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ARTICLE

Distinct functions of CAR-T cells possessing a dectin-1 intracellular signaling domain

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Abstract

Chimeric antigen receptor T (CAR-T) cell therapy has demonstrated remarkable efficacies in treating hematopoietic malignancies, but not in the solid tumors. Incorporating costimulatory signaling domains, such as ICOS or 4-1BB, can positively influence CAR-T cell functions and then the immune responses. These CAR-engineered T cells have showed their enhanced persistence and effector functions with improved antitumor activities, and provided a new approach for the treatment of solid tumors. Here, we designed novel 2nd generation CARs with a costimulatory signaling molecule, dectin-1. The impacts of dectin-1 signaling domain on CAR-T cells were evaluated in vitro and in vivo. Our data show that in vitro cytokine secretions by HER2 or CD19 specific CAR-T cells increase significantly via incorporating this dectin-1 signaling domain. Additional properties of these novel CAR-T cells are affected by this costimulatory domain. Compared with a popular reference (i.e., anti-HER2 CAR-T cells with 4-1BB), in vitro T cell functions and in vivo antitumor activity of the dectin-1 engineered CAR-T cells are similar to the 4-1BB based, and both are discrete to the mock T cells. Furthermore, we found that the CAR-T cells with dectin-1 show distinct phenotype and exhaustion marker expression. These collective results suggest that the incorporation of this new signaling domain, dectin-1, into the CARs may provide the clinical potential of the CAR-T cells through this signaling domain in treating solid tumors.

Introduction

Chimeric antigen receptor (CAR) T cell immunotherapy has achieved remarkable progress in treating hematologic malignancies, including non-Hodgkin lymphoma (NHL), B-cell acute lymphoblastic leukemia (ALL), multiple myeloma (MM) and chronic Lymphocytic leukemia (CLL). So far, the objective response rate (ORR) in CD19-specific or BCMA-specific CAR-T clinical trials ranges from 48 to 95% [1–6]. Two CAR-T cell products, specific to the B lymphoma, Axicabtagene Ciloleucel (KTE-C19, Kite Pharma) and Tisagenlecleuce (CTL019, Novartis), were approved by the U.S. Food and Drug Administration in 2017 [7, 8]. In a phase 1/2 trial with Axicabtagene ciloleucel, 2-year follow-up data, involving 108 patients with the refractory large B-cell lymphoma, showed that 83% of patients had an objective response, and 58% of them had a complete response, with a median follow-up of 15.4 months (IQR 13.7–17.3) [9]. This suggests that CAR-T cell therapy can maintain a long-term remission.

However, limited success was observed in the CAR-T cell therapy in treating solid tumors. There are a lot of challenges in the application of CAR-T cell therapy in the solid tumors, such as lack of appropriate tumor-specific antigen, inhibition of tumor microenvironment, and insufficient CAR-T cell localization and persistence [10–12]. In addition, continuous antigen exposure can result in CAR-T cell exhaustion, and then compromising the effectiveness of CAR-T cells. Therefore, new approaches are necessary to design CARs in the treatment of solid tumors.

One costimulatory signaling molecule, 4-1BB, has utilized in the 2nd generation CARs, and revealed its potential in the CAR-T cell immunotherapy [13, 14]. In recent work, a new molecule, dectin-1, one of the characterized C-type

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lectin receptors (CLRs) was identified and investigated [15, 16]. The pattern recognition receptor dectin-1 is a type-II transmembrane protein, expressed on neutrophils, macrophages and dendritic cells [17]. The dectin-1 is specific for β-glucans, which are glucose polymers consisting of β-1,6-glucan and β-1,3-glucan expressed on the cell wall of fungi [18]. Dectin-1 consists of the extracellular domain, transmembrane domain and intracellular domain [16]. Here, we designed this study to evaluate functions and antitumor activity of novel 2nd generation CAR-T cells engineered with the dectin-1 costimulatory signaling domain. We found that the incorporation of this dectin-1 signaling domain into HER2 specific CARs can impact in vitro cytokine secretion and cytotoxicity effects, and in vivo antitumor activity of CAR-T cells. These results provide not only additional knowledge in understanding CAR-T cell responses with signaling domain, but also insights in the potential applications of CAR-T cell therapy in patients with solid tumors.

Materials and methods

Cell lines

All cell lines were obtained from the American Type Culture Collection (ATCC). The K562 (myelogenous leukemia) and NALM6 (lymphoblastic leukemia) were cultured in RPMI-1640 with heat-inactivated 10% fetal bovine serum (FBS) (PAN, Germany. Cat: ST30-3302), penicillin (Gibco, Thermo Fisher, Waltham, MA. Cat: SV30010) (100 U/mL) and streptomycin (Gibco, Thermo Fisher, Waltham, MA. Cat: SV30010) (100 ug/mL). The human cancer cell lines SK-OV-3 (ovarian cystadenocarcinoma) and MDA-MB-468 (breast cancer) were cultured in DMEM with heat-inactivated 10% FBS, penicillin (100 U/mL) and streptomycin (100 ug/mL). SK-OV-3 cells were also engineered to express luciferase as a fluorescent reporter gene as SK-OV-3-luc cells (luciferase [Luc-GFP]-transduced SK-OV-3 cells) for some in vitro and in vivo experiments.

Construction of plasmids encoding CARs

Anti-CD19 or anti-HER2 CARs include a single-chain fragment variable (scFv) specific to CD19 (clone FMC63) [19, 20] or HER2 (clone 4D5) [21]. The scFv was followed by a human CD8α hinge region, then either a human CD8α transmembrane domain (TM), 4-1BB and CD3ζ intracellular domains (ICDs), (h198-BBz [anti-CD19scFv-CD8αTM-4-1BB-CD3ζICD], hH8-BBz [anti-HER2scFv-CD8αTM-4-1BB-CD3ζICD]), or a human dectin-1 TM, dectin-1 and CD3ζ signaling ICDs (h19D-Dz [anti-CD19scFv-Dectin-1TM-Dectin-1-CD3ζICD], hHD-Dz [anti-HER2scFv-Dectin-1TM-Dectin-1-CD3ζICD]).

All the sequences of scFvs above, were synthesized by the Beijing Genomics Institute, and spliced using overlapping PCR to form the CAR sequences. Individual lentiviral plasmid encoding each CAR sequence was constructed using double enzymes digestion with the PCLK lentiviral vector as detailed previously [22] (Addgene, Cambridge, Massachusetts).

Generation of lentiviral particles

HEK-293T cells (embryonic kidney cells) from ATCC were culture in DMEM with heat-inactivated 10% FBS (PAN, Germany. Cat: ST30-3302), penicillin (100 U/mL) and streptomycin (Gibco, Thermo Fisher, Waltham, MA. Cat: SV30010) (100 ug/mL).

To produce lentivirus-containing supernatant, HEK-293T cells were transfected with the following plasmids as detailed previously [22]: the appropriate CAR-coding plasmids, psPAX2 and pMD2.0 G (Invitrogen). The medium was changed 12 h after transfection. The supernatant was harvested and spun to get rid of cell debris. The supernatant was filtered and concentrated by ultracentrifugation at 19,700 rpm for 2 h. The supernatant was discarded. The lentivirus pellet was dissolved in PBS medium and the concentrated lentivirus was stored at −80 °C. The concentrated lentivirus titers were measured by quantitative real time polymerase chain reaction.

Isolation, transduction, production and expansion of human CAR-T cells

Peripheral Blood Mononuclear Cells (PBMCs) were isolated from healthy donor blood by the Ficoll-hypaque density gradient (Lonza, Cat:04-418Q). All samples were obtained after informed consent and approval by the Ethics Committee of the State Key Laboratory of Biotherapy.

PBMCs were cultured in X-VIVO 15 medium (Sigma-Aldrich, Cat:10771) with 5% human serum (Sigma-Aldrich, H4522) and 100 U/ml recombinant human IL-2 (rhIL-2) (PeproTech, NJ, USA. Cat: 200-02-10). PBMCs were stimulated with anti-CD3/CD28 magnetic beads (Gibco, Thermo Fisher, Waltham, MA. Cat: 11131D). After 24 h, T cells were cultured with the lentivirus at a MOI of 5 for 48 h, then the cells were washed and cultured in the T cell medium. Transduction efficiency was determined by CAR expressions measured by a flow cytometry assay.

Flow cytometry

All flow cytometry assays were performed on a Novocyte flow cytometer (ACEA Biosciences, Inc.) and data were analyzed with novocyte express (ACEA Biosciences, Inc.).

Transduction efficiency and associated CAR protein expression were evaluated using biotin-SP-conjugated AffiniPure Goat Anti-Mouse IgG, F(ab') 2 Fragment Specific (Cat: 120962, Jackson Immune Research) with PE-streptavidin (Cat: 405203, BD Biosciences).

The following antibodies were used for differentiation phenotype, and exhaustion marker assays: anti-CD3-FITC (clone: HIT3a, Cat: 300306, Biolegend), anti-CD8-APC (clone: HIT8a, Cat: 300912, Biolegend), anti-CD4-PE (clone: RPA-T4, Cat: 300508, Biolegend), anti-CD45RO-PE (BD Biosciences, clone: UCHL1, Cat: 555493), anti-CD62L-APC (BD Biosciences, clone: DREG-56, Cat: 559772), anti-PD-1-APC (clone: EH12.2H7, Cat: 329908, Biolegend), anti-CTLA-4-APC (Cat: 369612, Biolegend), anti-LAG3-APC (Cat: 369212, Biolegend), and anti-TIM3-APC (Cat: 345012, Biolegend).

In vitro cytokine experiments

Target cells (NALM6, K562, SK-OV-3. or MDA-MB-468, at 1×10^4 cells/well) were seeded in 96-well plates and incubated at 37 °C with 5% CO_2 overnight. After that, CAR-T cells were added at an effector/target ratio [E:T] of 5 or 10. The CAR-T cell number was normalized by transduction efficiency. Supernatants were collected 24 h after co-culture with target cells. ELISA kits for cytokine assay from Invitrogen (IFN-γ Cat: 88-7316-88, TNF-α Cat: 88-7346-88, and IL-6 Cat: 88-7066-88) were used to quantify IFN-γ, TNF-α and IL-6 according to manufacturer protocols.

Real-time cytotoxicity assays (RTCA)

The cytotoxic effect of CAR-T cells was measured by the real-time cytotoxicity assay (ACEA Bioscience, Inc. xCELLigence RTCA SP) as previously described [23]. SK-OV-3 or MDA-MB-468 cells at 1×10^4 cells/well were cultured in an E-plate 96 (ACEA Bioscience) for ~24 h. CAR-T cells (hH8-BBz and hHD-Dz) or mock T cells were added to the plates at an E:T ratio of 10. Data were acquired and analyzed according to the protocols specified by the manufacturers (ACEA Bioscience, Inc. RTCA Software 2.1).

In vivo xenograft studies

Six-week old female B-NSG (NOD- PrkdcscidIL2rgtm1/Bcgen) mice used in this study were purchased from Biocytogen. Each mouse received an i.p. injection of 2×10^6 SK-OV-3-luc cells. Tumors were allowed to grow for 3 days, and then each mouse received an i.p. injection of 1×10^7 human CAR-T cells (hH8-BBz or hHD-Dz, or mock). After 3 additional days, another i.p. injection of CAR-T cells was given to individual mouse. Bioluminescent imaging (BLI) for tumors was performed on scheduled days (day 3, day 10, day 17, day 24, day 31 and day 55) by IVIS (in vivo imaging system) (Caliper Life Science). Tumor fluxes (photons/s/cm^2/steradian) were quantified by measuring the photon signal within a delineated region of interest encompassing. Living Image software (v2.50, Xenogen; Caliper Life Sciences) was used to demonstrate the BLI data. The data for survival analysis was established at the death of each mouse.

Statistics

Statistical plotting and analysis were performed using GraphPad Prism v6.01 (GraphPad Software Inc.) and SPSS v17. Data were expressed as the mean ± SD. One-way ANOVA was used for comparison of three groups in a single condition. Kaplan–Meier survival data were analyzed using a log rank (Mantel–Cox) test. Data were transformed when needed to normalize variance. Symbols indicate statistical significance as follows: $*P < 0.05$; $**P < 0.01$, and $***P < 0.001$.

Results

Novel CAR constructs with the dectin-1 costimulatory signaling domain

Recently, a pattern recognition receptor, dectin-1, belonging to the C-type lectin family has been identified and evaluated [24]. In this study, we designed novel 2nd generation CAR constructs through the combination of scFv domains targeting either CD19 or HER2 epitope with the dectin-1 TM, dectin-1 and CD3ζ ICDs (Fig. 1A). Two more CAR constructs were generated with the components of the most popular 2nd generation CAR structure (human CD8α TM with 4-1BB and CD3ζ ICDs) [25–27].

All CAR constructs were well expressed on the surface of T cells (Fig. 1B). The hH8-BBz CAR expression ratio was 49.61%, and the hHD-Dz CAR expression ratio was 46.17%. As to the anti-CD19 CARs, the h198-BBz CAR expression ratio was 92.95%, and the h19D-Dz CAR expression ratio was 95.07%. Although the expression of anti-HER2 CARs was lower than anti-CD19 CARs, there was no clear difference in the expression level of anti-HER2 or anti-CD19 CARs containing different costimulatory signaling domains.

Effector functions of novel CAR-T cells through the dectin-1 signaling domain

Typically, a later effector function by CAR-T cells can be assessed by cytokine secretion.

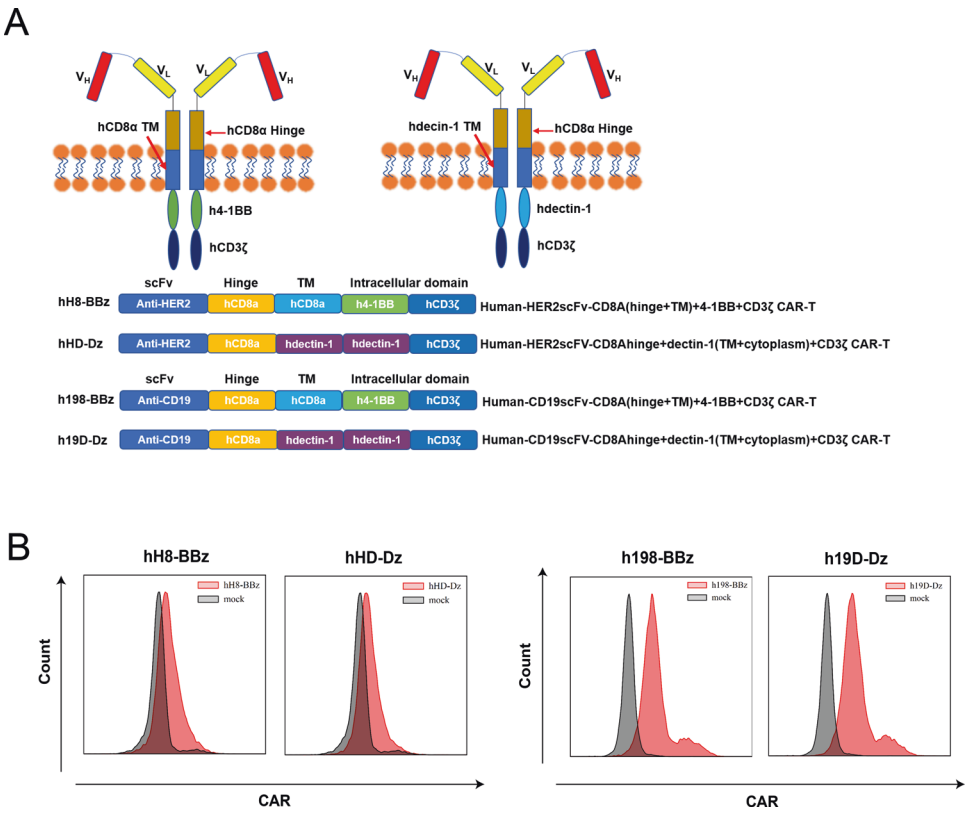

Fig. 1 CAR constructs and expression. A Schematic representation of chimeric receptors that contain the single-chain fragment that binds to HER2 or CD19 and differ in the transmembrane and intracellular domains. **B** Surface expression of the hH8-BBz CAR and the hHD-Dz CAR, the h198-BBz CAR and the h19D-Dz CAR on the human T cells.

Therefore, the impacts of dectin-1 as a costimulatory signaling molecule on cytokine release from the new CAR-T cells were evaluated following exposure to tumor cells that express either HER2 or CD19. Target positive (SK-OV-3 and NALM6) and negative (MDA-MB-468 and K562) tumor cell lines were confirmed by flow cytometry (data on file), which is consistent with literature reported.

The released effector cytokines, including IFN-γ, TNF-α and IL-6, by anti-HER2 or anti-CD19 CAR-T cells increased significantly with incorporating the dectin-1 signaling domain in the target positive cell lines (Fig. 2A, B and C). Anti-HER2 CAR-T cells in this study showed no significant increase of cytokine production against MD-MB-468 (Fig. 2A, B and C). In SK-OV-3 cell line, the hHD-Dz CAR-T cells showed higher levels of IFN-γ than hH8-BBz CAR-T cells; in contrast, the hH8-BBz CAR-T cells secreted more TNF-α (Fig. 2A, B and C). In NALM6 cell line, the h19D-Dz CAR-T cells produced similar levels of IFN-γ and TNF-α to the h198-BBz CAR-T cells (Fig. 2A, B and C).

The cytotoxic function of anti-HER2 CAR-T cells was further investigated to illustrate antigen engagement and CAR-T cell activation. After co-culture of anti-HER2 CAR-T cells with either SK-OV-3 or MDA-MB-468 tumor cell lines, it was observed that both hHD-Dz and hH8-BBz CAR-T cells effectively lysed the SK-OV-3 tumor cells, and hHD-Dz CAR-T cells showed a much different lytic cytotoxicity function from the hH8-BBz CAR-T cells (Fig. 2D).

Phenotype and exhaustion marker expression of the anti-HER2 CAR-T cells

The phenotype and exhaustion marker expression of both anti-HER2 CAR-T cells and mock T cells were analyzed after a 7-day period of cell expansion (Fig. 3).

The hHD-Dz, hH8-BBz CAR-T and mock T cells showed similar percentage of $CD4^+$ or $CD8^+$ T cells (Fig. 3A). Although comparable percentage of effector memory T (T_{EM}, CD45RO + CD62L−) was observed in the hHD-Dz and hH8-BBz CAR-T cells, the hHD-Dz CAR-T cells showed a higher percentage of central memory T cells (T_{CM}, CD45RO + CD62L +) than the hH8-BBz CAR-T cells (Fig. 3A).

The expression pattern of inhibitory receptors, including PD-1, CTLA-4, TIM3, and LAG3, was assessed for the anti-HER2 CAR-T cells (Fig. 3B). It was showed that there were ~10% less PD-1 or LAG3 positive cells in the hHD-Dz CAR-T cells than in the hH8-BBz CAR-T cells. In terms of TIM3 and CTLA-4 positive cells, a similar percentage was observed in the hHD-Dz and hH8-BBz CAR-T cells (Fig. 3B).

We further explored the time-course of anti-HER2 CAR expression. Although there was a decrease of CAR expression within 96 h, the CAR expression reached to >90% after additional 48 h (Fig. 3C).

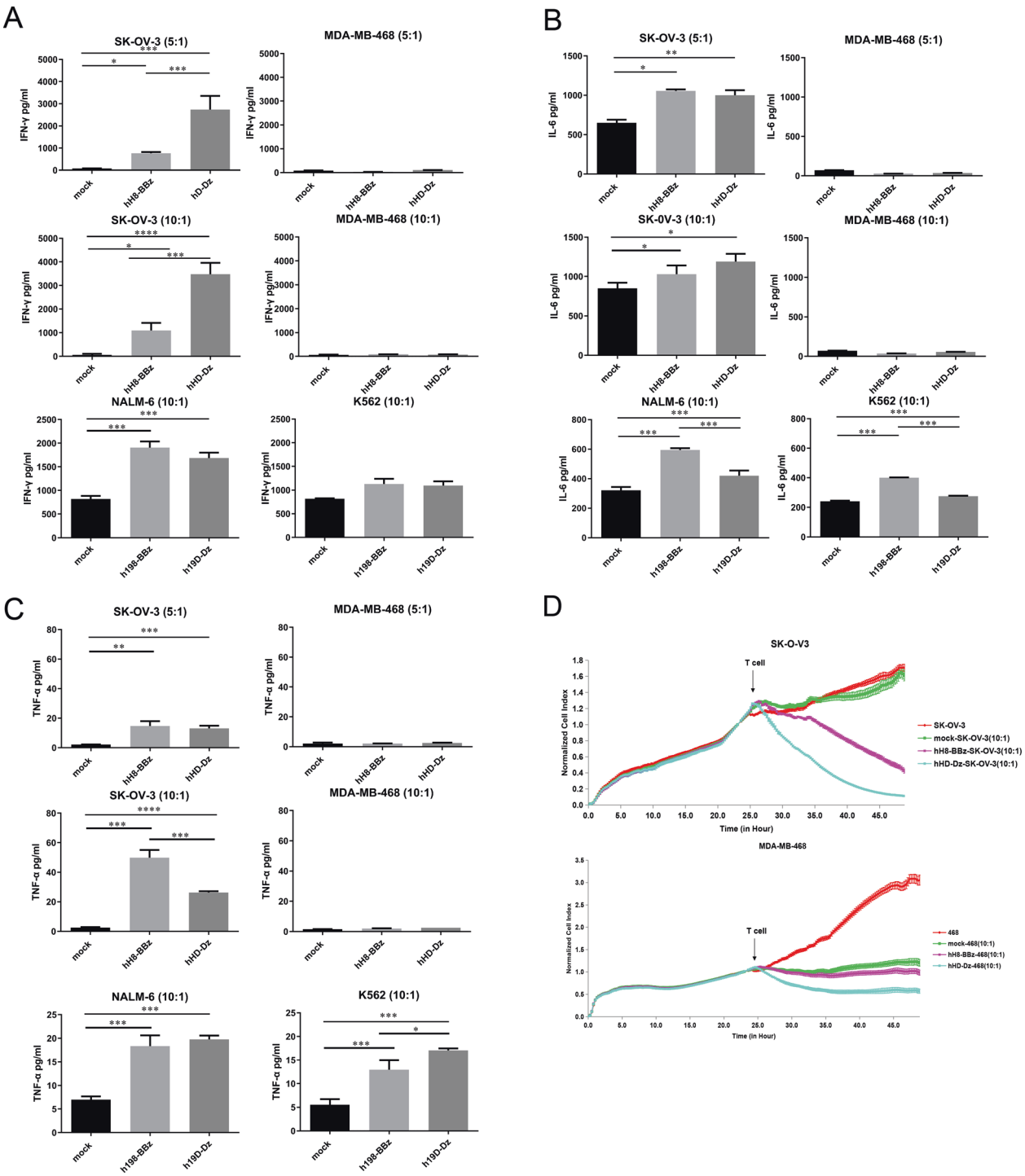

Fig. 2 In vitro cytokine production and cytotoxicity of the CAR-T cells. A IFN-γ, (**B**) IL-6, and (**C**) TNF-α production quantified by ELISA in supernatants from four different CAR-T cells or mock T cells co-cultured overnight with target positive or negative tumor cells (E:T ratio: 10:1 or 5:1) ($n = 3$ per group). Statistical analysis was performed by one-way ANOVA followed by Tukey's posttest analysis. Significance is considered $P < 0.05$. **D** RTCA analysis to show T cell lytic capacity ($n = 4$ per group).

Overall, the above results suggested that the dectin-1 signaling domain in the novel CAR-T cells may result in distinct phenotype and exhaustion marker expression, and discrete T cell proliferation potential.

In vivo antitumor activity of the anti-HER2 CAR-T cells

Using NSG mice bearing xenograft SK-OV-3-luc tumor cells, we investigated in vivo antitumor activity of the

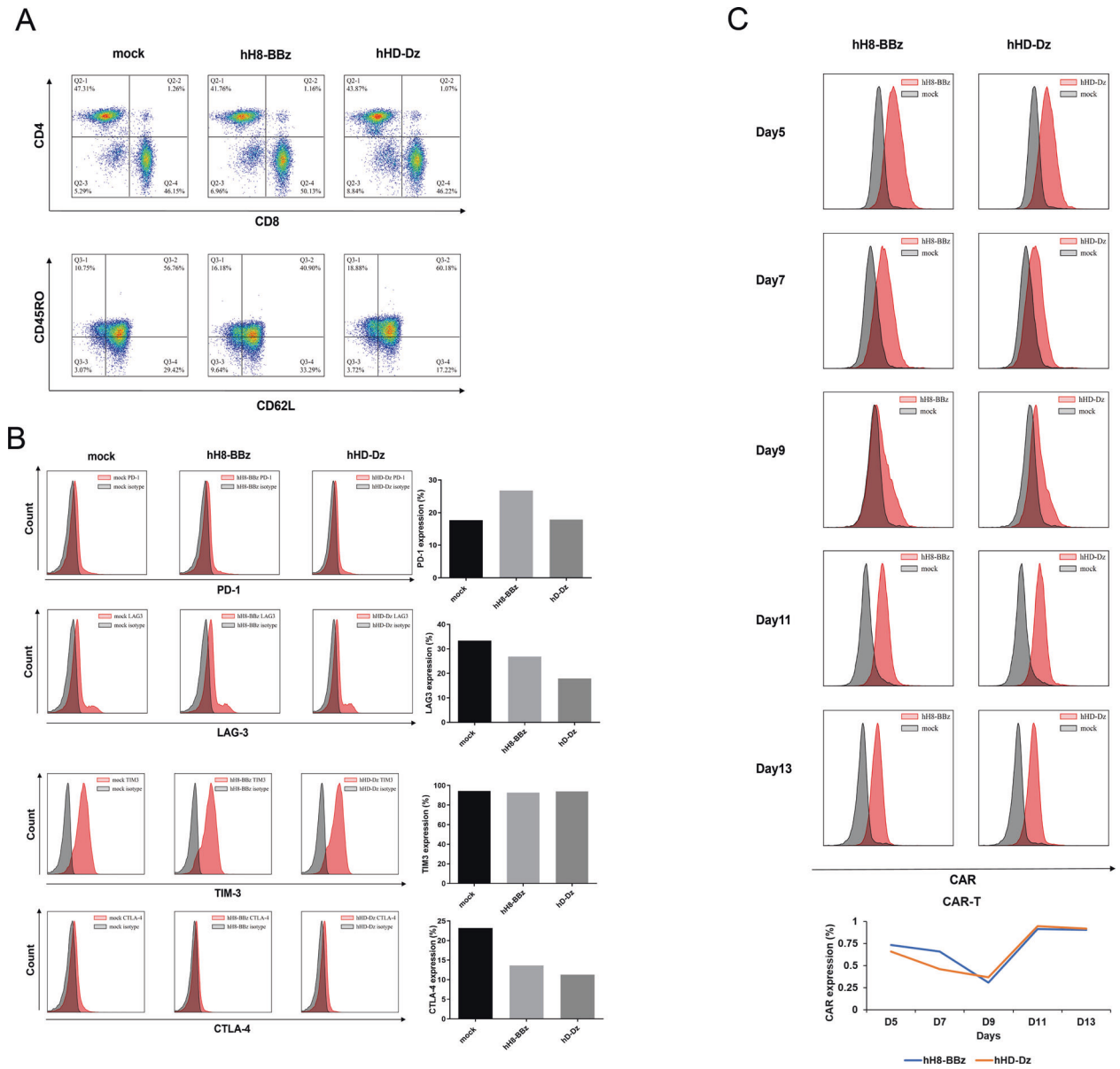

Fig. 3 Phenotype, exhaustion marker expression, and CAR expression of the HER2 specific CAR-T cells. A Flow cytometry density plots of phenotypic profile of each CAR-T cell: cells with either CD3, or CD4, or CD8, or a naive (T_N)(CD45RA-/CD62L-) central memory (T_{CM}) (CD45RO + /CD62L +) or effector memory (T_{EM}) (CD45RO + /CD62L−). **B** Flow cytometry density plots of inhibitory molecules of each CAR-T cell: cells with either PD-1, or LAG3, or CTLA-4, or TIM3. **C** The CAR expression of the hH8-BBz CAR-T cells and the hHD-Dz CAR-T cells with time as measured by flow cytometry.

anti-HER2 CAR-T cells (the hH8-BBz or hHD-Dz CAR-T cells). Overall survival and tumor volume were evaluated (Fig. 4). Treatment with the anti-HER2 CAR-T cells resulted in a delayed tumor progression compared to the mock T cell treated mice (Fig. 4A), and the anti-HER2 CAR-T cell groups at least doubled the median survival of tumor bearing mice (Fig. 4B). The log rank (Mantel–Cox) test demonstrated a statistically significant difference in the survival rates between either anti-HER2 CAR-T cell group and mock T cell group. Moreover, 100% of mice in the hHD-Dz CAR-T cell group were alive by day 55, and the hH8-BBz CAR-T group showed longer overall survival (Fig. 4B).

Discussion

The CAR-T cell immunotherapy demonstrated remarkable clinical efficacies in the hematological malignancies, including NHL, B-cell ALL, MM and CLL [1, 2, 7–9]. However, the applicability of CAR-T cell therapy is in part limited in the solid tumors, due to many challenges, such as

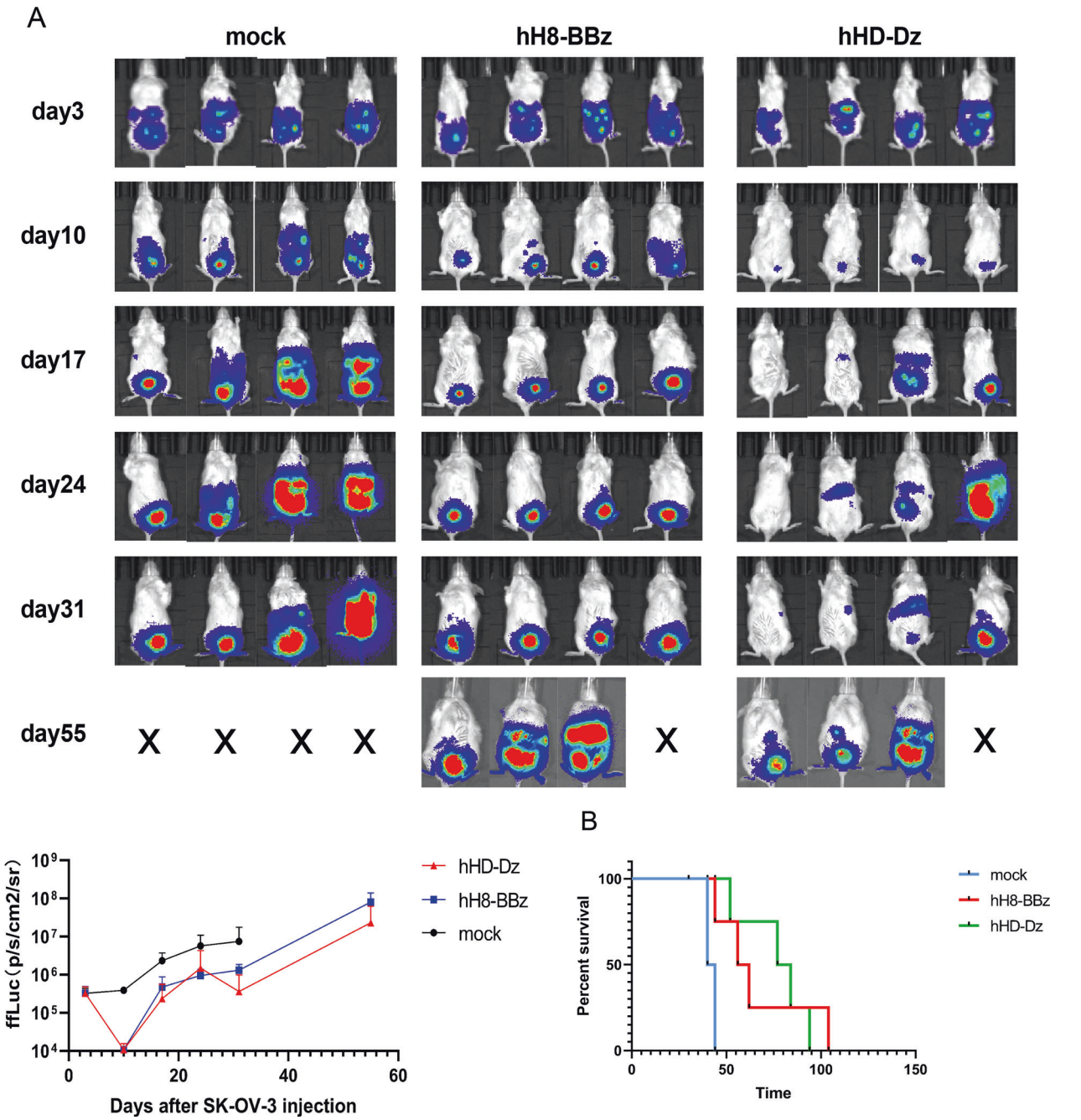

Fig. 4 In vivo antitumor activity of the HER2 specific CAR-T cells. A In vivo live imaging of the SK-OV-3-luc tumor bearing model ($n = 4$ per group). **B** Kaplan–Meier analysis of the overall survival for each group.

lack of appropriate tumor-specific antigen, inhibition of tumor microenvironment, and insufficient CAR-T cells localization and persistence [10–12, 28].

A typical CAR mainly consists of three key components, including a scFv to recognize antigen, a hinge and transmembrane domain (TM), such as CD3, CD28 or CD8 protein, and intracellular signaling domains (ICDs), such as CD3ζ or FcRγ [29–31]. The 2nd generation CARs include one or more intracellular costimulatory signaling domains, such as CD28, 4-1BB, CD27, OX40, ICOS, DAP10, IL-15Rα, MyD88/CD40 and TLR2, to transmit activation signals [14, 32–39]. It has been showed that different TMs or/and ICDs affect T cell expansion, persistence and other functions [40]. Recently, several 2nd generation CARs have been tested in patients with solid tumors, such as metastatic colorectal cancers and sarcoma [41, 42]. The results from these trials were far from exciting compared with that achieved in treating hematological malignancies [43–45].

Therefore, exploring different costimulatory domains may provide a new approach to improve antitumor effects of CAR-T cells in the solid tumors.

C-type lectin receptors (CLRs), highly expressed on myeloid cells, show the essential functions in homeostasis and immunity [46]. One function of CLRs is to identify the ligands by pattern recognition receptors to mediate the immunity activity. Recently, dectin-1 (i.e., CLEC-7A), a type-II transmembrane molecule was found to be a new subgroup of CLRs, and well investigated [15, 16]. Dectin-1 consists of the extracellular domain, transmembrane domain, and intracellular domain. The extracellular portion of dectin-1 has been utilized as the scFv of CAR-T cells to target fungus [47]. Dectin-1 is not only predominantly expressed on myeloid cells, including neutrophils, monocytes, dendritic cells and macrophages [48], but also on some subsets of human T and B cells [46]. Dectin-1 plays an important role in tumor growth and metastasis by activating the NK cells [49]. Dectin-1 can also regulate various cell responses, such as DC maturation, antigen presentation and the production of cytokines and chemokines [48]. In addition, dectin-1 can directly induce innate immune memory, and influence the development of CD8, CD4 T and B cells [46, 50, 51]. In our study, we used dectin-1 TM and its ICD as signaling domain in the CAR-T cells, and assessed whether this specific costimulatory design in the CARs can affect T cell functions, such as immunotherapeutic properties.

CD19 and HER2 were widely used as common tumor-associated antigen targets to represent hematological malignancy and solid tumor [13, 52]. A popular costimulatory signaling domain, 4-1BB, was naturally chosen as an effective reference [26, 31]. In this study, CD19 or HER2-targeting scFv domains were coupled to 4-1BB or dectin-1 signaling ICDs to construct four different 2nd generation CARs.

Our data revealed that the novel CAR design influenced T cell functions through dectin-1 signaling domain in both in vitro and in vivo experiments, such as enhanced cytokine secretion and lytic capacity, reduced exhaustion potential, increased cell expansion, and distinct antitumor activity.

In this study, we confirmed previous study results showing enhanced CAR-T cell functions with 4-1BB costimulatory signaling domains [53, 54]. Interestingly, in vitro T cell functions (e.g., increased cytokine production) of the hHD-Dz CAR-T cells are comparable to the 4-1BB based, and both are superior to the mock T cells. As to the HER2 specific CAR-T cells, the IFN-γ secretion of the hHD-Dz CAR-T cells was higher than the hH8-BBz, suggesting possible predominantly Th1 phenotype; while the hH8-BBz CAR-T cells released more TNF-α, consistent with Th1/Th2 phenotype. Similar cytokine production pattern by the anti-CD19 CAR-T cells indicated comparable phenotype irrespective to the costimulatory signaling domain. In the RTCA, we illustrated the cytotoxic ability of the hHD-Dz CAR-T cells, distinct to the hH8-BBz CAR-T cells. The above results may suggest that the dectin-1 signaling domain provides a new mechanistic approach in CAR-T cell immunotherapy in treating solid tumors.

Due to the immune resistance and T cell exhaustion, one of the biggest challenges in CAR-T cell therapy for solid tumors is the inhibition of tumor microenvironment [55, 56]. However, it has been showed that different T cell phenotype may play an important role in the antitumor immunity, for example, T_{CM} cells are more important than T_{EM} cells in the adoptive immunotherapies [57]. In our study, more T_{CM} and distinct exhaustion maker expression in the hHD-Dz CAR-T cells may suggest that the new CAR-T cells can be less influenced by tumor immunosuppressive microenvironment through the dectin-1 costimulatory signaling in the solid tumors.

It has been well published that the 2nd generation CARs surpass the 1st generation in preclinical and clinical studies [58–60]. Here, we demonstrated the distinct antitumor effects of the 2nd generation CAR-T cells through either dectin-1 or 4-1BB signaling domain in the established tumor xenograft model. However, the CARs with the different costimulatory signaling domains here showed discrete antitumor activity trend on in vivo survival. The hHD-Dz CAR-T cells showed increased effector functions at early timepoints, and the hH8-BBz CAR-T cells demonstrated a later antitumor activity. These observations suggested that different costimulatory signaling domains may result in distinct T cell phenotype. In the majority of recent clinical trials, the CAR-T cell therapy was based on the products from the pooled T cells, and here we also used this unselected "bulk" T cell approach to investigate the functions of this novel hHD-Dz CAR-T cells. However, there are some limitations with this approach as literature showed that some T cell subtypes (e.g., CD4 + and CD8 +) exhibited distinct properties, such as proliferative capacity and persistence in the CAR-T therapy [40, 61–63]. Therefore, the other signaling pathway in CARs should be further explored and optimized for the enhanced effector functions and improved persistence of T cells.

In summary, we extended our knowledge on the new costimulatory signaling domain, dectin-1 in the CARs. The dectin-1 engineered novel CARs demonstrated discrete CAR-T cell properties, such as effector functions, T cell phenotype and exhaustion marker expression, and in vivo antitumor effects. All collective results suggest that the incorporation of this new signaling domain, dection-1, into the CARs may provide the clinical potential of the CAR-T cells through this signaling domain for the treatment of solid tumors.

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Compliance with ethical standards

Conflict of interest WW has submitted a patent concerning the methodology and application. WW is one of the scientific co-founders of Cygenpeutics and CarEne and holds the equity of the company. The authors declare no competing interests.

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