Abstracts

130 IMMUNOGENIC POTENTIAL OF CHIMERIC ANTIGEN RECEPTOR (CAR)-ENGINEERED T CELLS EXPRESSING INDUCIBLE NUCLEASE-DEACTIVATED SPCAS9 (DCAS9)

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Background The application of CRISPR-Cas9 for personalized medicine is potentially revolutionary for the treatment of several diseases including cancer. However, the bacterial origin of the Cas9 protein raises concerns about immunogenicity. Recent ELISA-based assays detected antibodies against Cas9 from Streptococcus pyogenes (SpCas9) and Staphylococcus aureus (SaCas9) in 5–10% of sera from 343 normal healthy individuals.1,2 SpCas9-specific memory CD8 T cell responses were not demonstrated in those individuals. To date, there are no conclusive studies assessing whether CRISPR-Cas9-modified CAR-T could raise CD8 T cell-mediated immunogenicity in humans. Refuge CAR-T cell platform employs an inducible, non-gene editing, nuclease deactivated Cas9 (dCas9) to modulate gene expression in response to external stimuli such as antigen-dependent CAR signaling to suppress PD-1 expression.

Methods In the present study, we analyzed two putative HLA-A*02:01 and two HLA-B*07:02-associated SpCas9 T cell epitopes. The candidate epitopes were derived from a prediction algorithm that incorporates T cell receptor contact residue hydrophobicity and HLA binding affinity. We engaged in-vitro sensitization (IVS) assay to identify immunogenic potential of dCas9 peptides.

Results Autologous IVS assay of T cells in two healthy donor PBMCs identified CD8-T cell responses after two rounds of stimulation against only one HLA-A*02:01-associated Cas9 peptide (sequence NLIALSLGL) P1– while the other candidate epitopes did not elicit any response. Dextramer analysis demonstrated that 15% of CD8+ T cells were specific for P1 and − 11% of CD8+ cells produced INFγ upon challenge with P1-loaded T2 cells.

Conclusions Our in-vitro sensitization assay was able to demonstrate that dCas9 epitope P1 is immunogenic and may elicit adaptive immune response against gene edited CAR-T cells. Endogenous processing and presentation of P1 and other putative epitopes by Refuge CAR-T cells are currently being analyzed.

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REFERENCES


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131 COUPLED CAR® TECHNOLOGY STRENGTHENS ADOPTIVE T CELL THERAPY BY PROMOTING RAPID EXPANSION

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Background CAR T therapy has achieved remarkable results in the treatment of hematological tumors such as leukemia, lymphoma, and multiple myeloma. However, there remains challenges in treating solid tumors. These challenges include physical barriers, tumor microenvironment immunosuppression, tumor heterogeneity and target specificity. Especially, due to tumor microenvironmental barriers, CAR T cells are not effectively exposed to tumor antigens and cannot activate co-stimulation signals on CAR molecules, thus conventional CAR T cell therapy has thus far shown weak cell expansion in solid tumor patients, achieved little or no therapeutic responses.

Here, we developed CAR T cells based on a novel CoupledCAR® technology to overcome the lack of persistence of solid tumor CAR T cells in vivo.

Methods We designed a ‘CoupledCAR’ lentivirus vector containing a single-chain variable fragment (scFv) targeting human TSHR. The lentivirus was produced by transfecting HEK-293T cells with ‘CoupledCAR’ lentiviral vectors and viral packaging plasmids. Patient’s CD3 T cells were cultured in X-VIVO medium containing 125U/mL Linterleukin-2 (IL-2), and transduced with ‘CoupledCAR’ lentivirus at certain MOI. Transduction efficiency and was evaluated at 7 to 9 days after ‘CoupledCAR’ lentivirus transduction, and quality controls for fungi, bacteria, mycoplasma, chlamydia, and endotoxin were performed. After infusion, serial peripheral blood samples were collected, and the expansion and the cytokine release of CAR-T cells were detected by FACS and QPCR. The evaluation of response level for patients were performed at month 1, month 3, and month 6 by PET/CT.

Results We used prostatic acid phosphatase (PAP) as an exemplary CAR target for prostate cancer and demonstrated that our CoupledCAR® significantly enhanced the expansion of PAP CAR T cells in vitro and in vivo. Further, we observed that this expansion showed more memory-like phenotypes, and caused little exhaustion of PAP CAR T cells. Also, we find coupled solid tumor CAR T cells have stronger tumor killing ability. We demonstrated this simple expansion to enable the persistence of solid tumor CAR T cells and can be further applied to other kinds of T cell therapy like TCR T and TILs.

Conclusions We developed a novel platform technology (CoupledCAR®) that allows solid tumor CAR T cells to rapidly expand. This initial CAR T cell expansion enabled enhanced trafficking and infiltration of the tumor whereby further cell expansion occurred and thereby achieved tumor clearance. We have carried clinical trials and obtained early promising clinical data. We will further verify the safety and efficacy of this technology in the treatment of different kinds of solid tumors in the clinic research.

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132 CAR MACROPHAGES (CAR-M) ELICIT A SYSTEMIC ANTI-TUMOR IMMUNE RESPONSE AND SYNERGIZE WITH PD1 BLOCKADE IN IMMUNOCOMPETENT MOUSE MODELS OF HER2+ SOLID TUMORS

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Background Despite the remarkable efficacy achieved by CAR-T therapy in hematologic malignancies, application in solid tumors has been challenging. We previously developed human CAR-M and demonstrated that adoptive cell transfer of CAR-M into xenograft models of human cancer controls tumor progression and improves overall survival [1]. Given that
CAR-M are professional antigen presenting cells, we developed an immunocompetent animal model to evaluate the potential for induction of a systemic anti-tumor immune response.

Methods Murine bone marrow-derived macrophages were engineered to express an anti-HER2 CAR using the chimeric adenoviral vector Ad5f35. CAR-M were phenotypically and functionally evaluated in vitro and in syngeneic models. To evaluate CAR-M efficacy in an immunocompetent animal model, BALB/c mice were engrafted with CT26-HER2+ tumors (single-tumor model) and were treated with intratumoral CAR-HER2 or untransduced (UTD) macrophages. To evaluate epitope spreading, we simultaneously engrafted BALB/c mice with CT26-HER2+ and CT26-Wt tumors on opposite flanks (dual-tumor model), and treated mice with CAR-M or controls into the CT26-HER2+ tumor only. Peripheral and tumor-infiltrating immune cells were phenotypically and functionally characterized.

Results In addition to efficient gene delivery, Ad5f35 transduction promoted a pro-inflammatory (M1) phenotype in murine macrophages. CAR-M, but not control UTD macrophages, phagocytosed HER2+ target cancer cells. Anti-HER2 CAR-M eradicated HER2+ murine CT26 colorectal and human AU565 breast cancer cells in a dose-dependent manner. CAR-M increased MHC-I and MHC-II expression on tumor cells and promoted tumor-associated antigen presentation and T cell activation. In vivo, CAR-M treatment led to tumor regression and improved overall survival in the CT26-HER2+ single-tumor model. In the dual-tumor model, CAR-M treatment cleared 75% of CT26-HER2+ tumors and inhibited the growth rate of contralateral CT26-WT tumors, demonstrating an abscopal effect. CAR-M treatment led to increased infiltration of intratumoral CD4+ and CD8+ T, NK, and dendritic cells – as well as an increase in T cell responsiveness to the CT26 MHC-I antigen gp70, indicating enhanced epitope spreading. Given the impact CAR-M had on endogenous T-cell immunity, we evaluated the combination of CAR-M and anti-PD1 in the CT26-HER2 model and found that the combination further enhanced tumor control and overall survival.

Conclusions These results demonstrate that CAR-M therapy induces epitope spreading via activation of endogenous T cells, orchestrating a systemic immune response against solid tumors. Moreover, our findings provide rationale for the combination of CAR-M with immune checkpoint inhibitors. The anti-HER2 CAR-M CT-0508 will be evaluated in an upcoming Phase I clinical trial.