Development of Highly Effective Anti-Mesothelin hYP218 Chimeric Antigen Receptor T Cells With Increased Tumor Infiltration and Persistence for Treating Solid Tumors

Sakshi Tomar[1], Jingli Zhang[1], Manakamana Khanal[1], Jessica Hong[2], Abhilash Venugopalan[1], Qun Jiang[1], Manjistha Sengupta[1], Markku Miettinen[3], Nan Li[2], Ira Pastan[2], Mitchell Ho[2], and Raffit Hassan[1]

ABSTRACT

Mesothelin targeting CAR T cells have limited activity in patients. In this study, we sought to determine if efficacy of anti-mesothelin CAR T cells is dependent on the mesothelin epitopes that are recognized by them. To do so, we developed hYP218 (against membrane-proximal epitope) and SS1 (against membrane-distal epitope) CAR T cells. Their efficacy was assessed *in vitro* using mesothelin-positive tumor cell lines and *in vivo* in NSG mice with mesothelin-expressing ovarian cancer (OVCAR-8), pancreatic cancer (KLM-1), and mesothelioma patient-derived (NCI-Meso63) tumor xenografts. Persistence and tumor infiltration of CAR T cells was determined using flow cytometry. hYP218 CAR T cells killed cancer cells more efficiently than SS1 CAR T cells, with a two- to fourfold lower ET_{50} value (effector-to-target ratio for 50% killing of tumor cells). In mice with established tumors, single intravenous administration of hYP218 CAR T cells lead to improved tumor response and survival compared with SS1 CAR T cells, with complete regression of OVCAR-8 and NCI-Meso63 tumors. Compared with SS1 CAR T cells, there was increased peripheral blood expansion, persistence, and tumor infiltration of hYP218 CAR T cells in the KLM-1 tumor model. Persistence of hYP218 CAR T cells in treated mice led to antitumor immunity when rechallenged with KLM-1 tumor cells. Our results show that hYP218 CAR T cells, targeting mesothelin epitope close to cell membrane, are very effective against mesothelin-positive tumors and are associated with increased persistence and tumor infiltration. These results support its clinical development to treat patients with mesothelin-expressing cancers.

Introduction

Adoptive cellular therapy utilizing CAR T cells has shown remarkable success for the treatment of several hematologic malignancies. It has led to the FDA approval of CD19-targeting CAR T cells for the treatment of large B-cell lymphoma and acute lymphoblastic leukemia (1–7) as well as CAR T cells targeting BCMA in multiple myeloma (8). However, CAR T-cell therapy has had limited success in the treatment of solid tumors (9, 10).

One of the primary challenges for the development of CAR T-cell therapy for solid tumors is the identification of antigens that are highly expressed only on the tumor cells and not on essential normal tissue to avoid on-target off-tumor toxicities. Mesothelin remains one of the highly targeted tumor-associated antigens, and many anti-mesothelin drugs are in clinical trials for treatment of solid tumors (11). It is a glycosyl phosphatidyl inositol (GPI)-anchored protein present on the surface of normal mesothelial cells lining the peritoneum, pericardium, and pleura (12, 13). However, it is overexpressed by a variety of solid tumors, including mesothelioma, pancreatic, lung, and ovarian cancers (14–16), highlighting it as an attractive target for the development of cancer therapeutics (11, 17).

Several mesothelin-directed CAR T-cell therapies are currently being evaluated in clinical trials (18–22), but thus far have not resulted in significant antitumor efficacy. This lack of efficacy could be due to well-defined mechanisms such as tumor antigen expression heterogeneity, limited tumor penetration, lack of T-cell persistence, and their exhaustion (9, 10, 23). However, the role of tumor antigen epitope selection on CAR T-cell efficacy has not been well studied.

Previous studies of monoclonal and bispecific T cell-engager (BiTE) antibodies targeting diverse epitopes on the same antigen have clearly shown that distance from the target cell membrane influences the effector function of antibody-redirected cytotoxic T cells (24–27). Targeting membrane-proximal epitopes results in enhanced antitumor activity, which is attributed to effective formation and function of the cytolytic immune synapse between T cell and the target cell (24, 28, 29). Previous research by Mackall and colleagues at the NCI has shown that anti-CD22 CAR T cells targeting a membrane-proximal epitope had superior antitumor activity compared with those incorporating other binding domains (30).

Because antibodies targeting membrane-distal N-terminal region of mesothelin, region I, can compete with other cellular factors like MUC16 for binding (**Fig. 1A**) (31), focusing antibody design to target membrane-proximal region III of mesothelin is an attractive option. We recently described development of a rabbit mAb, YP218, which targets a membrane-proximal epitope of mesothelin (32). The humanized YP218 mAb, hYP218, was developed using computational tools that allow grafting of the complementarity determining regions of the rabbit antibody sequence into human germline sequences (33). In this study, we describe the development of CAR T-cell therapy using hYP218 single-chain variable fragments (scFvs) and show its superior antitumor activity *in vitro* and *in vivo* when compared with the

[1]Thoracic and GI Malignancies Branch, CCR, NCI, NIH, Bethesda, Maryland. [2]Laboratory of Molecular Biology, CCR, NCI, NIH, Bethesda, Maryland. [3]Laboratory of Pathology, CCR, NCI, NIH, Bethesda, Maryland.

Note: Supplementary data for this article are available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).

Corresponding Author: Raffit Hassan, Thoracic and GI Malignancies Branch, CCR, NCI, 10-CCR Rm. 4E-5330 Bethesda, MD 20892. Phone: 240-760-6232. E-mail: hassanr@mail.nih.gov

Mol Cancer Ther 2022;21:1195–206

doi: 10.1158/1535-7163.MCT-22-0073

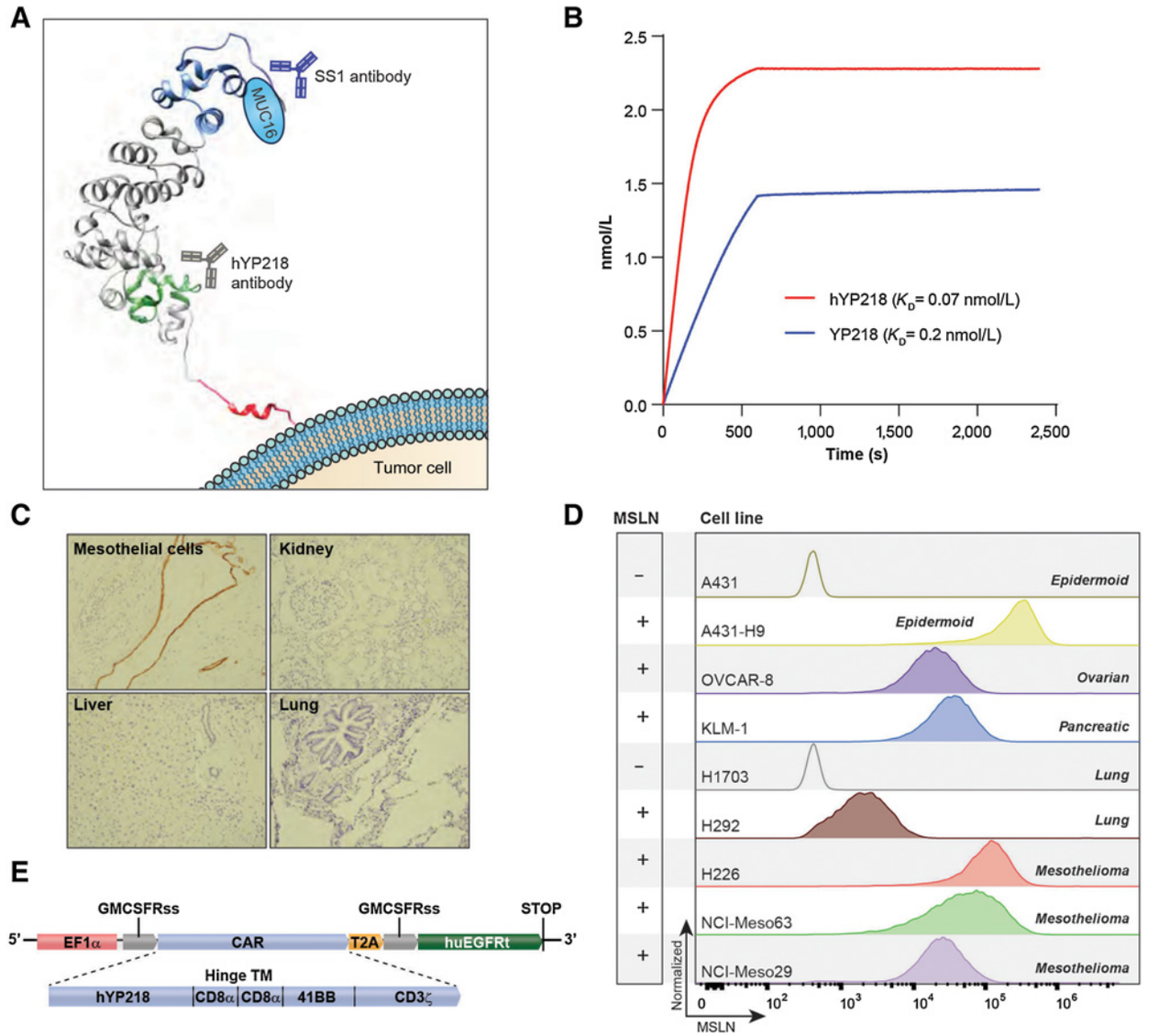

Figure 1.

Mesothelin recognition by YP218 antibody and the development of hYP218 CAR T cells. **A,** SS1 and hYP218 CAR T cells targeting distinct epitopes of mesothelin. Binding site for MUC16 is also indicated. (**B**) Binding affinity of YP218 and hYP218 for mesothelin. hYP218 has higher binding affinity than YP218. **C,** Examples of YP218 antibody reactivity to normal human tissues by IHC. Shown are representative staining of normal mesothelial cells that express mesothelin while as normal tissues of liver, lung, and kidney are negative for mesothelin expression. **D,** Cell surface mesothelin expression on human tumor cell lines by flow cytometry using YP218 antibody. MSLN expression indicated as + or −. A431-H9 is the A431 epidermoid carcinoma cell line transduced for high expression of human mesothelin. **E,** Representation of CAR vector design. GMCSFRss, GMCSF receptor signal sequence, directs surface expression; T2A: self-cleaved peptide sequence; huEGFRt, human EGFR-truncated polypeptide.

membrane-distal targeting SS1 CAR T cells in several different models of mesothelin-expressing cancers.

Materials and Methods

Mice, established tumor cell lines, and patient-derived cell lines

All animal studies were approved by Animal Care and Use Committee of the NCI, NIH (Bethesda, MD). NOD *scid* gamma (NSG) mice were obtained from the Jackson Laboratory. All mice were maintained in a dedicated pathogen-free environment following NIH guidelines approved by the Animal Care and Use Committee of the NCI. Established tumor cell lines OVCAR-8 (received from Dr. Hisataka Kobayashi, Center for Cancer Research, NCI, NIH, Bethesda, MD) and KLM-1 (received from Dr. Christine Alewine, Center for Cancer Research, NCI, NIH, Bethesda, MD) were maintained in RPMI1640 medium with 10% FBS, 2 mmol/L L-glutamine, and 100 U penicillin–streptomycin. Early-passage mesothelioma cell lines, NCI-Meso29 and NCI-Meso63, were established from ascites or pleural fluid obtained from patients with mesothelioma treated at the NCI (Bethesda, MD) on Institutional Review Board (IRB)–approved

protocol (ClinicalTrials.gov NCT 01950572). The methods for establishment of primary culture cell lines have been described previously (34). These cell lines were authenticated using short tandem repeat profiling (35) and regularly tested for the absence of *Mycoplasma*. PBMCs from healthy donors were obtained from the NIH Clinical Center Department of Transfusion Medicine under their IRB approved and consented healthy donor program. PBMCs from patients with mesothelioma were also obtained under IRB-approved protocol (ClinicalTrials.gov NCT 01950572). Studies were conducted in accordance with the Declaration of Helsinki, and informed written consent was obtained from each subject.

Mesothelin epitope recognition and binding affinity of hYP218 mAb

Epitope mapping of hYP218 was performed by ELISA assay. An ELISA plate (Thermo Scientific) was coated with rabbit Fc overnight. Full-length (aa 296–580), recombinant (aa 501–599), and region III (aa 487–580) of MSLN were plated at the concentration of 1 mcg/mL, in the first set of the experiment. Appropriate negative controls (Rabbit IgG and BSA) were used. For narrowing down the binding domain, the mesothelin peptide fragments (501–535, 525–560, 550–581) were plated at the concentration of 1 mcg/mL and further two fold serial dilutions were used. The plate was incubated for 1 hour. hYP218 was subsequently added at 1 mcg/mL, incubated for 1 hour, and binding was detected using goat anti-human kappa light chain HRP conjugate. Results were read using a spectrophotometer at 450-nm wavelength. Binding kinetics of hYP218 and YP218 mAb and cross-competition assays were performed using the Octet RED96 system (FortéBio) at the Biophysics core at the National Heart, Lung, and Blood institute (NHLBI, Supplementary Methods).

Reactivity of YP218 antibody with normal human tissues

To determine the reactivity of YP218 antibody with normal human tissues, we used tissue arrays containing 28 normal human tissues. Immunostaining was done on a Leica Bond automated system. Rabbit mAb YP218 (0.8 mcg/mL) was diluted 1:500. Epitope retrieval was performed with ER2 buffer for 25 minutes, and primary antibody was incubated for 30 minutes. Detection was performed by polymer Refine, and diaminobenzidine was used as the chromogen. Slides were counterstained with hematoxylin. The slides were evaluated for the presence of mesothelin immunoreactivity and intensity (weak or strong). All slides were read by a pathologist with expertise in IHC.

CAR plasmid design, lentivirus transduction, and *ex vivo* expansion of CAR T cells

Antibodies SS1 and YP218 were used to design the scFvs that would target membrane-distal and -proximal epitopes on mesothelin, respectively, and the YP218 sequence was humanized (hYP218) to reduce the immunogenicity for future clinical applications. Second-generation CAR vectors were designed by inserting the scFv sequences in the lentivirus plasmid backbone under the control of EF1α promoter, with CD8α hinge and transmembrane domains, followed by 41BB costimulatory domain and the CD3ζ signaling domain (36). A T2A self-cleaved sequence separated the CAR sequence from a truncated human EGFR polypeptide (hEGFR), which can be used for CAR T-cell tracking *in vitro* and *in vivo*, and CAR T cell ablation in patients if needed for safety. High-titer VSVG pseudotyped lentivirus was produced by Cellomics Technology or Genecopoeia. T cells were expanded *ex vivo* by activating healthy donor or patient-derived PBMCs with anti-CD3/CD28–coated Dynabeads in presence of IL2. Cells were transduced with CAR lentivirus at MOI∼6 in the presence of protamine sulphate. On day 9 posttransduction, the CAR T cells (or untransduced T cells) were used for *in vitro* and *in vivo* studies (Supplementary Methods). Surface expression of human T-cell markers, hEGFR and MSLN was determined by flow cytometry (Supplementary Methods).

Determination of mesothelin expression on tumor cell lines for *in vitro* CAR T-cell functional assays

We used both established tumor cell lines and primary mesothelioma patient-derived tumor cell lines to assess *in vitro* antitumor activity of CAR T cells. Tumor cell lines from a variety of tissue origins were used, including ovarian cancer (OVCAR-8), pancreatic cancer (KLM-1, KLM-1 MSLN-knockout), lung cancer (H292), mesothelioma (H226, NCI-Meso63, NCI-Meso29), and human epidermoid carcinoma cells (A431) transduced for high mesothelin expression (A431-H9). All tumor cells had been transduced with luciferase-expressing lentivirus (Frederick National Laboratory, clone R980-M03–663) and selected with puromycin to express GFP and luciferase, with more than 90% transduction efficiency. Surface mesothelin expression of these tumor cell lines was determined by flow cytometry using either the mouse MN antibody (Rockland) or rabbit YP218 antibody (Supplementary Methods). Quantitation of mesothelin molecules per cell was performed by generating a standard curve using the PE quantikine beads (BD Biosciences, catalog No. 340495).

In vitro assays to assess effector function of CAR T cells

CAR T-cell effector function was determined *in vitro* in a coculture of CAR T cells and tumor cells by (i) measuring direct killing (cytotoxicity) of mesothelin-expressing tumor cells by the CAR T cells and (ii) quantifying the cytokines released by the CAR T cells. For cytotoxicity assays, 100 μL of complete RPMI media containing 5,000 tumor cells were seeded in a 96-well plate. Four to six hours after seeding the tumor cells, 50 μL of media containing CAR T cells or untransduced T cells at a range of effector-to-target (E:T) ratios were added to initiate the co-culture. Wells with tumor cells only, without the CAR T cells, were used as internal plate references. After 20- to 24-hour coculture, luciferase signal from the remaining live tumor cells was determined (Promega, Luciferase Assay System, catalog No. E1501). Percent killing was calculated using the formula: % killing = 100 × [1- relative light units (RLUs) from coculture wells/RLU from target-alone wells]. ELISA kits were used to quantify cytokines IL2, IFNγ, and TNFα (R&D Systems, catalog No. D2050, DIF50C, DTA00D) secreted by the stimulated CAR T cells in the culture supernatant after 20 to 24 hours of coculture with the tumor cells.

In vivo antitumor activity of CAR T cells

Three different human xenograft models were used to assess the *in vivo* antitumor efficacy of CAR T cells, including luciferase-expressing OVCAR-8 (ovarian cancer), luciferase-expressing KLM-1 (pancreatic cancer), and NCI-Meso63 (mesothelioma patient-derived primary tumor). Briefly, 5×10^6 OVCAR-8 tumor cells or 3×10^6 KLM-1 tumor cells were implanted i.p., while 1×10^7 NCI-Meso63 non–luciferase-expressing mesothelioma cells were implanted subcutaneously into 5- to 6-week-old female NSG mice. For luciferase-expressing OVCAR-8 and KLM-1 tumor models, tumor growth was monitored weekly via bioluminescence imaging (PerkinElmer, IVIS Lumina III *In vivo* Imaging System) after intraperitoneal injection of the substrate D-luciferin (Syd Labs, catalog No. MB000102-R70170). NCI-Meso63 subcutaneous tumors were measured weekly using calipers, and the tumor volume was calculated using the formula: tumor volume = length × $(\text{width})^2$ × 0.4. A single intravenous

infusion of 1×10^7 CAR T cells was administered either 2 weeks post-tumor implantation in the OVCAR-8 and KLM-1 tumor–bearing mice, or when tumor volume reached ~180 mm^3 for NCI-Meso63 tumor–bearing mice.

For the tumor rechallenge experiment, 3×10^6 luciferase-expressing KLM-1 tumor cells were implanted intraperitoneally (i.p.) into 5- to 6-week-old female NSG mice, and tumor growth was monitored weekly via bioluminescence imaging. After 14 days, seven mice were treated with 1×10^7 hYP218 CAR T cells. Thirty-five days post CAR T-cell treatment, three of seven mice were rechallenged with i.p. administration of 3×10^6 KLM-1 tumor cells. The other four mice who had complete tumor regression were excluded because they were sick due to graft-versus-host disease, which is often seen in immunodeficient mice receiving human T cells (37, 38). Concurrently, three age-matched mice were also injected with the same number of KLM-1 tumor cells i.p. to compare tumor growth.

Statistical analysis

Unpaired Student *t* test was used to compare the differences between the SS1 and hYP218 CAR T cells in the *in vitro* cytotoxicity and cytokine release assay. Mouse survival differences were compared using log-rank (Mantel-Cox) test. Statistical analysis was performed using GraphPad Prism 9.0. $P < 0.05$ was considered statistically significant.

Data availability statement

The data generated in this study are available within the article and its Supplementary Data files.

Results

Epitope recognition by mAb YP218 and its humanization

YP218 antibody binds to the membrane-proximal region III of mesothelin, while SS1 recognizes a membrane-distal region of mesothelin in the highly immunogenic region I (**Fig. 1A**). For our *in vitro* studies, we generated a humanized version of YP218 (hYP218) mAb. We mapped the epitope of MSLN that is recognized by hYP218 mAb. We used full-length MSLN protein (aa 296–580), recombinant Fc-MSLN (aa 501–599), and region III of MSLN (aa 487–580) in an ELISA assay and found maximum binding of hYP218 to region 501–599 (Supplementary Table S1A). We further narrowed down the binding epitope by using newly synthesized mesothelin fragments (fragments 501–535, 525–560 and 550–581). Our data show that hYP218 binds most strongly around region 525–560 of mesothelin (Supplementary Table S1B). However, hYP218 mAb binding is highly conformation specific; hence, it is difficult to map the exact binding epitope using protein fragments. We next evaluated whether hYP218 competes with MUC16 or HN1. Like SS1, HN1 is also a region I binder that blocks the interaction of MUC16 and mesothelin (39). Cross-competition assays showed hYP218 does not compete with MUC16 or a region I binder (HN1) for mesothelin binding. (Supplementary Table S1C). We also compared the mesothelin binding affinities of hYP218 versus YP218 antibodies. After humanization, hYP218 affinity improved from $K_D = 0.2$ nmol/L (YP218) to $K_D = 0.07$ nmol/L (hYP218; **Fig. 1B**; Supplementary Table S2).

Reactivity of mAb YP218 with normal human tissues and tumor cell lines

Although mesothelin expression on normal human tissues has been previously described, we wanted to be sure that this new anti-mesothelin antibody, YP218, which is being developed for clinical use in patients, did not have different reactivity to human tissues. Except for the normal mesothelial cells, YP218 antibody did not show nonspecific binding to parenchymal organs, indicating the safety of YP218 for clinical use. Immunoreactivity was seen in squamous epithelia of tonsil and thymus, prostatic urethral epithelium, and minimally in bronchial and pancreatic duct epithelia as has been previously described with other anti-mesothelin monoclonal antibodies (**Fig. 1C**; Supplementary Table S3; refs. 13, 15, 16). Next, we tested established and mesothelioma patient-derived solid tumor cell lines for surface mesothelin expression via flow cytometry using YP218 antibody. As shown in **Fig. 1D**, most of our selected tumor cell lines express mesothelin, albeit at different levels. Two cell lines, A431 and H1703, that do not show mesothelin expression, as well as KLM-1 MSLN knock-out cell line were used as negative controls in *in vitro* assays.

Generation of hYP218 and SS1 CAR T cells

We used the scFvs of YP218 and SS1 antibodies to compare the effect of membrane-proximal or membrane-distal targeting of mesothelin, respectively, by the CAR T cells. scFv of SS1 antibody and the humanized version of YP218 antibody were cloned into a second-generation CAR lentivirus vector containing the CD8α hinge and transmembrane domains, 41BB costimulatory domain, and CD3ζ signaling domain (**Fig. 1E**). Transduction efficiency of the *ex vivo* expanded CAR T cells was determined via flow cytometry by staining with the anti-EGFR antibody cetuximab. Using PBMCs from six healthy donors, we performed an initial assessment of the transduction efficiency and *in vitro* antitumor activity of *ex vivo* expanded hYP218 CAR T cells. Transduction efficiency and the percentage of $CD3^+$ T cells in the *ex vivo* culture was assessed on day 8 posttransduction. More than 98% of all the *ex vivo* expanded cells showed surface expression of the pan T-cell marker CD3 (Supplementary Fig. S1A). Transduction efficiency ranged from 32% to 46% between *ex vivo* expanded hYP218 CAR T cells derived from six different healthy donors (Supplementary Fig. S1B). Although the viability of PBMCs posttransduction was similar among all donors (Supplementary Fig. S1C), donor No. 1–derived hYP218 CAR T cells displayed the highest *ex vivo* expansion, and transduction efficiency (Supplementary Fig. S1B and S1D). The *in vitro* activity of hYP218 CAR T cells obtained from these different donors were tested by coculturing them with the mesothelin-expressing cell lines H226, KLM-1, and A431-H9. All the six healthy donor-derived CAR T cells showed cytotoxicity at low E:T ratios (Supplementary Fig. S1E). On the basis of these comparable results, we selected donor 1–derived hYP218 and SS1 CAR T cells for further *in vitro* and *in vivo* studies.

hYP218 CAR T cells have superior antitumor activity *in vitro* than SS1 CAR T cells

Both hYP218 CAR T and SS1 CAR T cells displayed cytolytic activity toward a variety of mesothelin-expressing tumor cells, including patient-derived primary mesothelioma cell lines, NCI-Meso63 and NCI-Meso29 (**Fig. 2A** and **B**). However, hYP218 CAR T cells displayed superior cytolytic activity, compared to SS1 CAR T cells, even at low E:T ratios (**Fig. 2B**). ET_{50}, defined as the E:T ratio to achieve 50% killing of the tumor cells, was significantly lower for the hYP218 CAR T cells than SS1 CAR T cells (Supplementary Table S4), indicating more efficient killing of tumor cells by the hYP218 CAR T cells. In addition, the CAR T cells displayed minimal nonspecific killing of the mesothelin-negative tumor cell lines A431, H1703 and KLM-1 MSLN k/o (**Fig. 2B**). Consistently, untransduced T cells, without the CAR expression, did not show any nonspecific killing of MSLN-positive

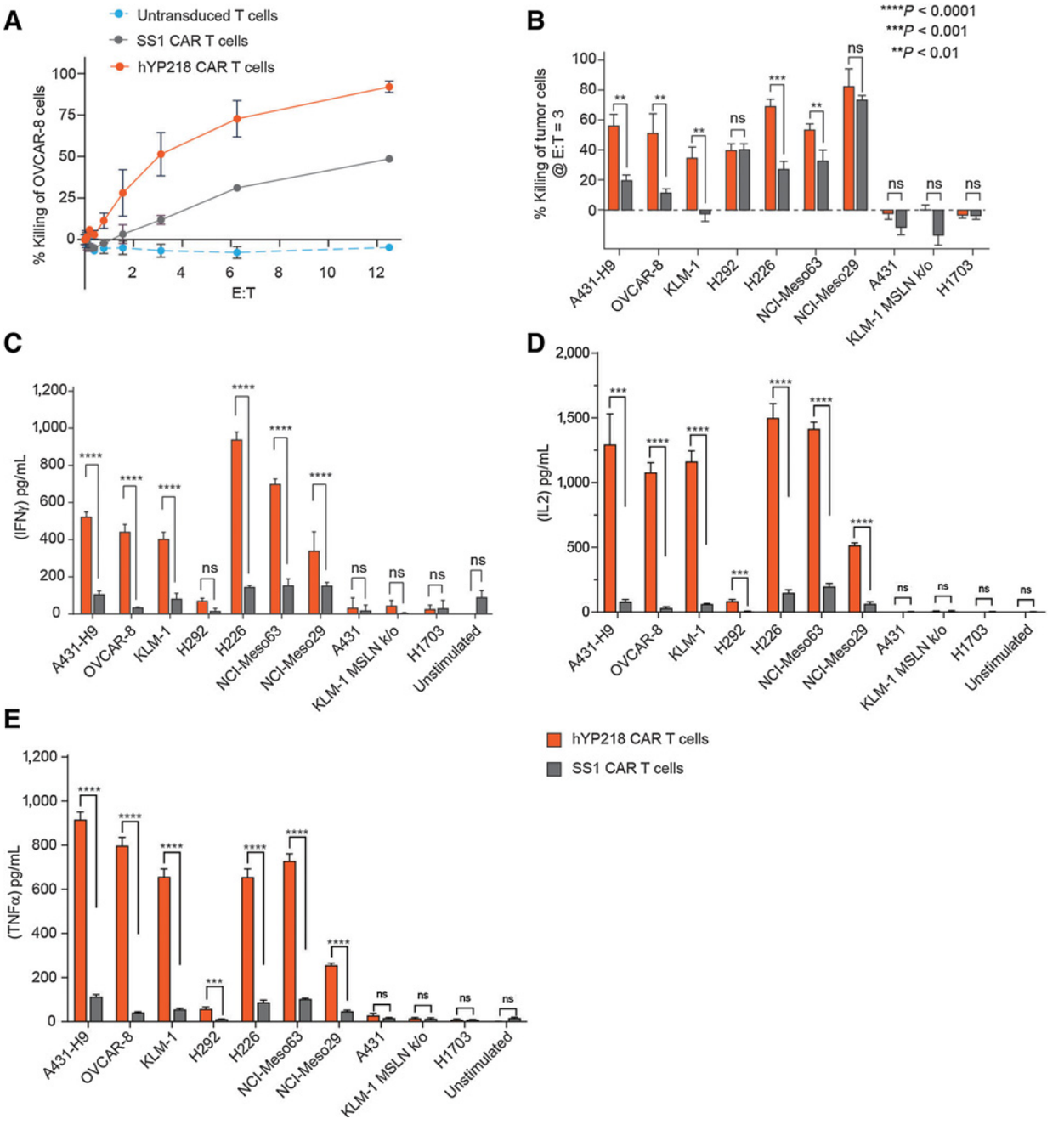

Figure 2.

Comparison of *in vitro* antitumor activity of hYP218 and SS1 CAR T cells. **A,** Killing of mesothelin-expressing OVCAR-8 cells upon coculture with untransduced, SS1 CAR or hYP218 CAR T cells at different E:T ratios. **B,** Percent killing of different mesothelin-expressing tumor cells upon coculture with SS1 CAR or hYP218 CAR T cells at E:T of 3. Mesothelin-negative cell lines A431, KLM1 MSLN k/o, and H1703 were used as negative controls. Release of cytokines IFNγ (**C**), IL2 (**D**), and TNFα (**E**) upon coculture of tumor cells with SS1 CAR or hYP218 CAR T cells.

tumor cells (**Fig. 2A**). Next, we determined the level of cytokines released in the cell culture supernatant upon stimulation of the CAR T cells by the tumor cells. We observed significantly higher levels of all three cytokines (IFNγ, IL2, TNFα) released only upon CAR T-cell stimulation with the mesothelin-positive tumor cells, while unstimulated CAR T cells did not show any cytokine release (**Fig. 2C–E**). In addition, in agreement with the cytolytic activity, the level of cytokines released by the stimulated hYP218 CAR T cells was significantly higher than the stimulated SS1 CAR T cells (**Fig. 2C–E**). hYP218 CAR T cells did not show higher binding to soluble mesothelin (10 mcg/mL of MSLN-hFc) compared with SS1 CAR T cells in a flow cytometry–based assay, suggesting higher cytolytic activity of hYP218 CAR T cells is not

a result of higher binding affinity of hYP218 CAR to mesothelin (Supplementary Fig. S2).

Because several prior studies have shown that intrinsic deficits in T cells of patients with cancer can impair CAR T-cell efficacy (40, 41), we evaluated the efficacy of CAR T cells manufactured using PBMCs from six patients with malignant mesothelioma. As shown in Supplementary Fig. S3A and S3B, for all patient PBMC-derived CAR T cells, hYP218 CAR T cells displayed higher cytolytic activity than SS1 CAR T cells toward mesothelin-expressing OVCAR-8 and KLM-1 tumor cells.

hYP218 CAR T cells lead to significant tumor regression of ovarian and pancreatic cancer cell line–derived xenografts in mice

We used two i.p. tumor models to test the *in vivo* antitumor efficacy of hYP218 and SS1 CAR T cells. Mesothelin-positive OVCAR-8 ovarian cancer cells and KLM-1 pancreatic ductal adenocarcinoma cells were implanted i.p. in 6-week-old female NSG mice. Tumors were allowed to engraft for 2 weeks, followed by a single intravenous infusion of CAR T cells. Tumor burden was monitored weekly using noninvasive bioluminescence imaging (BLI). While the SS1 CAR T-cell treatment only delayed the tumor growth, we observed complete tumor regression within 2 weeks of the hYP218 CAR T-cell treatment in the OVCAR-8 tumor–bearing mice (**Fig. 3A**, **i** and **ii**). BLI imaging done at day 49 posttreatment showed that all hYP218 CAR T-cell–treated mice remained tumor free. Complete OVCAR-8 tumor regression with the hYP218 CAR T cells also provided survival advantage (**Fig. 3A**, **iii**). In the KLM-1 (pancreatic cancer) tumor-bearing mice, although complete tumor regression was not observed in all mice after the hYP218 CAR T-cell treatment, significant tumor regression, delay in tumor growth, and survival benefit was observed until the experiment was terminated (**Fig. 3B**, **i–iii**). BLI imaging done at day 42 showed that in the five mice treated with hYP218 CAR T cells, there was persistent complete tumor regression in two mice, no significant increase in tumor burden in two mice, and tumor progression in one mouse that was euthanized. These results provide clear evidence that targeting of a membrane-proximal epitope on mesothelin by the hYP218 CAR T cells induces a better antitumor response when compared with mesothelin targeting at a membrane-distal epitope by the SS1 CAR T cells.

hYP218 CAR T cells lead to complete tumor regression of mesothelioma patient-derived NCI-Meso63 tumor xenografts in mice

Because differences exist between established tumor cell lines and patient-derived tumor cell lines, we also used mesothelioma patient-derived tumor cell lines as a more patient relevant tumor model to test the antitumor efficacy of hYP218 CAR T cells. First, we made CAR T cells using PBMCs from a patient with mesothelioma and tested the efficacy of these CAR T cells against the patient's own tumor cells, NCI-Meso63. As shown in **Fig. 4A**, both hYP218 and SS1 CAR T cells displayed autologous tumor cell killing at different E:T ratios. The cytolytic activity of hYP218 CAR T cells was significantly higher than the SS1 CAR T cells against these autologous tumor cells.

Next, the patient-derived NCI-Meso63 tumors were implanted subcutaneously in 6-week-old female NSG mice, and the tumors were allowed to grow until the average tumor size reached $\sim$180 mm^3. When the tumors reached the desired volume, CAR T cells were infused intravenously. Three weeks after the hYP218 CAR T-cell treatment, we observed significant reduction in the tumor burden (**Fig. 4B**). Within 4 weeks, hYP218 CAR T-cell–treated mice showed complete regression of the tumors, and these mice remained tumor free until the experiment was terminated. Significant reduction in tumor growth was also observed in the SS1 CAR T-cell treatment group; however, unlike the hYP218 treatment group, complete tumor regression was not observed in all SS1 CAR T-cell–treated mice.

Robust expansion and higher tumor infiltration of hYP218 CAR T cells *in vivo*

Because poor CAR T-cell expansion and infiltration into the solid tumors limit the antitumor efficacy of CAR T cells, we aimed to investigate enhanced CAR T-cell expansion and infiltration as potential mechanisms for higher *in vivo* antitumor efficacy of hYP218 CAR T cells. We assessed peripheral blood and tumors for $hCD45^+$ $hCD3^+$ cells on day 2 and day 7, post CAR T-cell treatment in the KLM-1 tumor model. **Fig. 5A** shows flow cytometry analysis of peripheral blood (left) and tumor cells (right) obtained from a representative mouse in saline, untransduced T cell, SS1 CAR T cell, and hYP218 CAR T-cell treatment groups. We detected a higher proportion of $CD45^+$ $CD3^+$ human T cells in the blood of hYP218 CAR T-cell–treated mouse (8.5%) compared with SS1 CAR T-cell–treated mouse (1.0%) on day 7 posttreatment. There was a several-fold increase in this cell type on day 7 (8.5%) compared with day 2 (0.8%) in hYP218 CAR T-cell–treated mice, showing higher expansion. In contrast, SS1 CAR T-cell–treated mice had almost the same percentage of $hCD45^+$ $hCD3^+$ cells on day 2 (1.1%) and day 7 (1.0%) in peripheral blood. In addition, there was an increase in tumor-infiltrating $hCD45^+$ $hCD3^+$ cells on day 7 (25.7%) in hYP218 CAR T cell–treated mouse versus SS1 CAR T cell–treated mouse (7.4%). Utilizing anti-EGFR antibody in the flow cytometric analysis, we also confirmed that most of the $CD45^+CD3^+$ human T cells detected in the tumor tissues are CAR T cells, with more than 80% cells being hEGFR positive in SS1 CAR T cells as well as hYP218 CAR T-cell–treated mice (**Fig. 5A** right). **Figure 5B** and **C** show combined data of all three mice from each treatment group. There is a significant increase in the absolute number of $CD45^+CD3^+$ human T cells per 100 μL of peripheral blood from day 2 to day 7 in mice treated with hYP218 CAR T cells in comparison to the SS1 CAR T-cell–treated group (**Fig. 5B**, $P < 0.0001$), showing increased cell expansion. Similarly, there was an increase in the percentage of tumor-infiltrating $hCD45^+$ $hCD3^+$ cells in hYP218 CAR T-cell–treated group on day 7 as compared with the SS1 CAR T-cell–treated group, $P < 0.0001$ (**Fig. 5C**). These results suggest peripheral expansion and increased tumor infiltration as a potential mechanism for superior antitumor activity of hYP218 CAR T cells *in vivo*.

Persistence of hYP218 CAR T cells in mice prevents tumor growth upon tumor rechallenge

We evaluated the persistence of hYP218 CAR T cells using the peripheral blood of mice bearing KLM-1 tumors who were treated with hYP218 CAR T cells. On day 35 day posttreatment, we detected the presence of hYP218 CAR T cells in all mice ($n = 5$) ranging from 14% to 61%. **Fig. 6A** shows an example of circulating CAR T cells that were detected in the peripheral blood of a mouse 7 weeks after hYP218 CAR T-cell infusion. To determine whether these persisting hYP218 CAR T cells were still functioning, we evaluated their ability to prevent tumor formation on rechallenge of these mice with KLM-1 tumor cells. The study is shown in **Fig. 6B**. On day 35, two of the hYP218 CAR T-cell–treated mice showing no detectable tumors and one having an incomplete response via BLI imaging were rechallenged with 3×10^6 KLM-1 tumor cells implanted i.p. In addition, NSG mice of the same age were also implanted with KLM-1 tumor cells as a control to

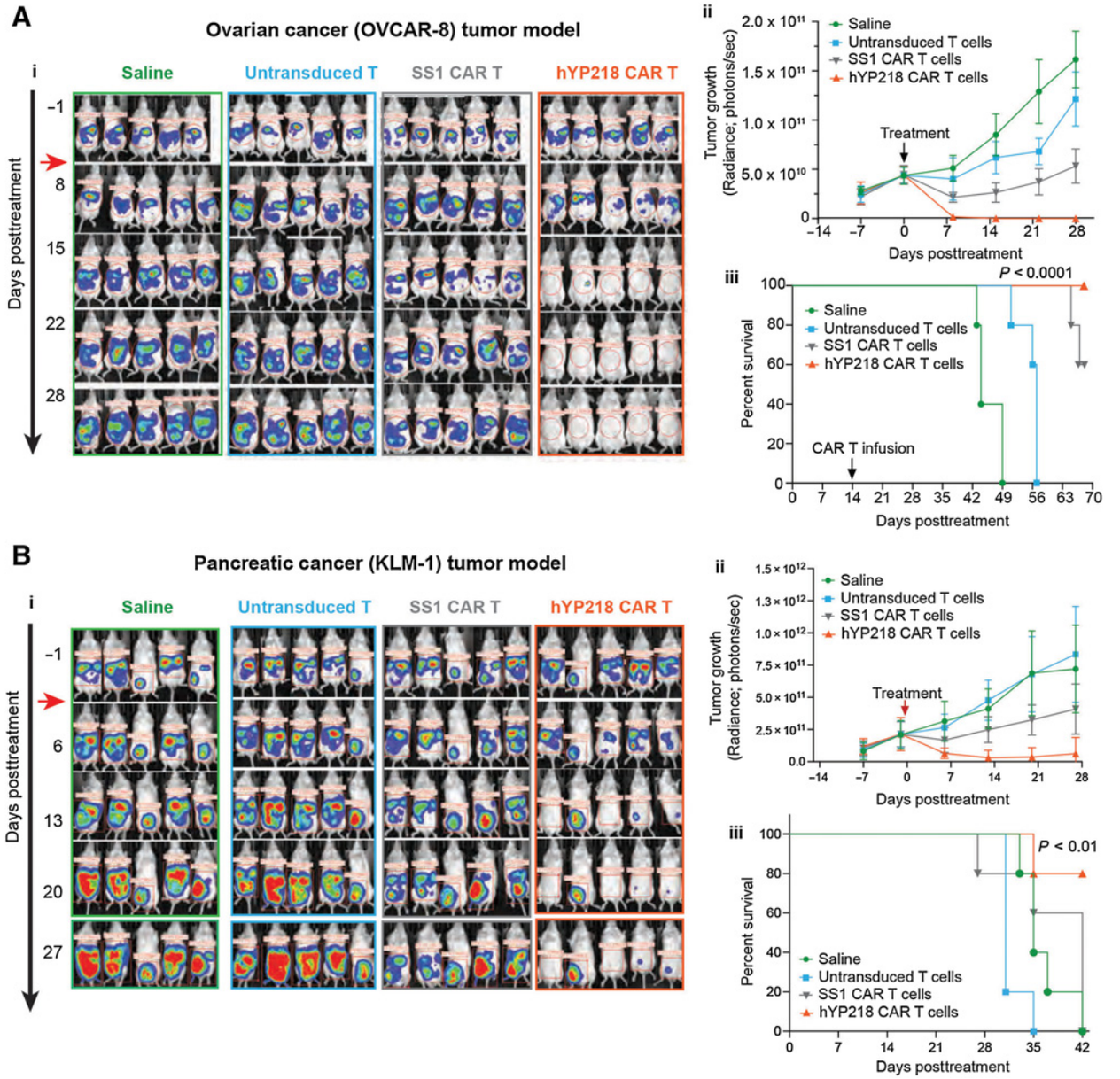

Figure 3.

In vivo antitumor efficacy of hYP218 and SS1 CAR T cells using ovarian and pancreatic cancer mesothelin-expressing tumor models. **A,** Ovarian cancer, OVCAR-8 tumor model. (i) Bioluminescence imaging (BLI) of OVCAR-8 tumor growth in NSG mice treated with saline, untransduced T cells, SS1 CAR T and hYP218 CAR T cells, red arrow indicates CAR T-cell infusion on day 14 after tumor implantation. (ii) Kinetics of OVCAR-8 tumor growth. (iii) Overall survival of OVCAR-8 tumor–implanted mice in different treatment groups ($P \leq 0.0001$ hYP218 CAR T cell versus SS1 CAR T-cell–treated mice). **B,** Pancreatic cancer, KLM-1 tumor model. (i) BLI of KLM-1 tumor growth in NSG mice treated with saline, untransduced T cells, SS1 CAR T, and hYP218 CAR T cells, red arrow indicates CAR T-cell infusion on day 14 after tumor implantation. (ii) Kinetics of KLM-1 tumor growth. (iii) Overall survival of KLM-1 tumor–implanted mice in different treatment groups ($P \leq 0.01$ hYP218 CAR T cell vs. SS1 CAR T-cell–treated mice).

monitor tumor growth. As shown in **Fig. 6C** and **D**, two of the hYP218 CAR T-cell–treated mice with prior complete tumor regression had no tumor growth when rechallenged with KLM-1 tumor cells, and there was also no significant increase in the tumor burden of the mouse with residual tumor on rechallenge with KLM-1 tumor cells. As expected, there was tumor growth in the age-matched NSG mice who had not received prior hYP218 CAR T cells. These results show that persisting hYP218 CAR T cells can inhibit further tumor implantation.

Discussion

In this study, we showed that hYP218 CAR T cells targeting a membrane-proximal epitope of the tumor antigen mesothelin are

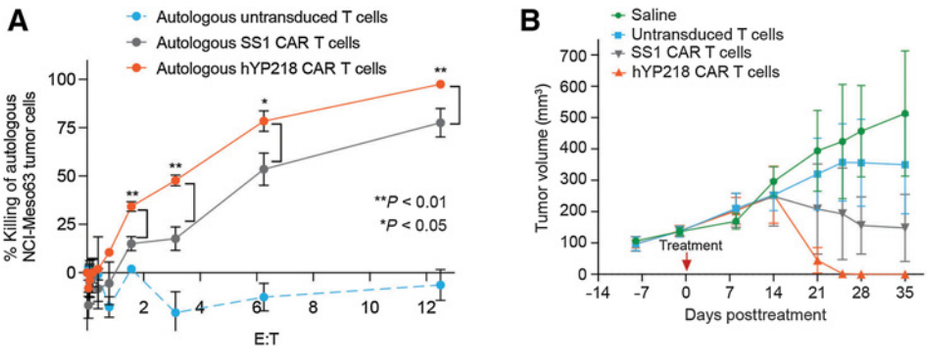

Figure 4.

Antitumor efficacy of hYP218 and SS1 CAR T cells using a mesothelioma patient–derived cell line xenograft model. **A,** Killing of mesothelioma patient-derived NCI-Meso63 tumor cells upon coculture with autologous untransduced, SS1 CAR or hYP218 CAR T cells derived from the PBMCs from the same patient at different E:T ratios. **B,** Kinetics of NCI-Meso63 mesothelioma tumor growth in mice implanted with subcutaneous NCI-Meso63 cells. Untransduced T cells, hYP218 CAR, or SS1 CAR T cells were infused on day 14 after tumor implantation. $n = 5$ mice per treatment group.

highly effective and can mediate complete tumor regression in several mesothelin-expressing solid tumors. Identical CAR T cells, SS1 CAR T, that target a membrane-distal epitope of mesothelin were less effective. In addition, treatment with hYP218 CAR T cells was associated with increased tumor infiltration, persistence, and antitumor immunity. These results clearly show that where the antibody fragment binds to mesothelin has a major impact on the efficacy of CAR T-cell therapy.

Although multiple mechanisms for the lack of efficacy of CAR T-cell therapy have been described (9, 10, 23), we focused our investigation to determine whether selection of scFvs directed to different epitopes of mesothelin could affect CAR T-cell efficacy. We hypothesized that CAR T cells targeting a membrane-proximal epitope of mesothelin could be more efficacious as has been previously described for CD22- and GPC3-targeting CAR T cells (30, 36). However, to do so, it is important to generate scFvs that bind the region of mesothelin close to the cell membrane. The distal N-terminal part of membrane-bound mesothelin, region I is the most immunogenic part of the protein, and it is very rare to get antibodies that bind the proximal portion, region III of mesothelin, close to cell membrane. Because rabbit mAbs can recognize poorly immunogenic epitopes of human antigens, we immunized rabbits with rabbit Fc-human mesothelin fusion protein and identified 223 mAbs that bind region I and only three that bind region III, close to the cell membrane (32). We therefore used one of these three antibodies, YP218 for further preclinical development. To make the YP218 antibody useful for the clinic, we generated the humanized version, hYP218 and showed that it increased the affinity of the antibody (**Fig. 1B**). Although the expression of mesothelin on normal human tissues has been well described, we decided to further study it in case this new antibody reacted to other normal tissues in addition to what has been reported (13, 15, 16). As described in this article, YP218 shows a limited reactivity to normal mesothelial cells, some squamous epithelia, minimal reactivity with bronchial, prostatic urethral, and pancreatic duct epithelia supporting its development for clinical use.

Having generated the humanized rabbit mAb, hYP218, binding the membrane-proximal region of mesothelin, we decided to evaluate whether this could lead to increased efficacy of CAR T cells. In addition, we wanted to directly compare it with similar CAR T cells generated, using the SS1 anti-mesothelin scFv that binds to the membrane-distal region I of mesothelin (32). We chose the SS1 scFv, as CAR T cells using this scFv have been evaluated in patients but have shown very little efficacy (18–20, 22, 42). In contrast to SS1 CAR T cells, hYP218 CAR T cells displayed an increased cytotoxic activity against a variety of mesothelin-expressing cell lines as well as mesothelioma patient–derived cell lines. In comparison to SS1 CAR T cells, hYP218 CAR T cells were highly effective against mesothelin-expressing ovarian and pancreatic *in vivo* tumor models as well a mesothelioma patient-derived xenograft tumor model. This antitumor efficacy was accompanied by expansion of hYP218 CAR T cells in the peripheral blood as well as increase in tumor infiltration, both of which were significantly greater than with SS1 CAR T cells. In addition, the persistence of hYP218 CAR T cells in treated mice prevented tumor growth upon tumor rechallenge.

Our work is supported by another recent report, using our published YP218 antibody sequence, which showed CAR T cells to the membrane-proximal epitope of mesothelin are more effective in a mouse model of gastric cancer (43, 44). Their results using a different CAR construct and different experimental conditions confirm our work that CAR T cells against a membrane-proximal epitope are more effective than CAR T cells targeting a distal epitope. In addition, our studies provide a mechanistic basis of their efficacy including increased tumor infiltration.

It is possible that at higher dose levels in patients, there could be potential side effects due to the increased potency of the hYP218 CAR T cells. However, the hYP218 CAR construct contains a truncated human EGFR, that can be targeted using the anti-EGFR antibody cetuximab to ablate the CAR T cells in case of unexpected serious toxicity (33, 45). On the other hand, increased potency of the hYP218 CAR T cells could result in rapid tumor clearance and therefore prevent chronic antigen exposure and upregulation of exhaustion markers in the infused CAR T cells (46, 47). However, this will need to be evaluated in well-designed clinical trials that include study of T-cell persistence, exhaustion, and function in patients.

Although we show superior activity of hYP218 CAR T cells in different tumor xenograft models, these studies are limited by the fact that we used immunodeficient mice. It is well known that the tumor immune environment has significant effect on CAR T-cell efficacy (10, 48). Given the lack of such models, we are currently developing mouse models of mesothelin-expressing tumors to evaluate the efficacy of anti-mesothelin CAR T cells. Similarly, it will be important to determine whether increased persistence and

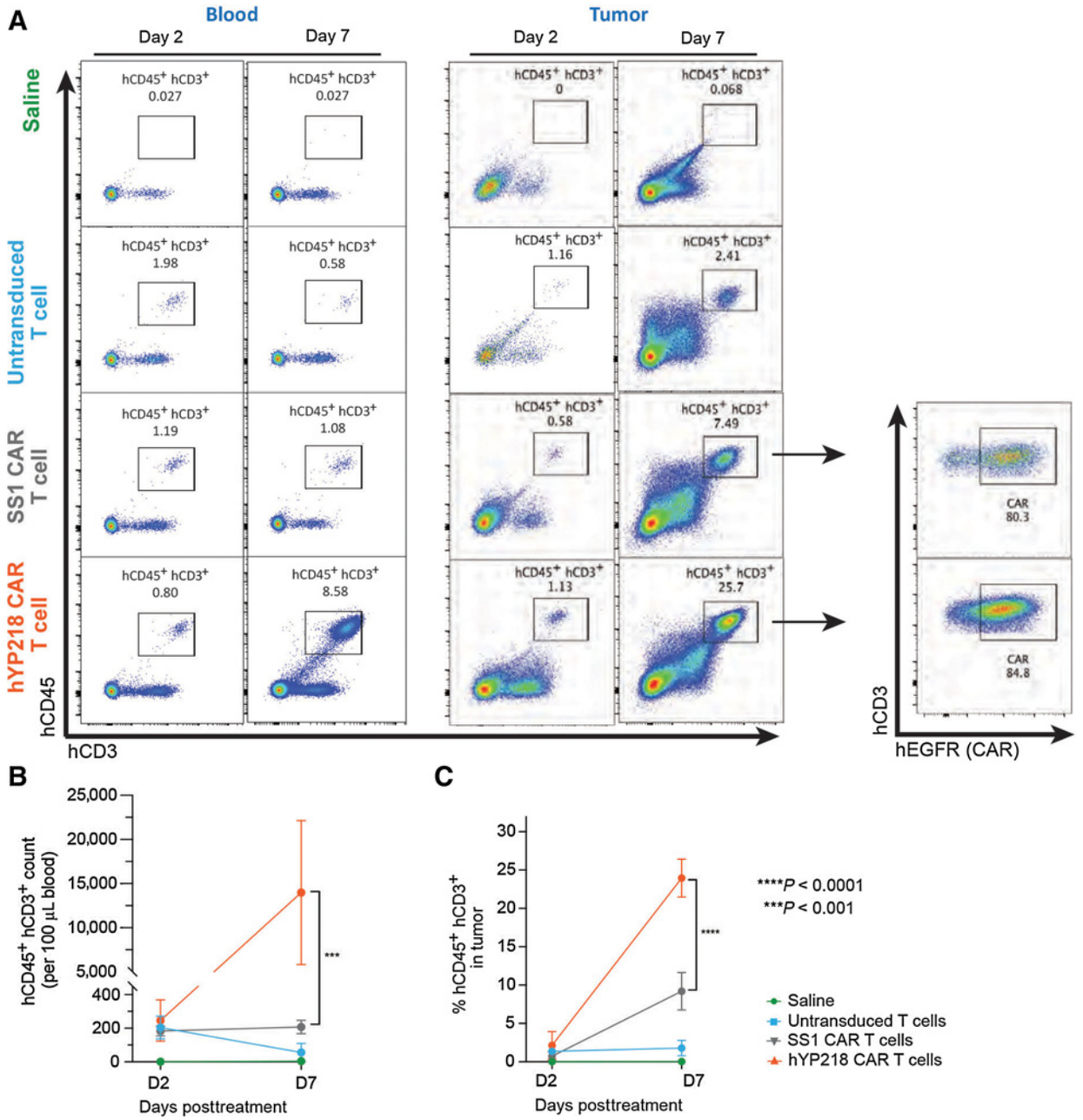

Figure 5.

In vivo tumor infiltration and persistence of CAR T cells in the blood and tumors of KLM-1 tumor-bearing mice. **A,** Representative flow cytometry analysis ($n = 1$ mouse) detecting the presence of hCD45$^+$ hCD3$^+$ double-positive human T cells in the blood (left) and tumors (center) of NSG mice implanted with KLM-1 tumors, on days 2 and day 7 after CAR T-cell infusion. Gating strategy: live cells → single cells → live/dead stain → hCD45$^+$ hCD3$^+$. Percentage of CAR$^+$ T cells within the hCD45$^+$ hCD3$^+$ double-positive population from KLM-1 tumors of NSG mice on day 7 after CAR T-cell infusion (right). **B,** Change in the number of hCD45$^+$ hCD3$^+$ human T cells in 100 µL of blood from day 2 to day 7 after CAR T-cell infusion ($n = 3$). **C,** Change in the percentage of tumor-infiltrating hCD45$^+$ hCD3$^+$ human T cells from day 2 to day 7 after CAR T-cell infusion ($n = 3$).

tumor accumulations is also seen in patients treated with hYP218 CAR T cells. The precise molecular mechanism that leads to the expansion and persistence of hYP218 CAR T cells, which differ from SS1 CAR T cells only in the mesothelin epitope recognized, is unclear and needs further study.

Adoptive T-cell therapy using anti-mesothelin CAR T cells is an area of active investigation to treat solid tumors, but thus far, most of these studies have shown limited efficacy in patients (18–20, 22). A recently completed study reported on the results of mesothelin targeted CAR T cells using a CAR construct consisting

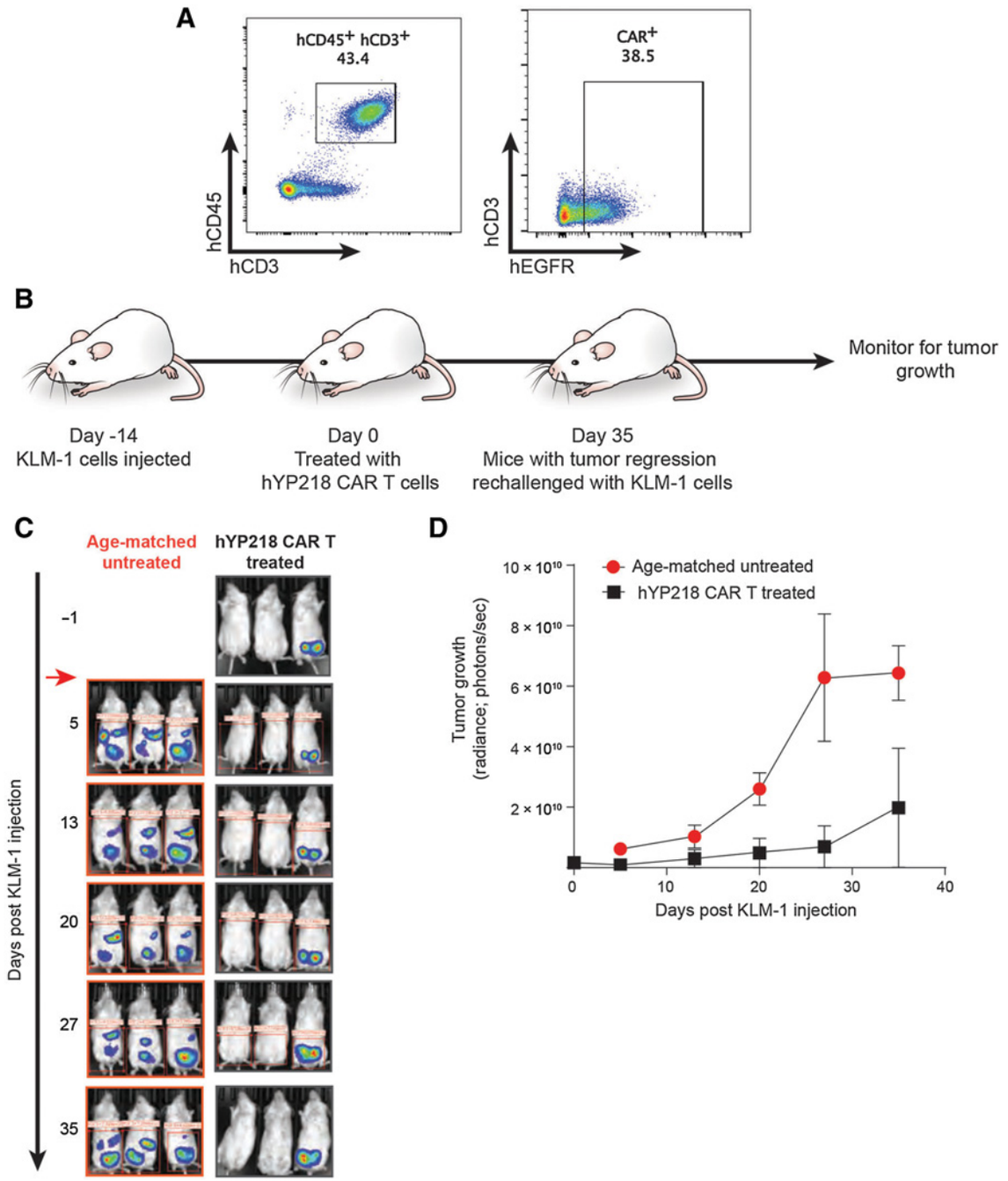

Figure 6.

hYP218 CAR T-cell therapy provides antitumor immunity. **A,** Representative flow cytometry of peripheral blood cells obtained from a mouse with KLM-1 tumor that was treated with hYP218 CAR T cells and stained with hCD45, hCD3, and hEGFR to detect hYP218 CAR T cells. At 7 weeks posttreatment, 38.5% of CAR T cells were present in the peripheral blood. **B,** Representation of the tumor rechallenge experiment. Mice that responded to hYP218 CAR T cells at day 35 were rechallenged with KLM-1 tumor cells and followed for tumor growth. *In vivo* imaging (**C**) and total radiance (**D**) measured in photons/second in age matched untreated mice as well as mice previously treated with hYP218 CAR T cells. There was tumor progression in the age-matched untreated group but not in mice previously treated with hYP218 CAR T cells.

of the human anti-mesothelin scFv m912, CD28/CD3ζ and iCaspase-9 (21). Using an innovative approach of intrapleural administration, the authors showed it to be safe and well tolerated. Interestingly, in some patients who received pembrolizumab off protocol after administration of intrapleural CAR T cells, there appeared to be increased antitumor activity. These results warrant further studies of anti-mesothelin CAR T cells with PD-1–blocking agents.

In conclusion, our results show that the mesothelin epitopes recognized by CAR T cells has a significant impact on their efficacy. In addition, our preclinical development of hYP218 CAR T cells shows that it has several properties that could make it useful in the clinic: (i) use of the humanized YP218 to decrease allergic reactions in patients, (ii) increased potency that may decrease the number of CAR T cells administered to patients and therefore side effects, and (iii) increased persistence in blood and better accumulation in tumors. Whether these favorable properties translate to increased activity in patients will be evaluated in an upcoming clinical trial of hYP218 CAR T cells.

Authors' Disclosures

R. Hassan reports other support from Bayer AG and other support from TCR^2 Therapeutics outside the submitted work; in addition, R. Hassan has a patent for WO2014031476 issued, licensed, and with royalties paid to NIH. N. Li reports a patent for WO2019094482 Chimeric antigen receptors for targeting tumor antigens issued to the NIH. I. Pastan reports a patent for WO2014031476 (Ho, M., Pastan, I., Phung, Y., Zhang, Y., Gao, W., and Hassan, R.) Mesothelin domain-specific monoclonal antibodies and use thereof issued to the NIH. M. Ho reports a patent for WO2014031476 (Ho, M., Pastan, I., Phung, Y., Zhang, Y., Gao, W., and Hassan, R.) Mesothelin domain-specific monoclonal antibodies and use thereof issued to NIH and a patent for WO2019094482 (Ho, M., Li, N., and Li, D.) Chimeric antigen receptors for targeting tumor antigens issued to the NIH. No disclosures were reported by the other authors.

Authors' Contributions

S. Tomar: Conceptualization, methodology, writing–original draft, writing–review and editing. **J. Zhang:** Methodology, writing–review and editing. **M. Khanal:** Methodology, writing–review and editing. **J. Hong:** Methodology, writing–review and editing. **A. Venugopalan:** Methodology, writing–review and editing. **Q. Jiang:** Methodology, writing–review and editing. **M. Sengupta:** Writing–review and editing. **M. Miettinen:** Methodology, writing–review and editing. **N. Li:** Methodology, writing–review and editing. **I. Pastan:** Conceptualization, funding acquisition, methodology, writing–review and editing. **M. Ho:** Conceptualization, funding acquisition, methodology, writing–review and editing. **R. Hassan:** Conceptualization, resources, supervision, funding acquisition, validation, investigation, visualization, methodology, writing–review and editing.

Acknowledgments

This work was supported by the Intramural Research Program of the NIH, NCI, Center for Cancer Research (CCR; Z01-BC-006150), and by the Cancer Moonshot program through the NCI CCR Center for Cell-based Therapy.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received January 25, 2022; revised March 9, 2022; accepted April 18, 2022; published first May 2, 2022.

References

1. Park JH, Riviere I, Gonen M, Wang X, Senechal B, Curran KJ, et al. Long-term follow-up of CD19 CAR therapy in acute lymphoblastic leukemia. N Engl J Med 2018;378:449–59.
2. Neelapu SS, Locke FL, Bartlett NL, Lekakis LJ, Miklos DB, Jacobson CA, et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. N Engl J Med 2017;377:2531–44.
3. Schuster SJ, Svoboda J, Chong EA, Nasta SD, Mato AR, Anak O, et al. Chimeric antigen receptor T cells in refractory B-cell lymphomas. N Engl J Med 2017;377: 2545–54.
4. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. N Engl J Med 2018;378:439–48.
5. Schuster SJ, Bishop MR, Tam CS, Waller EK, Borchmann P, McGuirk JP, et al. Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. N Engl J Med 2019;380:45–56.
6. Shah BD, Ghobadi A, Oluwole OO, Logan AC, Boissel N, Cassaday RD, et al. KTE-X19 for relapsed or refractory adult B-cell acute lymphoblastic leukaemia: phase 2 results of the single-arm, open-label, multicentre ZUMA-3 study. Lancet 2021;398:491–502.
7. Abramson JS, Palomba ML, Gordon LI, Lunning MA, Wang M, Arnason J, et al. Lisocabtagene maraleucel for patients with relapsed or refractory large B-cell lymphomas (TRANSCEND NHL 001): a multicentre seamless design study. Lancet 2020;396:839–52.
8. Raje N, Berdeja J, Lin Y, Siegel D, Jagannath S, Madduri D, et al. Anti-BCMA CAR T-cell therapy bb2121 in relapsed or refractory multiple myeloma. N Engl J Med 2019;380:1726–37.
9. Long KB, Young RM, Boesteanu AC, Davis MM, Melenhorst JJ, Lacey SF, et al. CAR T cell therapy of non-hematopoietic malignancies: detours on the road to clinical success. Front Immunol 2018;9:2740.
10. Schmidts A, Maus MV. Making CAR T cells a solid option for solid tumors. Front Immunol 2018;9:2593.
11. Hassan R, Thomas A, Alewine C, Le DT, Jaffee EM, Pastan I. Mesothelin immunotherapy for cancer: ready for prime time? J Clin Oncol 2016;34:4171–9.
12. Chang K, Pastan I. Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers. Proc Natl Acad Sci U S A 1996;93:136–40.
13. Chang K, Pai LH, Pass H, Pogrebniak HW, Tsao MS, Pastan I, et al. Monoclonal antibody K1 reacts with epithelial mesothelioma but not with lung adenocarcinoma. Am J Surg Pathol 1992;16:259–68.
14. Hassan R, Bera T, Pastan I. Mesothelin: a new target for immunotherapy. Clin Cancer Res 2004;10(12 Pt 1):3937–42.
15. Ordonez NG. Application of mesothelin immunostaining in tumor diagnosis. Am J Surg Pathol 2003;27:1418–28.
16. Weidemann S, Gagelmann P, Gorbokon N, Lennartz M, Menz A, Luebke AM, et al. Mesothelin expression in human tumors: a tissue microarray study on 12,679 tumors. Biomedicines 2021;9:397.
17. Morello A, Sadelain M, Adusumilli PS. Mesothelin-targeted CARs: driving T cells to solid tumors. Cancer Discov 2016;6:133–46.
18. Maus MV, Haas AR, Beatty GL, Albelda SM, Levine BL, Liu X, et al. T cells expressing chimeric antigen receptors can cause anaphylaxis in humans. Cancer Immunol Res 2013;1:26–31.
19. Beatty GL, Haas AR, Maus MV, Torigian DA, Soulen MC, Plesa G, et al. Mesothelin-specific chimeric antigen receptor mRNA-engineered T cells induce anti-tumor activity in solid malignancies. Cancer Immunol Res 2014;2:112–20.
20. Beatty GL, O'Hara MH, Lacey SF, Torigian DA, Nazimuddin F, Chen F, et al. Activity of mesothelin-specific chimeric antigen receptor T cells against pancreatic carcinoma metastases in a phase 1 Trial. Gastroenterology 2018; 155:29–32.
21. Adusumilli PS, Zauderer MG, Riviere I, Solomon SB, Rusch VW, O'Cearbhaill RE, et al. A phase I trial of regional mesothelin-targeted CAR T-cell therapy in patients with malignant pleural disease, in combination with the anti-PD-1 agent pembrolizumab. Cancer Discov 2021;11:2748–63.
22. Haas AR, Tanyi JL, O'Hara MH, Gladney WL, Lacey SF, Torigian DA, et al. Phase I study of lentiviral-transduced chimeric antigen receptor-modified T cells recognizing mesothelin in advanced solid cancers. Mol Ther 2019;27: 1919–29.
23. Watanabe K, Kuramitsu S, Posey AD Jr., June CH. Expanding the therapeutic window for CAR T cell therapy in solid tumors: the knowns and unknowns of CAR T cell biology. Front Immunol 2018;9:2486.
24. Bluemel C, Hausmann S, Fluhr P, Sriskandarajah M, Stallcup WB, Baeuerle PA, et al. Epitope distance to the target cell membrane and antigen size determine the potency of T cell-mediated lysis by BiTE antibodies specific for a large melanoma surface antigen. Cancer Immunol Immunother 2010;59: 1197–209.
25. Cleary KLS, Chan HTC, James S, Glennie MJ, Cragg MS. Antibody distance from the cell membrane regulates antibody effector mechanisms. J Immunol 2017;198: 3999–4011.

26. Hatterer E, Chauchet X, Richard F, Barba L, Moine V, Chatel L, et al. Targeting a membrane-proximal epitope on mesothelin increases the tumoricidal activity of a bispecific antibody blocking CD47 on mesothelin-positive tumors. MAbs 2020; 12:1739408.
27. Qi J, Li X, Peng H, Cook EM, Dadashian EL, Wiestner A, et al. Potent and selective antitumor activity of a T cell-engaging bispecific antibody targeting a membrane-proximal epitope of ROR1. Proc Natl Acad Sci U S A 2018;115: E5467-E76.
28. Xiong W, Chen Y, Kang X, Chen Z, Zheng P, Hsu YH, et al. Immunological synapse predicts effectiveness of chimeric antigen receptor cells. Mol Ther 2018; 26:963–75.
29. Li J, Stagg NJ, Johnston J, Harris MJ, Menzies SA, DiCara D, et al. Membrane-proximal epitope facilitates efficient T cell synapse formation by anti-FcRH5/ CD3 and is a requirement for myeloma cell killing. Cancer Cell 2017;31:383–95.
30. Haso W, Lee DW, Shah NN, Stetler-Stevenson M, Yuan CM, Pastan IH, et al. Anti-CD22-chimeric antigen receptors targeting B-cell precursor acute lymphoblastic leukemia. Blood 2013;121:1165–74.
31. Kaneko O, Gong L, Zhang J, Hansen JK, Hassan R, Lee B, et al. A binding domain on mesothelin for CA125/MUC16. J Biol Chem 2009;284:3739–49.
32. Zhang YF, Phung Y, Gao W, Kawa S, Hassan R, Pastan I, et al. New high affinity monoclonal antibodies recognize non-overlapping epitopes on mesothelin for monitoring and treating mesothelioma. Sci Rep 2015;5:9928.
33. Zhang YF, Ho M. Humanization of rabbit monoclonal antibodies via grafting combined Kabat/IMGT/Paratome complementarity-determining regions: Rationale and examples. MAbs 2017;9:419–29.
34. Zhang J, Khanna S, Jiang Q, Alewine C, Miettinen M, Pastan I, et al. Efficacy of anti-mesothelin immunotoxin RG7787 plus nab-paclitaxel against mesothelioma patient-derived xenografts and mesothelin as a biomarker of tumor response. Clin Cancer Res 2017;23:1564–74.
35. Rathkey D, Khanal M, Murai J, Zhang J, Sengupta M, Jiang Q, et al. Sensitivity of mesothelioma cells to PARP inhibitors is not dependent on BAP1 but is enhanced by temozolomide in cells with high-schlafen 11 and low-O6-methylguanine-DNA methyltransferase expression. J Thorac Oncol 2020;15:843–59.
36. Li D, Li N, Zhang YF, Fu H, Feng M, Schneider D, et al. Persistent polyfunctional chimeric antigen receptor T cells that target glypican 3 eliminate orthotopic hepatocellular carcinomas in mice. Gastroenterology 2020;158:2250–65.
37. Covassin L, Laning J, Abdi R, Langevin DL, Phillips NE, Shultz LD, et al. Human peripheral blood CD4 T cell-engrafted non-obese diabetic-scid IL2rγ(null) H2-Ab1 (tm1Gru) Tg (human leucocyte antigen D-related 4) mice: a mouse model of human allogeneic graft-versus-host disease. Clin Exp Immunol 2011; 166:269–80.
38. Ali R, Babad J, Follenzi A, Gebe JA, Brehm MA, Nepom GT, et al. Genetically modified human CD4(+) T cells can be evaluated in vivo without lethal graft-versus-host disease. Immunology 2016;148:339–51.
39. Ho M, Feng M, Fisher RJ, Rader C, Pastan I. A novel high-affinity human monoclonal antibody to mesothelin. Int J Cancer 2011;128:2020–30.
40. Das RK, Vernau L, Grupp SA, Barrett DM. Naïve T-cell deficits at diagnosis and after chemotherapy impair cell therapy potential in pediatric cancers. Cancer Discov 2019;9:492–9.
41. Arcangeli S, Falcone L, Camisa B, De Girardi F, Biondi M, Giglio F, et al. Next-generation manufacturing protocols enriching TSCM CAR T cells can overcome disease-specific T cell defects in cancer patients. Front Immunol 2020;11.
42. Ko AH, Jordan AC, Tooker E, Lacey SF, Chang RB, Li Y, et al. Dual targeting of mesothelin and CD19 with chimeric antigen receptor-modified T cells in patients with metastatic pancreatic cancer. Mol Ther 2020;28:2367–78.
43. Zhang Z, Jiang D, Yang H, He Z, Liu X, Qin W, et al. Modified CAR T cells targeting membrane-proximal epitope of mesothelin enhances the antitumor function against large solid tumor. Cell Death Dis 2019;10:476.
44. Zhang Z, Jiang D, Yang H, He Z, Liu X, Qin W, et al. Correction: Modified CAR T cells targeting membrane-proximal epitope of mesothelin enhances the antitumor function against large solid tumor. Cell Death Dis 2020;11:235.
45. Wang X, Chang W-C, Wong CW, Colcher D, Sherman M, Ostberg JR, et al. A transgene-encoded cell surface polypeptide for selection, in vivo tracking, and ablation of engineered cells. Blood 2011;118:1255–63.
46. Good CR, Aznar MA, Kuramitsu S, Samareh P, Agarwal S, Donahue G, et al. An NK-like CAR T cell transition in CAR T cell dysfunction. Cell 2021;184: 6081–100.e26.
47. Blank CU, Haining WN, Held W, Hogan PG, Kallies A, Lugli E, et al. Defining 'T cell exhaustion'. Nat Rev Immunol 2019;19:665–74.
48. Labanieh L, Majzner RG, Mackall CL. Programming CAR-T cells to kill cancer. Nat Biomed Eng 2018;2:377–91.