levels in an array of malignancies including mesothelioma, ovaria, non-small cell lung cancer, and pancreatic cancers and is an attractive target antigen for immune-based therapies. Early clinical evaluation of autologous MSLN-targeted chimeric antigen receptor (CAR)-T cell therapies for malignant pleural mesothelioma has shown promising acceptable safety1 and have recently evolved with incorporation of next-genera-

REFERENCES


BACKGROUND

Adoptive immunotherapy using chimeric antigen receptor (CAR) is currently one of the most promising cancer therapy. Especially CAR-T cell therapy targeting CD19 antigen in B-cell tumors have shown impressive clinical results and CAR-T cell products targeting CD19 have already approved. However as the high relapse rate is still the problem and the clinical efficacy of CAR-T cell therapy for solid tumors is currently inadequate, further improvement of CAR design is required. It is known that the design of CAR construct affects the function of CAR-T cells. For example co-

STUDY DESIGN

In this study, we characterized ATA3271 both in vitro and in vivo. ATA3271 show stable and proportional CAR and anti-PD1DN expression. Functional studies show potent antitu-

METHODS

We generated allogeneic MSLN CAR+ EBV T cells (ATA3271) using retroviral transduction of EBV T cells. ATA3271 includes a novel 1XX CAR signaling domain, previ-

RESULTS

In this study, we described the first preclinical evaluation of ATA3271, a new-generation allogeneic CAR EBV T cell targeting MSLN and incorporating PD1DNR, designed for the treatment of solid tumor indications.

CONCLUSIONS

Overall, ATA3271 shows potent antitumor activity without evidence of alloreactivity, both in vitro and in vivo, suggesting that allogeneic MSLN-CAR-engineered EBV T cells are a promising approach for the treatment of MSLN-positive cancers and warrant further clinical investigation.

99 STRUCTURAL OPTIMIZATION OF ANTI-CEA-GITR-CAR TO REDUCE TONIC SIGNALING AND IMPROVE ANTIGEN-SPECIFIC REACTIVITY

1. Yasunori Amaihi*, 1Yu Okubo, 2Yizheng Wang, 1Linan Wang, 2Takuma Kato, 1Sachiko Okamoto, 2Hiroshi Shiku, 1Junichi Mineno. 3Takara Bio Inc., Kusatsu, Shiga, Japan; 2Mie University Graduate School of Med., Tsu, Japan; 3Mie University, Tsu, Japan

BACKGROUND

Adaptive immunotherapy using chimeric antigen receptor (CAR) is recently reported as one of the effective cancer therapy. Especially CAR-T cell therapy targeting CD19 antigen in B-cell tumors have shown impressive clinical results and CAR-T cell products targeting CD19 have already approved. However as the high relapse rate is still the problem and the clinical efficacy of CAR-T cell therapy for solid tumors is currently inadequate, further improvement of CAR design is required. It is known that the design of CAR construct affects the function of CAR-T cells. For example co-

STUDY DESIGN

In this study, we characterized ATA3271 both in vitro and in vivo. ATA3271 show stable and proportional CAR and anti-PD1DN expression. Functional studies show potent antitu-

RESULTS

In this study, we described the first preclinical evaluation of ATA3271, a new-generation allogeneic CAR EBV T cell targeting MSLN and incorporating PD1DNR, designed for the treatment of solid tumor indications.

CONCLUSIONS

Overall, ATA3271 shows potent antitumor activity without evidence of alloreactivity, both in vitro and in vivo, suggesting that allogeneic MSLN-CAR-engineered EBV T cells are a promising approach for the treatment of MSLN-positive cancers and warrant further clinical investigation.

REFERENCES


http://dx.doi.org/10.1136/jitc-2020-SITC2020.0098
specific response with reduced tonic signaling, potentially indicating that our novel CAR-T cells may show improved clinical efficacy on solid tumor.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0099

Background Bioengineered T cell treatments for acute myeloid leukemia (AML) are challenged by near universal expression of leukemia antigens on normal hematopoietic stem/progenitor cells,1-2 on target/off tumor activity may cause myelosuppression while sustained antigen exposure can lead to T cell exhaustion.3 In addition, splicing variants may allow antigen escape. We hypothesize that by using a novel CD33-C2-specific single domain VHH antibody as the antigen targeting domain in dimerizing agent-regulated immunoreceptor complex T cells (DARIC T cells), we will enable pharmacologically-controllable targeting of CD33, allowing eradication of leukemia expressing either of the major splice variants of CD33: i.e., full-length CD33 or CD33ΔE2.

Methods We engineered DARIC-expressing lentiviral vectors containing encoding separated CD33-C2-specific antigen binding and 41BB-CD3ζeta signaling chains that heterodimerize following addition of rapamycin via embedded FKBP12 and FRB* domains.4 Peripheral blood mononuclear cells were stimulated with IL-2, anti-CD3, and anti-CD28 antibodies 24h prior to transduction with DARIC33 lentivector. Surface expression of antigen binding or signaling chains was assessed using biotinylated CD33, or antibodies to VHH-domains or FRB* respectively. Rapamycin-dependent in vitro activity was measured by IFNγ release. To evaluate in vivo activity, NSG mice injected with 1 x 10^3 MOLM-14/luc cells were treated 5-7 days later with 1 x 10^6 DARIC33 T cells in the presence or absence of rapamycin and tumor progression followed by luciferase activity.

Results DARIC33+ T cells bound biotinylated-CD33, anti-VHH and anti-FRB* antibodies. Rapamycin addition increased expression of both signaling and antigen-recognition chains, suggesting augmented receptor stability in the presence of dimerizing drug. In the presence of rapamycin, coculture of engineered gamma/delta T cells with DARIC33 T cells resulted in potent anti-leukemic activity: they are activated by AML cell lines in vitro as demonstrated by cytokine release and cytotoxicity, and significantly extend survival in an aggressive xenograft model.

Temporal control provided by the DARIC architecture promises to enhance safety and potentially efficacy of CAR T therapy for AML, for example by enabling hematopoietic recovery or providing T cell rest.

REFERENCES

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0100

Abstracts

DRUG-REGULATABLE ENGINEERED T CELLS ELIMINATE CD33+ AND CD33ΔE2+ AML
1Jacob Appelbaum*, 2Wai-Hang Leung, 1Unja Martin, 1Kaozi Oda, 1Giacomo Tampella, 2Dong Xia, 1Joy Zhang, 1Anne-Rachael Krostag, 1Rachael Logan, 1Claudya Evandy, 2Querrie Vog, 1John Jones, 1Timmer, 1Brendan Eckelman, 2Jim Rotman, 2Danielle Montt, 2Bryan Peguero, 2Mark Pogson, 2Alexander Astrakhin, 3Jordan Jarjur, 1Joshua Gustafson, 1Michael Jensen. 1University of Washington, Seattle, WA, USA; 2Seattle Childrens Research Institute, Seattle, WA, USA; 3Inhibrix, Inc, La Jolla, CA, USA.


DARIC33+ and CD33ΔE2+ AML

ENGINEERING GAMMA/DELTA T CELLS WITH THE T-CELL ANTIGEN COUPLER RECEPTOR EFFECTIVELY INDUCES ANTIGEN-SPECIFIC TUMOR CYTOTOXICITY IN VITRO AND IN VIVO

Sarah Asbury*, Seung Mi Yoo, Jonathan Branson. MMaster University, Hamilton, Canada

Background Engineered T cell therapies have revolutionized treatment of relapsed refractory haematological malignancies, however the cost of treatment for autologous products remains a significant challenge to their widespread use. The high cost is driven largely by the need for personalized manufacturing of autologous cell products. A non-conventional class of T cells, the gamma/delta T cell, can be safely transplanted into an unrelated recipient without inducing graft-versus host disease,1 making them an ideal candidate for mass-manufactured off-the-shelf T cell therapies. We have previously described a novel method of directing conventional alpha/beta T cells towards tumour targets by co-opting the T cell receptor using the T cell Antigen Coupler (TAC) receptor.2 Here, we describe the use of TAC receptors to engineer antigen-specific reactivity into gamma/delta T cells, resulting in highly potent anti-tumor cytotoxicity.

Methods Engineered gamma/delta T cells were manufactured by activating PBMCs with Zoledronate and IL-2. The TAC transgene was introduced into T cells using either VSVG pseudotype lentivirus or GALV-pseudotyped gamma-retrovirus vectors. Through optimization studies, we determined transduction was highest 24 hours post-activation for lentivirus and 72 hours post-activation for gamma-retrovirus. Cultures were fed with IL-2 supplemented media every 2 – 3 days and enriched on Day 14 to >99% gamma/delta T cell purity using CD4/CD8 magnetic-activated cell sorting depletion (Miltenyi Biotec).

Results Both methods of gene transfer tested for our pilot study yielded excellent gene transduction (40% - 70%). Using lentivirus-engineered gamma/delta T cells, we demonstrated that the TAC receptor re-directs gamma/delta T cells to attack tumors in an antigen-specific manner. The presence of the TAC receptor did not interfere with lysis of tumor cells via the natural tumor-reactive gamma/delta T cell receptors.

Abstracts

101

ENGINEERING GAMMA/DELTA T CELLS WITH THE T-CELL ANTIGEN COUPLER RECEPTOR EFFECTIVELY INDUCES ANTIGEN-SPECIFIC TUMOR CYTOTOXICITY IN VITRO AND IN VIVO

Sarah Asbury*, Seung Mi Yoo, Jonathan Branson. MMaster University, Hamilton, Canada

Background Engineered T cell therapies have revolutionized treatment of relapsed refractory haematological malignancies, however the cost of treatment for autologous products remains a significant challenge to their widespread use. The high cost is driven largely by the need for personalized manufacturing of autologous cell products. A non-conventional class of T cells, the gamma/delta T cell, can be safely transplanted into an unrelated recipient without inducing graft-versus host disease,1 making them an ideal candidate for mass-manufactured off-the-shelf T cell therapies. We have previously described a novel method of directing conventional alpha/beta T cells towards tumour targets by co-opting the T cell receptor using the T cell Antigen Coupler (TAC) receptor.2 Here, we describe the use of TAC receptors to engineer antigen-specific reactivity into gamma/delta T cells, resulting in highly potent anti-tumor cytotoxicity.

Methods Engineered gamma/delta T cells were manufactured by activating PBMCs with Zoledronate and IL-2. The TAC transgene was introduced into T cells using either VSVG pseudotype lentivirus or GALV-pseudotyped gamma-retrovirus vectors. Through optimization studies, we determined transduction was highest 24 hours post-activation for lentivirus and 72 hours post-activation for gamma-retrovirus. Cultures were fed with IL-2 supplemented media every 2 – 3 days and enriched on Day 14 to >99% gamma/delta T cell purity using CD4/CD8 magnetic-activated cell sorting depletion (Miltenyi Biotec).

Results Both methods of gene transfer tested for our pilot study yielded excellent gene transduction (40% - 70%). Using lentivirus-engineered gamma/delta T cells, we demonstrated that the TAC receptor re-directs gamma/delta T cells to attack tumors in an antigen-specific manner. The presence of the TAC receptor did not interfere with lysis of tumor cells via the natural tumor-reactive gamma/delta T cell receptors.