of 1:2 with or without addition of indicated MOI of OV19t. Green dots indicate T cells co-cultured with MDA-MB-468 cells stably expressing CD19t.

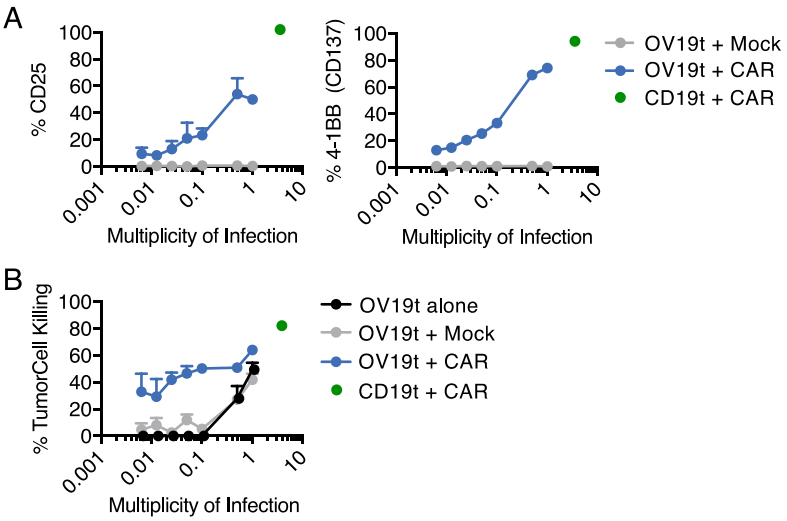

Fig. S3. CD19-CAR T cells activate and kill MDA-MB-231 cells infected with OV19t. A) Quantification of CD25 (left) and CD137 (right) expression on Mock or CD19-CAR T cells following a 24 h co-culture with MDA-MB-231 cells at an E:T ratio of 1:2 with or without addition of indicated MOI of OV19t. B) Tumor cell killing as assessed by flow cytometry comparing the effect of Mock T cells or CD19-CAR T cells on following 24 h co-culture with MDA-MB-231 cells treated with indicated MOIs of OV19t. Green dots indicate T cells co-cultured with MDA-MB-231 cells lentivirally transduced to stably express CD19t.

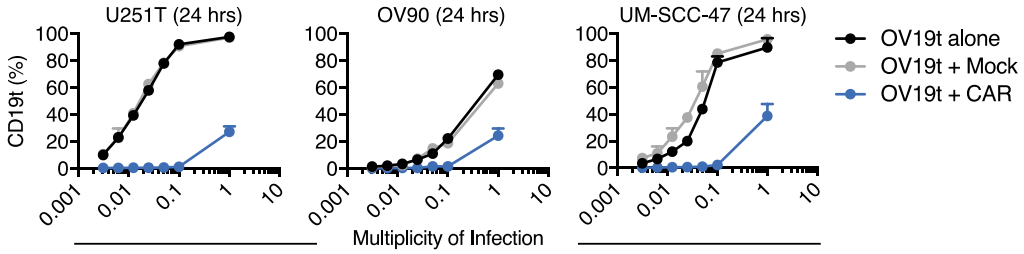

Fig. S4. OV19t-mediated expression of CD19t in tumor cells promotes tumor cell–targeted cytotoxicity of CD19-CAR T cells in vitro. Percent of CD19t positive U251T, OV90, or UM-SCC-47 cells exposed to the indicated MOIs of OV19t and cocultured for 24 h with Mock T cells or CD19-CAR T cells. Data presented are from duplicate wells and shown as mean + SD.

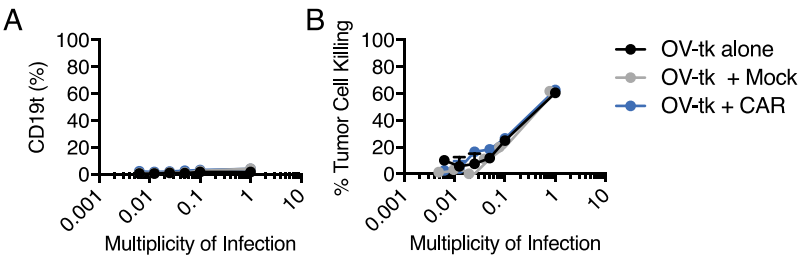

Fig. S5. OV carrying *tk* does not induce CD19-CAR T cell activity. (**A**) Quantification of percent of MDA-MB-468 cells positive for cell surface CD19t following 24 h coculture with indicated MOIs of OV-*tk* alone or with Mock T cells or CD19-CAR T cells. (**B**) Tumor cell killing of MDA-MB-468 treated with the indicated MOIs of OV-*tk* alone or with Mock T cells or CD19-CAR T cells. Viable cells were assessed by flow cytometry. Data presented are from duplicate wells and shown as mean + SD.

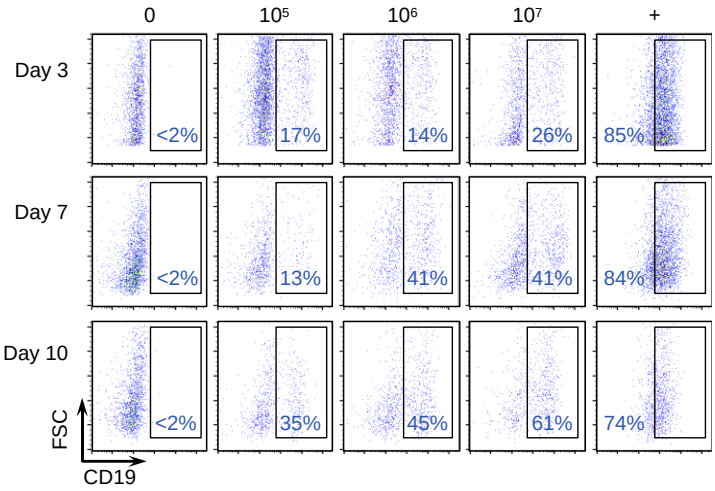

Fig. S6. Representative fluorescence-activated cell sorting plots of CD19t$^+$ MDA-MB-468 cells from harvested tumors (related to Fig. 3A). Representative FACS plots show percent of CD19t-positive viable EpCAM+ MDA-MB-468 tumor cells from harvested tumors at indicated timepoints and from mice treated by intratumoral injection with the indicated pfu of OV19t. Cells from harvested CD19t lentivirus stably-transduced tumors are indicated as '+'. Values indicate percent of cells in the boxed region.

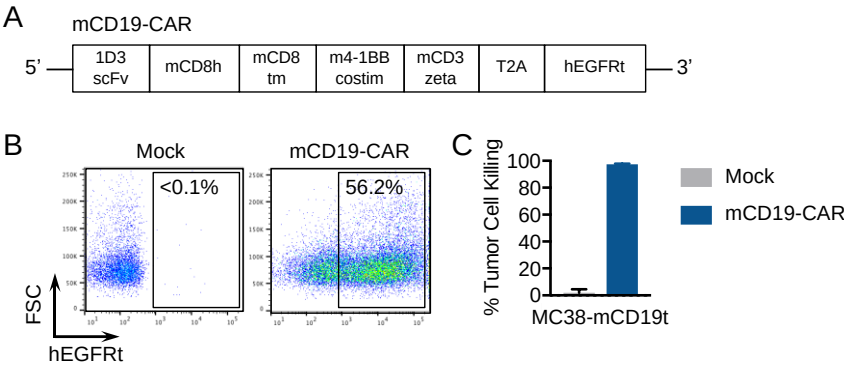

Fig. S7. Murine CD19-CAR T cells. (**A**) Diagram of the retroviral expression cassette with mCD19-CARs containing the murine scFv (1D3 clone) targeting CD19, a murine CD8 hinge and transmembrane domain, a murine cytoplasmic 4-1BB costimulatory domain, and a murine CD3ζ cytolytic domain. A human truncated, nonsignaling EGFR (hEGFRt), separated from the CAR with a T2A ribosomal skip sequence, was expressed for tracking CAR-expressing cells. (**B**) Representative FACS plots of cells with mCD19-CAR (detected by hEGFRt positivity) on the surface of ex vivo engineered murine T cells. (**C**) Tumor cell killing ability of mCD19-CAR T cells. Untransduced murine T cells (Mock) or mCD19-CAR T cells were cocultured for 24 h co-culture with MC38 tumor cells lentivirally-transduced to stably express murine CD19t (MC38-mCD19t). The ratio of T cells to tumor cells was 1:1. Tumor cell killing is compared to tumor cells cultured alone. Data presented are from duplicate wells and shown as mean + SD.

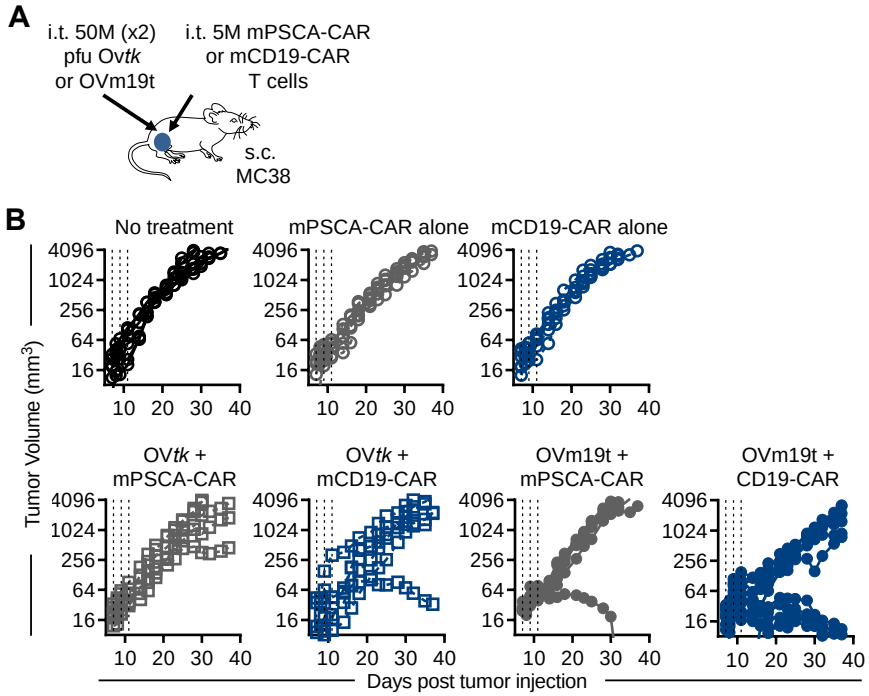

Fig. S8. Specificity of combination therapy in the murine MC38 tumor model. (**A**) Schematic of MC38 tumor-bearing mice treated with OV*tk* or OVm19t in combination with either mPSCA-CAR or mCD19-CAR T cells. Mice were intratumorally treated with 5×10^7 pfu of either OV*tk* or OVm19t on days 7 and 9. On day 11, mice were intratumorally treated with either mPSCA-CAR or mCD19-CAR T cells (5×10^6 cells). (**B**) Tumor volume (mm^3) was measured with calipers. Data for each mouse per group is shown, $n \geq 6$ per group. Dashed vertical lines indicate intratumoral injections.

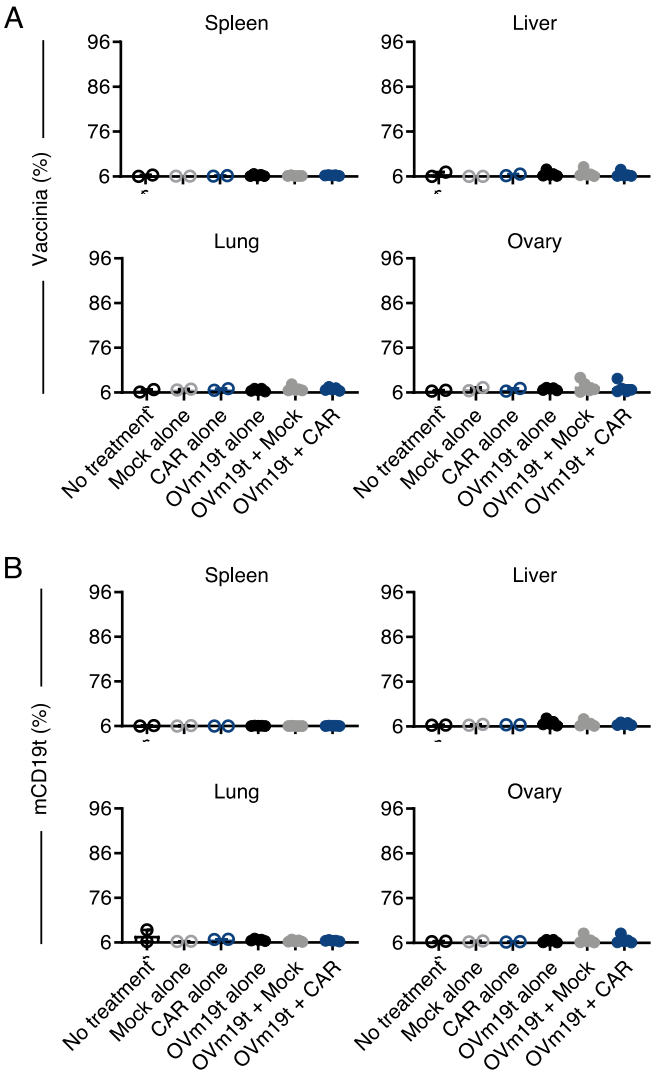

Fig. S9. Assessment of OVm19t tumor selectivity. Mice were subcutaneously engrafted with MC38 (5×10^5) cells and at days 14 and 16 mice were intratumorally treated with 5×10^7 pfu per mouse. On day 18 mice were treated intravenously (i.v.) with untransduced T cells (Mock) or mCD19-CAR T cells. Spleen, liver, lung, and ovary were harvested 5 days after OVm19t and 3 days after T cell treatments for quantification of percent cells positive for vaccinia (**A**) or mCD19t (**B**) by flow cytometry. Tissues from 2 - 5 mice for each group were evaluated and data for each mouse is shown.

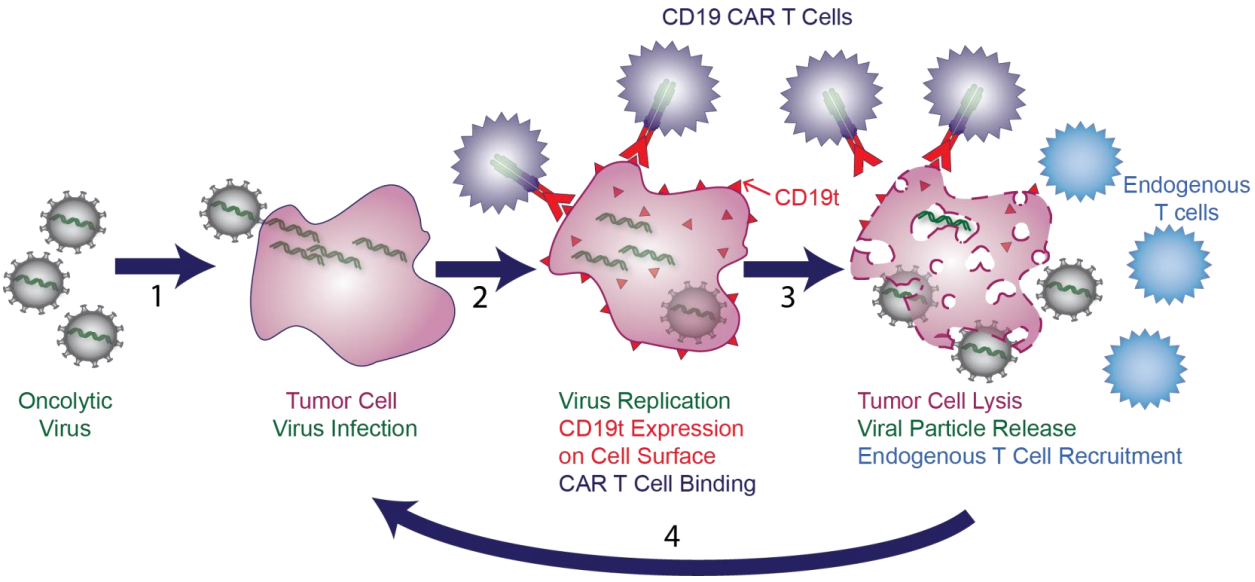

Fig. S10. Schematic of combination therapy concept using OV to introduce CAR targets to solid tumors. (1) Oncolytic virus (in this study, OV19t) infects tumor cells. (2) Virus replication and production of CD19t on the cell surface enables CD19-CAR T cell targeting. (3) Tumor cell lysis leads to viral particle release and the combination promotes endogenous immune cell recruitment to tumors. (4) Released viral particles re-initiate virus infection of surrounding tumor cells.