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14. ABSTRACT: In the previous report, most of Tasks 1, 2, and 3 (Aim 1) were completed. In brief, post approval for IACUC and IRB protocols, we completed the development of the 2nd and 3rd generation human CAR constructs, confirmed the sequences, generated the CAR T cells, and successfully completed cytolysis assays against TNBC cell lines in an antigen-specific manner without any toxic effects to normal epithelial cells. We initiated the in vivo experiments outlined in specific aim 2. Most of the data is now published. In this cycle, we report the progress made in Tasks 3 through 7. We show the efficacy of CAR T + anti-PD1 blocking antibody treatment in the NSG mouse model of human TNBC. We have successfully generated the murine CAR t cells and conducted in vitro functional assays using murine breast cancer cell lines. We also report on the efficacy of murine CAR t cells in an immune competent spontaneous PyVMT tumor model in vivo. Due to COVID related lockdown, we did face challenges that were beyond our control.

15. SUBJECT TERMS
None listed.

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1. **Introduction:** Antibody-derived chimeric antigen receptor (CAR) T cell therapy has achieved gratifying breakthrough in hematologic malignancies but has shown limited success in solid tumor immunotherapy. Monoclonal antibody, TAB004, specifically recognizes the aberrantly glycosylated tumor form of MUC1 (tMUC1) in all subtypes of breast cancer including 95% of triple-negative breast cancer (TNBC) while sparing recognition of normal tissue MUC1. We transduced human T cells with MUC28z, a chimeric antigen receptor comprising of the scFv of TAB004 coupled to CD28 and CD3ζ. MUC28z was well expressed on the surface of engineered activated human T cells. MUC28z CAR T cells demonstrated significant target-specific cytotoxicity against a panel of human TNBC cells. Upon recognition of tMUC1 on TNBC cells, MUC28z CAR T cells increased production of Granzyme B, IFN-γ and other Th1 type cytokines and chemokines. In this report, we found that a single dose of human MUC28z CAR T cells significantly reduced HCC70 TNBC tumor growth in a xenograft model. Murine MUC28z was successfully expressed on mouse primary $CD8^+$ T cells. In vitro, those mouse MUC28z CAR t cells recognized tMUC1, lysed mouse breast cancer cells, and released IFN-γ and IL-2 in a tMUC1-antigen highly specific manner. In vivo, mouse MUC28z CAR t cells control spontaneous MMT tumor growth. Thus, MUC28z CAR T cells have high therapeutic potential against tMUC1-positive TNBC tumors with minimal damage to normal breast epithelial cells.

2. **Keywords:** Triple-negative breast cancer, Immunotherapy, MUC28z CAR T cells

3. **Accomplishments**

   What were the major goals of the project?

The Specific Aims were:

Aim 1. Demonstrate TAB 004-CAR-T mediated killing *in vitro* of human and mouse TNBC cells (with and without anti-PD1 Ab).

Aim 2a. Demonstrate TAB 004-CAR-T + anti-PD1 Ab mediated killing *in vivo* in xenograft model of human metastatic TNBC.

Aim 2b. Demonstrate TAB 004-CAR-T + anti-PD1 Ab mediated killing *in vivo* in orthotopic model of mouse metastatic TNBC in human MUC1.Tg syngeneic – immune competent mice.

Aim 2c. Demonstrate TAB 004-CAR-T + anti-PD1 Ab mediated killing in spontaneous breast cancer model in the human MUC1-PyV MT bitransgenic (MMT) mice.

Aim 3. Demonstrate TAB 004-CAR-T + anti-PD1 Ab mediated killing in human tumor explant models of metastatic, treatment refractory TNBC.

What was accomplished under these goals?

Specific Aim 1. Demonstrate TAB 004-CAR-T mediated killing *in vitro* of human and mouse TNBC cells (with and without anti-PD1 Ab).

Task 1: Amendments to the existing UNCC IRB/IACUC for approval (1-2 months):

Task 2: TAB-CAR-T mediated TNBC killing in vitro (1-6 months):

Task 3: tMUC1-CAR-t mediated murine breast cancer cell killing in vitro (4-8 months).

Task 1 Progress: Amendments to the existing UNCC IRB/IACUC for approval (1-2 months):

We completed this task and received UNCC IRB number: #18-0227 and this study was also approved through HRPO. UNCC IUCUC number was also received #16-014 and the study in animal subjects was approved through ACURO.

Task 2 Progress: TAB-CAR-T mediated TNBC killing in vitro (1-6 months):

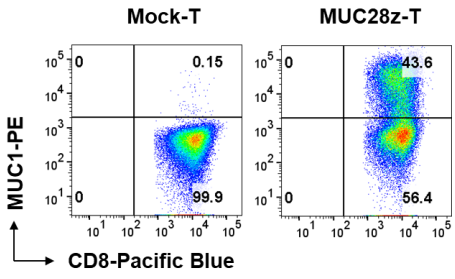
We have completed majority of this task to best of our abilities. The results are now published (Front. Immunol., 24 May 2019 | https://doi.org/10.3389/fimmu.2019.01149 and Front. Immunol., 07 December 2020 | https://doi.org/10.3389/fimmu.2020.628776.)

The publication abstract is provided below:

Antibody-derived chimeric antigen receptor (CAR) T cell therapy has achieved gratifying breakthrough in hematologic malignancies but has shown limited success in solid tumor immunotherapy. Monoclonal antibody, TAB004, specifically recognizes the aberrantly glycosylated tumor form of MUC1 (tMUC1) in all subtypes of breast cancer including 95% of triple-negative breast cancer (TNBC) while sparing recognition of normal tissue MUC1. We transduced human T cells with MUC28z, a chimeric antigen receptor comprising of the scFv of TAB004 coupled to CD28 and CD3ζ. MUC28z was well-expressed on the surface of engineered activated human T cells. MUC28z CAR T cells demonstrated significant target-specific cytotoxicity against a panel of human TNBC cells. Upon recognition of tMUC1 on TNBC cells, MUC28z CAR T cells increased production of Granzyme B, IFN-γ and other Th1 type cytokines and chemokines. A single dose of MUC28z CAR T cells significantly reduced TNBC tumor growth in a xenograft model. Thus, MUC28z CAR T cells have high therapeutic potential against tMUC1-positive TNBC tumors with minimal damage to normal breast epithelial cells.

During this report period (10/15/2019-10/14/2020), we have achieved the following:

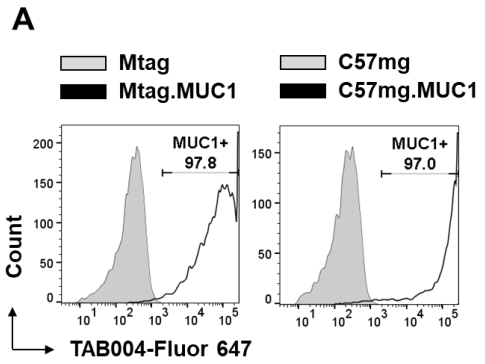
Task 3 Progress: tMUC1-CAR-t mediated murine breast cancer cell killing in vitro.



We successfully expressed mouse MUC28z CAR on the cell surface of primary mouse CD8+ T cells (Figure 1).

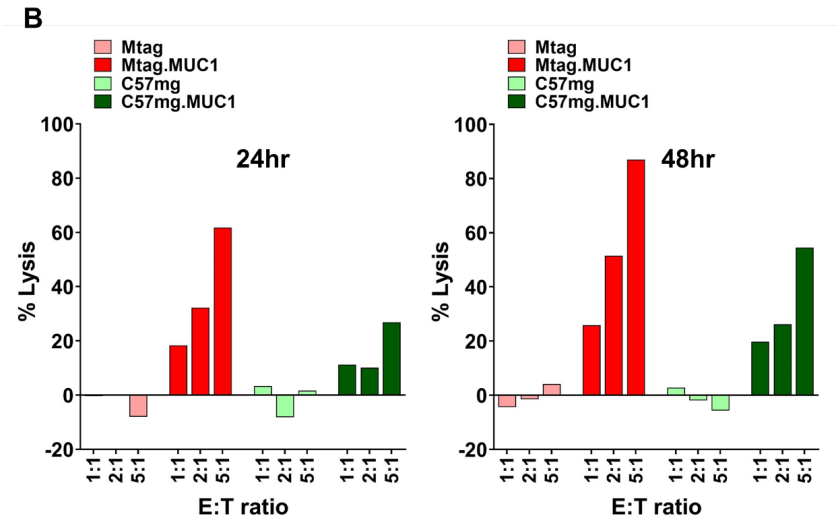
Figure 1. Mouse MUC28z CAR expression in activated mouse $CD8^+$ T cells after retrovirus transduction, as determined by flow cytometry analysis of MUC1-biotin binding in $CD8^+$ T cells. Dead cells were excluded by 7-AAD staining.

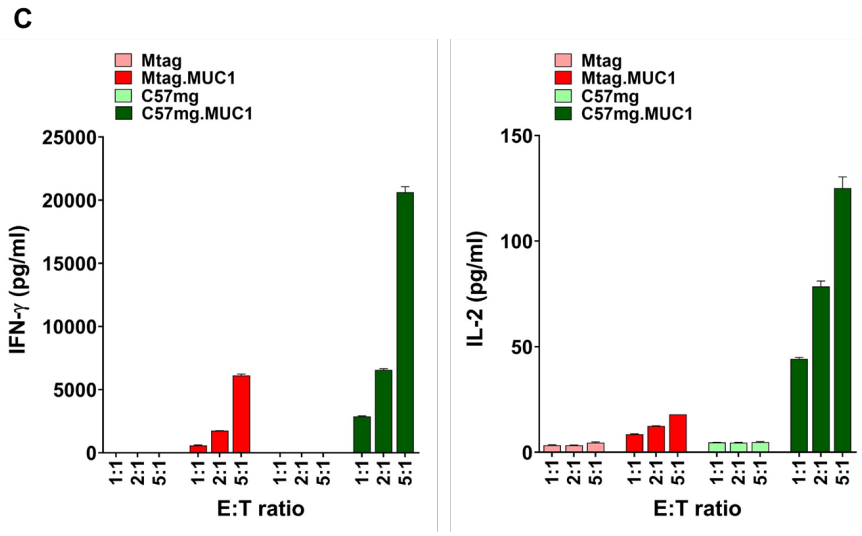
We next assessed the level of tMUC1 on the cell surface of a panel of mouse breast cancer cell lines by flow cytometry. The percentages of cells that express tMUC1 is shown in Figure 2A. The counterpart wildtype cell lines are shown in gray histograms to serve as human tMUC1-null controls. We had proposed to use MMT and MMT-Lung. Even though MMT tumor cells showed high expression of tMUC1 when freshly isolated, the MMT cells lost their MUC1 expression after in vitro passages (data not shown here). Thus, we stably transfected the MTag cell lines derived from the PyVMT tumors with the full-length MUC1 gene and designated the cell line Mtag.MUC1 cells. We also included another C57BL/6 mouse syngeneic mammary gland cell line, C57mg and C57mg.MUC1 cells that stably expresses full-length human MUC1.



We next assessed tMUC1-CAR-t mediated murine breast cancer cell killing in vitro.

The four cell lines (Mtag, Mtag.MUC1, C57mg, and C57mg.MUC1) were co-cultured with mouse MUC28z CAR t cells at E:T ratios of 1:1, 2:1, and 5:1 for 24h and 48h. There was a significant lysis of Mtag.MUC1 cells in vitro by MUC28z CAR t cells (Figure 2B). Even though only about 30% of the breast line C57mg.MUC1 were lysed by MUC28z CAR t cells within 24h co-culture, the lysis of C57mg.MUC1 was increase to approximately 60% after 48h co-culture with CAR t cells (Figure 2B). Importantly, the CAR t cell did not lyse tMUC1-null cell lines, suggesting the tumor killing was highly tMUC1 antigen dependent. All lysis data presented here was normalized to its own mock T cell lysis. We also included MMT cell line in the killing assay.





However, there was minimal cell death observed in MMT cells since they lost tMUC1 (data not shown).

Besides tumor cell lysis, the engagement of murine MUC28z CAR t cells with tMUC1-expressing murine tumor cell lines led to the IFN-γ and IL-2 production (Figure 2C) in an antigen-dependent manner.

Figure 2. The mouse MUC28z CAR t cells target on tMUC1-expressing tumor cells for lysis in vitro. (A) Percentage of cells expressing tMUC1, determined by TAB004-Fluor 647 staining and flow cytometry analysis. (B) Percentage of mouse tumor cell lysis by MUC28z CAR t cells. Cells were co-cultured at the indicated E:T ratio for 24hr and 48hr. The lysis of tumor cells was determined by MTT assay. Data are presented as the mean ± SD. (C) IFN-γ and IL-2 production by MUC28z CAR t cells. Data are presented as the mean ± SD.

Milestones for Aim 1: Tasks 1-3: Test 15 human breast cancer cell lines for tMUC1-CAR-T cell killing in vitro: 10 TNBC, 3 luminal and Her-2 type, and 2 normal mammary epithelial lines. Test 6 murine cell lines (syngeneic to C57BL/6) for tMUC1-CAR-t cell killing in vitro. This will determine the optimal breast cancer cell lines for *in vivo* experiment.

This was mostly accomplished. Instead of 6 murine cell lines, we tested 4 because couple cell lines lost their tMUC1 expression in vitro. Nevertheless, this did not change the direction of the overall goals. We were able to identify the cell lines to be used for in vivo studies.

Specific Aim 2a: Demonstrate TAB 004-CAR-T + anti-PD1 Ab mediated killing *in vivo* in xenograft model of human metastatic TNBC.
Task 4: Test the treatment efficacy of the 2nd and 3rd generation TAB-CAR-T cells in orthotopic implantation model in NSG mice (8-18 months).
Task 5: We therefore propose to use two cell lines that are resistant to CAR T cell alone to test the combination of CAR T cell + PD1 antibody (16-20 months).

Task 4 Progress: Test the treatment efficacy of the 2nd and 3rd generation TAB-CAR-T cells in orthotopic implantation model in NSG mice.

To determine the anti-tumor effect of MUC28z CAR T cells on TNBC tumor growth, the HCC70 cells were inoculated in NSG female mice, followed by a single injection of human MUC28z CAR T cells 6 days after tumor cell injection. Compared to the vehicle control, MUC28z CAR T cells effectively reduced the HCC70 tumor growth till the experiment endpoint on day 81 (Figure 3A). The insert in Figure 3A showed the wet weights of tumors resected from NSG mice at the endpoint. The tumor weights in the MUC28z CAR T cell-treated group was significantly lower than the vehicle group that received PBS. However, it must be noted that even though there was a significant difference between control and treated groups, the tumors treated with MUC28z CAR T cells did start to progress faster after ~60 days post treatment suggesting that a) a single injection of CAR T cells may not be sufficient, b) tMUC1 is lost in the remaining tumor that progressed, and c) blocks anti-tumor immune response and therefore a combination therapy together with CAR T cells is needed.

We investigated tMUC1 expression in tumors post MUC28z CAR T cell treatment in vitro and in vivo. The level of tMUC1 on HCC70 cells remained unchanged post co-culture with MUC28z CAR T cells or mock T cells in

vitro (Figure 3B). In addition, the tumor sections from MUC28z CAR T cells and vehicle treated mice were stained with TAB004 for tMUC1. Surprisingly, there was increased tMUC1 staining in the group treated with MUC28z CAR T cells than in the vehicle group (Figure 3C) suggesting that tMUC1 loss is not a factor for tumor out-growth post CAR T treatment.

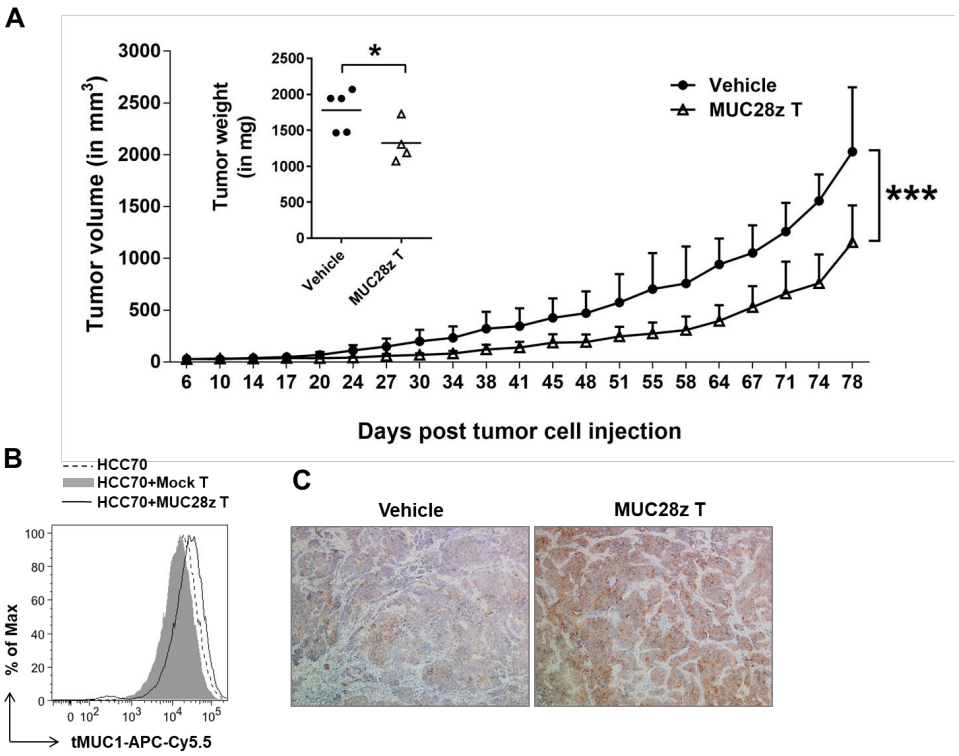
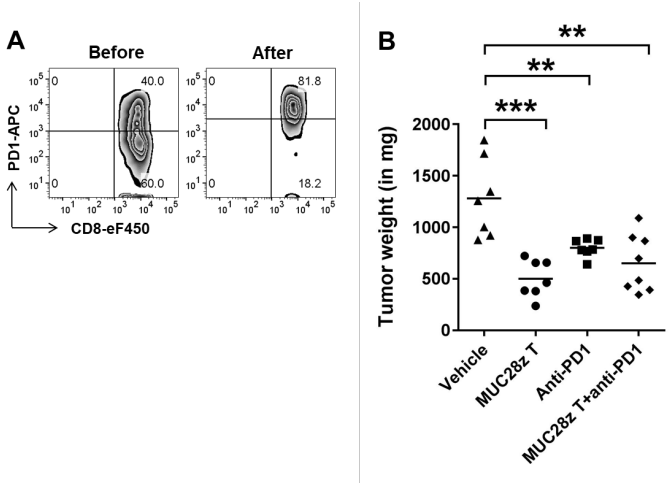


Figure 3. MUC28z CAR T cells have long-term efficacy for HCC70 tumor reduction in vivo. (A) Decrease of HCC70 tumor burden by a single injection of MUC28z CAR T cells in vivo. HCC70 cells were orthotopically injected into the mammary fat pad of female NSG mice. When tumors were palpable, mice were randomized and received a single i.v. injection of PBS as vehicle control, or MUC28z CAR T cells on day 6 post tumor cell challenge. Tumor growth was monitored by caliper measurement. Data are presented as mean ± SD. The statistical analysis was performed by two-way ANOVA. ***, $p<0.001$. The insert shows the wet weight of resected tumor mass on day 81 at endpoint. *$p<0.05$ (student t-test). (B) No tumor antigen loss while MUC28z CAR T cells were present in vitro. HCC70 cells were cultured alone or co-cultured with the mock T cells or MUC28z CAR T cells (E:T = 2:1) for 24hr. The viable HCC70 cells were analyzed for tMUC1 level. (C) Increased intensity of tMUC1 expression in MUC28z CAR T cells-treated HCC70 tumors. HCC70 tumor sections were prepared on day 81 at endpoint. Immunohistochemistry staining of tMUC1 was performed with TAB004 antibody. The brown staining shows tMUC1 positivity (100x magnification).

Some parts of Task 4 are now published in the Frontiers in Immunology paper. We used the 2nd generation CAR T cells for the in vivo experiments. We are working on the 3rd generation CAR T cells now (Front. Immunol., 24 May 2019 | https://doi.org/10.3389/fimmu.2019.01149 and Front. Immunol., 07 December 2020 | https://doi.org/10.3389/fimmu.2020.628776.)

Task 5 Progress: We therefore propose to use two cell lines that are resistant to CAR T cell alone to test the combination of CAR T cell + PD1 antibody (16-20 months).

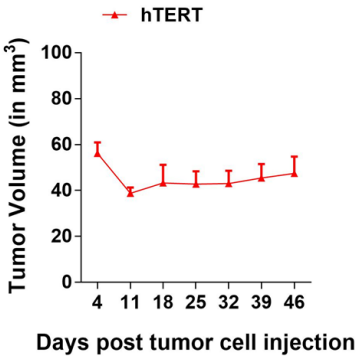
We checked the MUC28z CAR T cells for PD1 expression right before the adoptive transfer and on the day of the experimental endpoint. Data in Figure 4A showed the changes within CD8+ MUC28z CAR T cells. Approximately 50 days after surviving in vivo, the tumor-infiltrating CD8+ MUC28z CAR T cells expressed very high level of PD1 (Figure 4A), suggesting their further activation by in vivo tMUC1 tumor antigen stimulation.



To assess if in vivo combining MUC28z CAR T cells with anti-PD1 antibody may be a better strategy for tumor eradication, we i.p. injected anti-human PD1 antibody at 10mg/kg once weekly for about 8 weeks. Combing anti-PD1 antibody with human MUC28z CAR T cells did not show improvement for tumor reduction compared to CAR T cell alone (Figure 4B). We will optimize the PD1 blocking antibody dose and treatment schedule to see whether we can improve the outcome. Alternatively, we will block PD-L1 on TNBC tumor along the PD1-PD-L1 checkpoint signaling axis, which could possibly enhance our CAR T cell efficacy.

Figure 4. PD1 blockade did not enhance tumor reduction by human MUC28z CAR T cells under the indicated conditions. (A) Increase of PD1, an activation/exhaustion marker, on CD8+ MUC28z CAR T cells. MUC28z CAR T cells were stained right before i.v. injection and right after tumor infiltrating lymphocytes analysis from tumor mass. Cells were gated on $CD8^+$ T cells. (B) Combining anti-PD1 blocking antibody with MUC28z CAR T cells showed no synergistic effect for tumor reduction. HCC70 tumors were inoculated same as Figure 3A. When tumors were palpable, mice were randomized and received a single i.v. injection of PBS as vehicle control, or MUC28z CAR T cells. Anti-human PD1 antibody were i.p. injected once weekly till the endpoint. Tumor growth was monitored by caliper measurement. Tumors were resected and weighed at endpoint on Day 57. The statistical analysis was performed by Student t-test. **, $p<0.01$; ***, $p<0.001$.

Milestones for Aim 2a: Tasks 4 and 5: Test n=2 human TNBC and n=1 normal epithelial cell in vivo in NSG mice using 3 preparation of TAB-CAR T cells and two sources of T cells (one source from normal donor and one from TNBC patient).



We have completed most of the milestone with the 2nd generation CAR T cells. Experiments with the 3rd generation CAR T cells are still ongoing

We had proposed to use a normal mammary gland epithelial cells in vivo but we found that the normal hTERT-HME1 didn't form tumors that were capable of progress in the NSG mouse (Figure 5). Therefore, we will not use this cell line for in vivo control.

Figure 5. hTERT-HME1 cells were not able to form tumor in vivo. hTERT-HME1 cells were injected into mammary fat pad of NSG mice same as the procedure for HCC70 cells in Figure 3 and 4.

Specific Aim 2b: Demonstrate TAB-CAR-t mediated killing *in vivo* in orthotopic model of mouse metastatic TNBC in human MUC1.Tg syngeneic mice.

Task 6: Test the treatment efficacy of 2nd and 3rd generation TAB-CAR-t in orthotopic implantation model in MUC1.Tg mice (18-24 months).

Task 6 Progress: Test the treatment efficacy of 2nd and 3rd generation TAB-CAR-t in orthotopic implantation model in MUC1.Tg mice.

So far, we used 2nd generation mouse MUC28z CAR t cells for Task 6. We orthotopically injected Mtag.MUC1 cells into mammary fat pad of immune competent MUC1.Tg mice. A single dose of CAR t cells was i.v. injected to Mtag.MUC1 tumor bearing mice. We didn't observe any tumor control by mouse MUC28z CAR t cells as compared to mock t cell control or vehicle PBS control under current conditions (Figure 6). There is limited research using mouse CAR t cells in immune competent mice. We expected the difficulties we would encounter. To improve the outcome, we would like to precondition the host mice with cyclophosphamide before CAR t cell infusion to make recipient mice partially immune compromised, so to improve the acceptance, cell proliferation, and function of CAR t cells in vivo. Also, instead of one dose of CAR t cell treatment, we are planning to increase the treatment frequency to 2-3 times in total. The experiments with the 3rd generation CAR t cells are ongoing.

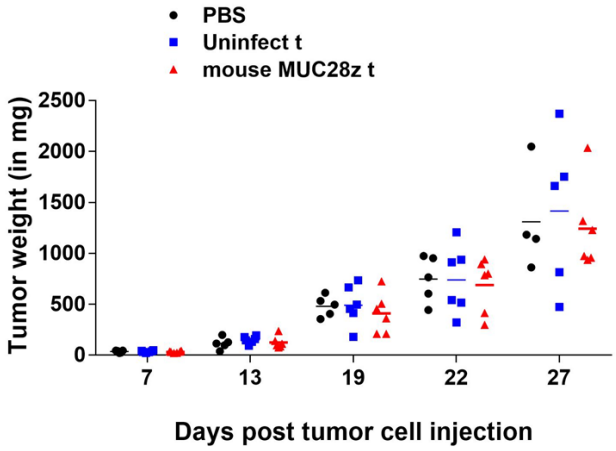


Figure 6. Mouse MUC28z CAR t cells showed no tumor reduction for mouse Mtag.MUC1 breast tumor in human MUC1.Tg syngeneic immune competent mice. The procedure for tumor inoculation and t cell treatment were carried out similarly as in Figure 3A.

Milestones for Aim 2b: Tasks 6: Test n=2 murine cell lines in vivo in MUC1.Tg mice. Identify the optimal dosing schedule. Confirm enhanced antitumor effect of the 3 CAR-T/t cell preparation (TAB28z, TAB28BBz, and TAB28OXz) in orthotopic implantation model.
We tested 1 cell line with 2nd generation CAR-t cells. The other experiments are ongoing.

Specific Aim 2c: Demonstrate tMUC1-CAR-T mediated killing in spontaneous breast cancer model in the human MUC1-PyV MT bitransgenic (MMT) mice.

Task 7: (Months 12-24). Two groups of MMT mice will be treated 1) starting at 8 weeks of age (at the mammary intraepithelial neoplasia (MIN), MIN stage), a second cycle at 12 weeks of age, and a third cycle at 15 weeks of age; and 2) starting at 12 weeks of age (early carcinoma) and a second cycle at 15 weeks of age. This will determine if treatment early during tumor progression is more efficacious. Three formulations of TAB-CAR-t cells will be injected **(12-30 months)**

Task 7 Progress: Demonstrate TAB-CAR-t cell mediated killing of tumors in MMT bitransgenic mice that develop spontaneous mammary gland tumors and express human MUC1.

So far, we had only used 2nd generation mouse MUC28z CAR t cells and without combination for Task 7. We used the immune competent MMT mice spontaneously developed mammary tumors. The tMUC1 expression on two freshly isolated MMT tumors were evaluated and the data in Figure 7A confirmed the tMUC1 presence for targeting by our tMUC1-specific mouse MUC28z CAR t cells. Then mouse MUC28z CAR t cells were i.v. injected into MMT mice starting at approximately 8-10 weeks of age to determine whether they could control the spontaneously arising breast tumor in immune competent mice. Two additional T cell injections were administered at 3 weeks interval. The kinetics of tumor growth among the MMT mice showed big variation (Figure 4B). However, the growth of MMT tumors in MUC28z CAR t cell treated mice were statistically slower than the age-matched mice receiving mock T cell control (Figure 4B, p=0.02). Since the spontaneous MMT mice are immune competent, we will precondition the MMT hosts with cyclophosphamide before CAR t cells infusion to improve the in vivo activity of mouse MUC28z CAR t cells.

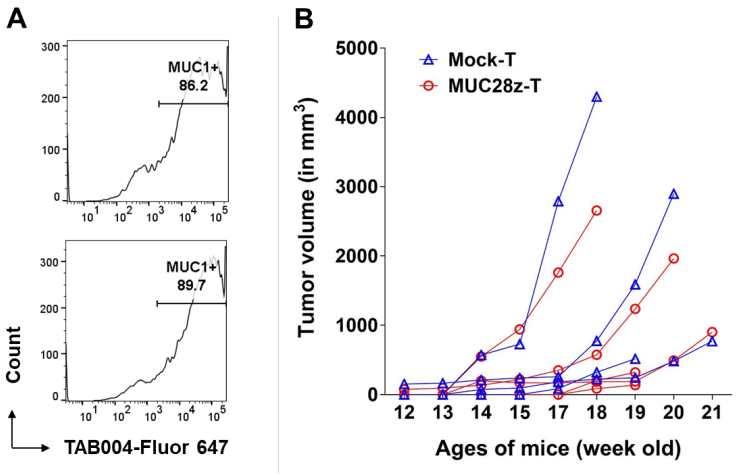


Figure 7. MUC28z CAR t cells control spontaneous MMT tumor growth. (A) Tumors from two female MMT mice (~20 weeks of age) were resected and single cell suspensions were prepared. Cells were then promptly stained with TAB004-Fluor 647 and analyzed for MUC1 level by flow cytometry. (B) Female MMT mice at approximately 10 weeks of age were pooled and randomized into two groups. One group received an i.v. injection of mock t cells as control, and the other group received mouse MUC28z CAR t cells. Two additional t cell injections were administered at a 3-weeks interval afterwards. N=4 mice for mock t cell group; N=5 mice for mouse MUC28z CAR t cells. *, p<0.05 (two-way ANOVA).

Milestones for Aim 2c: Tasks 7: Test the anti-tumor efficacy of TAB-CAR-t cells in immune competent MMT model that develop spontaneous mammary gland tumors and express human MUC1 and mimics the human disease. Early intervention and late intervention will be evaluated.

Thus far, we evaluated early intervention model. The experiment was conducted with the MMT model using the 2nd generation CAR T cells. Other experiments are being conducted now with alternatives to get a better outcome.

Specific Aim 3: Demonstrate TAB-CAR-T cell mediated killing in human tumor explant models of metastatic, treatment refractory TNBC.

Task 8: Receive cells generated from breast cancer specimens from patients with metastatic/treatment refractory breast cancer (**anticipated receipt of cells will be Month 24**).

No progress has been made on this task

Task 9: Expression of tMUC-1 on these human primary cell lines and determine TAB-CAR-T cell mediated killing of TNBC explant cells in vitro **(24-28 months).**

No progress has been made on this task

Task 10: Determine TAB-CAR-T cell mediated killing of TNBC explant cells in vivo **(28-36 months).**

No progress has been made on this task

Milestones: Duke University (Dr. Lyerly's group) will collect at least 15 breast cancer samples. They will establish least 10 breast cancer explant in NSG mice. We will test n=6 TNBC explants in vitro and n=2 in vivo in the orthotopic model for treatment.

No progress has been made yet---however, as the lock downs are being lifted, we anticipate progress on this this year.

What opportunities for training and professional development has the project provided?

We had PhD students and undergraduate students work on parts of this project. We also had the opportunity to train 2 undergraduate students belonging to underrepresented minority population. Dr. Ru Zhou was able to get her promotion from Research Assistant Professor to Research Associate Professor while working on this grant.

How were the results disseminated to communities of interest?

Front. Immunol., 24 May 2019 | https://doi.org/10.3389/fimmu.2019.01149 and Front. Immunol., 07 December 2020 | https://doi.org/10.3389/fimmu.2020.628776.)

Due to the pandemic, we did not present at any conferences.

4. **Impact:** The proposed research has the potential to lead to revolutionary therapies that will not only eliminate the mortality associated with metastatic TNBC but also replace interventions that have life threatening toxicities with ones that are safe and effective, i.e.: novel immunotherapeutic strategies targeting only the specific tumor associated antigen on TNBC while sparing normal organs. Such therapies have the potential of controlling disease progression, prolonging time to recurrence and ultimately, even serving as a preventive measure or cure. If successful, this project will have a major impact and accelerate progress toward a clinical trial for metastatic TNBC. The impact will be significant and move much beyond an incremental advancement.

   What was the impact on the development of the principal discipline(s) of the project?
   The fact that the murine CAR t cells did not cause toxicity on immune competent MUC1.Tg mouse suggests that the TAB004 CAR s will be safer than other CARs that are being developed and that these CAR T cells will not attack the normal epithelia that expresses normal MUC1. Targeting the tumor form of MUC1 was the overall innovation and goal of the project and thus far, it certainly seems that is the case.

   What was the impact on other disciplines?
   Nothing to report
   What was the impact on technology transfer?
   Nothing to report
   What was the impact on society beyond science and technology?
   Nothing to report

5. **Changes/Problems:**
   Actual or anticipated problems or delays and actions or plans to resolve them

   Established MMT cell line lost its MUC1. We will focus on stable Mtag.MUC1 cells. hTERT-HME1 cells were not able to form tumor in vivo. We will use other tMUC1 low expressing cells as replacement. In immune competent recipient mice, we will precondition animals with cyclophosphamide to improve the CAR t cells acceptance and activities in vivo. These changes will not affect the overall impact.

   Other delays are due to the COVID-related lockdown on conducting mouse experiments. Progress has been definitely hampered. Unfortunately, due to the lockdown from early March of UNCC and the vivarium, we were unable to conduct any mouse experiments. The vivarium staff were just maintaining the mouse colony. However, for a while even that became a challenge as one of the vivarium staff was infected with the CORONA virus and all vivarium staff were guaranteed and we were unable to resume

mouse experiments for a long time. The vivarium fully opened only in the end of October of 2020 for new experiments. One of my lab technicians was also exposed to COVID because her mother got COVID and everyone in my lab had to be quarantined and retested before entry into the lab. Therefore, there were many challenges this last year. However, we are certain that we will successfully complete the project.

Changes that had a significant impact on expenditures

Nothing to report. However, as with most other investigators, even though work was not being conducted due to COVID related shut downs, we had to keep all employees paid from the grant.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report

Significant changes in use of biohazards and/or select agents
Nothing to report

6. **Products:** murine CAR t cells were successfully generated and tested in vitro.

7. **Participants & Other Collaborating Organizations**: Nothing to report.

   Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period? Nothing to report

   What other organizations were involved as partners?
   Nothing to report

8. **Special Reporting Requirements:** Nothing to report

9. **Appendices:** publication attached