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14. ABSTRACT

The lack of effective therapies for the treatment of metastatic triple negative breast cancer (TNBC) has prompted us to develop a combinatorial strategy for the treatment of this highly malignant type of breast cancer. In this strategy, the tumor antigen chondroitin sulphate proteoglycan 4 (CSPG4) is used as a target, since it is expressed on both differentiated TNBC cells and TNBC cancer initiating cells (CICs) and has a restricted distribution in normal tissues. Therefore, immunotargeting of CSPG4 is not expected to cause major side effects because of targeting of normal tissues and is expected to eliminate not only differentiated TNBC cells, but also TNBC CICs. According to the cancer stem cell theory, CICs play a major role in disease recurrence and in metastatic spreading. The effector mechanism is represented by T cells transduced with CSPG4-specific chimeric antigen receptor (CAR), since i) this strategy allows rapid generation of polyclonal T cells with tumor antigen (TA)-specificity and ii) the recognition of tumor cells by CAR T cells does not depend on HLA class I antigen expression by target cells. CAR T cells are combined with strategies which counteract the escape mechanisms utilized by TNBC cells to avoid recognition and destruction by CSPG4 CAR T cells. The escape mechanisms, which are triggered by the changes induced by hypoxia in the tumor microenvironment, include i) reduced susceptibility of TNBC cells to the lytic activity of CAR T cells because of the upregulation of antiapoptotic molecules. The latter is caused by the activation of the Sonic Hedgehog Homolog (SHH) pathway triggered by hypoxia, a hallmark of tumor microenvironment; ii) the dysfunction of CAR T cells caused by the interaction of PD-1 they express with PD-L1 induced by hypoxia on tumor cells and on nonmalignant cells present in the tumor microenvironment and iii) the reduced viability of CAR T cells because of the unbalanced level of cytokines in the tumor microenvironment. Our combinatorial strategy includes i) sonidegib, an inhibitor of SHH pathway, ii) anti-PD-L1 monoclonal antibodies and iii) fusion proteins which target cytokines to the tumor microenvironment.

The therapeutic efficacy of our combinatorial immunotherapy is tested both *in vitro* and in mouse models. TNBC cell lines and TNBC PDXs are used as targets.

15. SUBJECT TERMS
CSPG4, TNBC, PD1, Chimeric antigen receptors, T cell immunotherapy

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Table of Contents

1. **INTRODUCTION:** The goal of this proposal is to test the anti-tumor activity of the combinatorial strategy which utilizes the individual approaches we have developed and shown to be effective in counteracting the corresponding escape mechanism triggered by hypoxia.

2. **KEYWORDS:**
   CSPG4
   TNBC
   PD1
   Chimeric antigen receptors
   T cell immunotherapy

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

Specific Aim 1. CSPG4 up-regulation induced by hypoxia in TNBC tumors is associated with a poor clinical course of the disease.

As indicated in the progress report, we have optimized the immunohistochemical assay (IHC) to analyze CSPG4 expression in 63 surgically removed TNBC tumors. We have correlated CSPG4 expression with the clinical characteristics of the patients. On the other hand, there is a delay in analyzing the role of hypoxia in the regulating the expression of CSPG4 in TNBC tumors since we have had difficulties in identifying a HIF1a-specific monoclonal antibody (mAb) which yields reliable results.

Specific Aim 2. T cells transduced with a CSPG4-specific CAR containing a PD-1 shRNA (CSPG4-specific CAR+ PD-1 shRNA-T cells) in combination with IL-2-anti-idiotypic (anti-id) mAb MK2-23 fusion protein and LDE225, an inhibitor of the SHH pathway, eradicate both differentiated TNBC cells and TNBC CICs incubated under hypoxic conditions *in vitro*.

As indicated in the progress report, we have now generated and validated a CAR from the CSPG4-specific mAb 225.28, since this mAb crossreacts with mouse vessels. Therefore, the results obtained with the CAR derived from this mAb in the proposed *in vivo* experiments are more likely to mimic the clinical setting. We have shown that T cells genetically engineered with CAR generated from mAb 225.28 are effective in eliminating CSPG4 expressing target cells. In addition, the concern that the IL-2-anti-idiotypic mAb MK2-23 fusion protein may inhibit the recognition of target cells by CSPG4 CAR T cells has prompted us to generate a fusion protein with a B7-H3-specific mAb and to utilize it instead of the IL-2-anti-idiotypic mAb MK2-23 fusion protein to target cytokines to the tumor microenvironment. As a first step, we have analyzed the expression of B7-H3 in normal tissues and in the 63 surgically removed TNBC tumors. The results we have obtained indicate that a fusion protein generated with a B7-H3-specific mAb is expected not to cause side effects since B7-H3 has a very restricted expression in normal tissues. Furthermore, the B7-H3-specific mAb is expected to target IL-2 to most of TNBC tumors.

Specific Aim 3. CSPG4-specific CAR+ PD-1 shRNA-T cells in combination with IL-2-anti-id mAb MK2-23 fusion protein and LDE225 suppress in an adjuvant setting metastatic spread and disease recurrence and prolong survival of NSG mice which are orthotopically grafted with the TNBC MDA-MB-231-Luc-D3H1 cell line and then subjected to surgical removal of their primary tumor.

Specific Aim 4. The results obtained with the TNBC cell lines have clinical significance, as they are reproduced in NSG mice orthotopically grafted with patient derived TNBC xenografts (PDX).

What was accomplished under these goals?

Task 1

CSPG4 up-regulation induced by hypoxia in TNBC tumors is associated with a poor clinical course of the disease.

Immunohistochemical staining (IHC) of TNBC tumors with CSPG4-specific mAb

Several antigen retrieval methods were tested to identify the most effective to revive CSPG4 in formalin fixed, paraffin embedded TNBC tumors. Incubation of slides in EDTA buffer, pH8.0, was found to be the most effective.

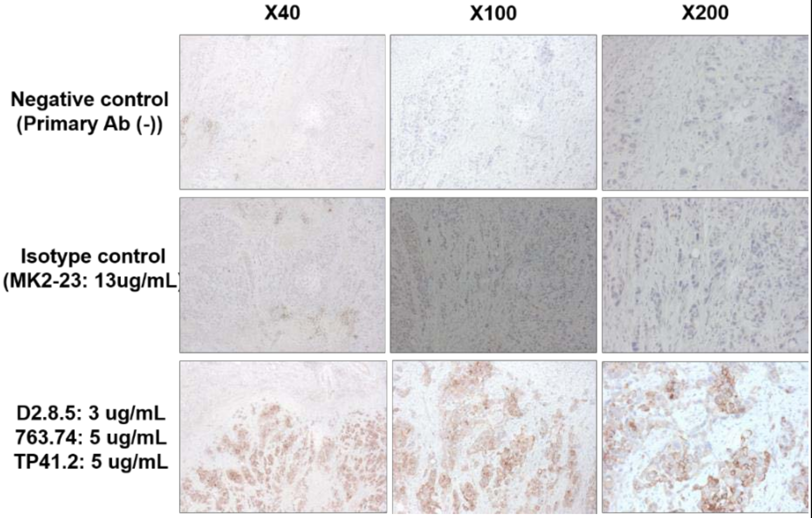


Figure 1. Specific staining of TNBC tumors by CSPG4-specific mouse mAbs. TNBC sections were IHC stained with mixed CSPG4-specific mouse mAbs (D2.8.5, 763.74 and D2.8.5).

In preliminary experiments, 10 of our mouse monoclonal antibodies which recognize distinct and spatially distant epitopes of CSPG4 have been purified from ascitic fluid by affinity chromatography on Protein G. The mAb preparations have been tested for purity and activity by SDS-PAGE and by binding assays, respectively. mAbs have been screened for their ability to stain individually and in combination formalin fixed, paraffin embedded tissues. Furthermore, the mAbs have also been tested for their ability to stain fixed cell pellets prepared with the melanoma Colo 38 cell line, which expresses CSPG4, and with the melanoma M14 cell line, which does not express CSPG4. The cell pellets have been used as a positive and negative control in the IHC assays. We have found that the combination of the mouse mAbs (D2.8.5: 3ug/mL, 763.74: 5ug/mL and TP41.2: 5ug/mL) yields the best results. **Fig. 1** shows staining patterns, which indicate the specific staining of TNBC tumors by the combination of the three CSPG4-specific mAbs D2.8.5, 763.74 and TP41.2. This combination of mAbs has been used to stain 63 TNBC lesions present in a TMA. This TMA has been prepared by Dr. E. Brachtel, a pathologist who is a member of our research team. The staining pattern shows that in the positive lesions all the cells were stained. Based on the intensity of staining, the tumor sections were scored on a scale from 0 to 3. Representative staining intensity patterns are shown in **Fig. 2. Fig.3** summarizes the results we have obtained. More than 85% of the TNBC

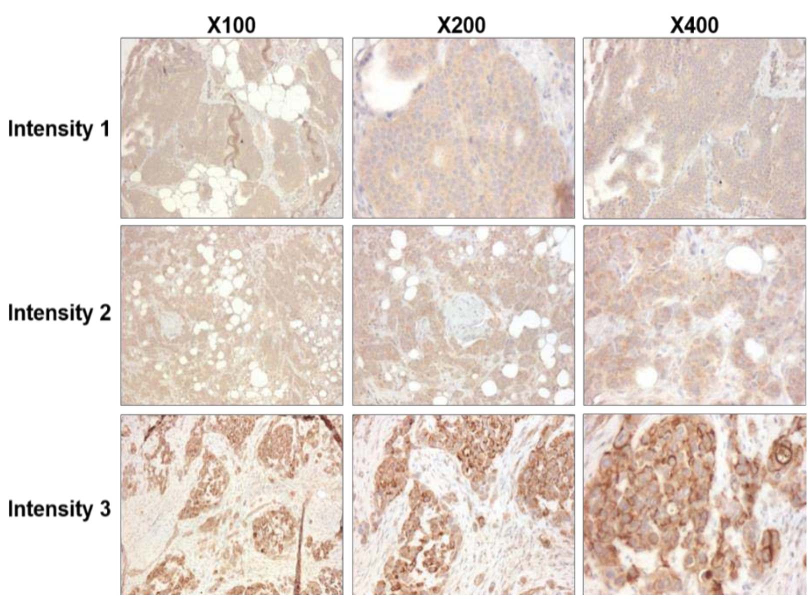
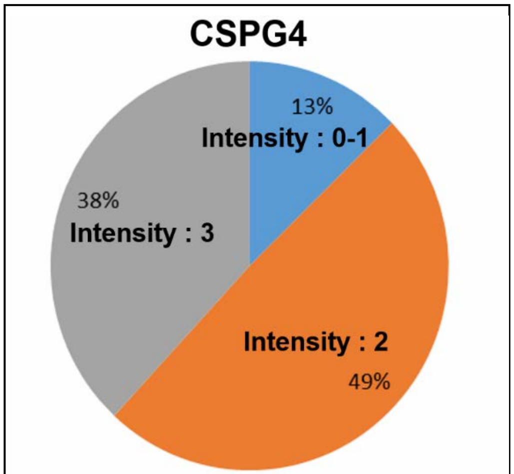


Figure 2. Representative staining pattern of TNBC tumors with CSPG4-specific mouse mAbs. TNBC lesions in TMA were IHC stained with mixed CSPG4-specific mouse mAbs (D2.8.5, 763.74 and D2.8.5).



Intensity	No. of Patients	Percentage
0-1	8	13%
2	31	49%
3	24	38%

Figure 3. CSPG4 expression in TNBC tumors.

tumors tested displayed a strong reactivity with the combination of the three CSPG4-specific mAbs. Only 8 (13%) of the tumors tested displayed a low reactivity with the combination of the CSPG4-specific mAbs. When we submitted the grant application we had found that the rabbit anti-human HIF1 alpha antibody [EP1215Y], C-term, GTX61608 company sold by GeneTex, Inc. Irvine, CA yielded reliable results in IHC with formalin fixed tissues. A representative example is shown in **Fig. 4**. However, this mAb has been discontinued at GeneTex. We have screened other anti-human HIF1 alpha antibodies and we have found that the rabbit anti-human HIF1 alpha antibody [EP1215Y] (ab51608) yields reliable results in IHC with formalin fixed tissues. At present, we are optimizing the use of this mAb. If we succeed in the optimization in the next 10 days, we should be able to test the TNBC TMAs by the end of April 2018.

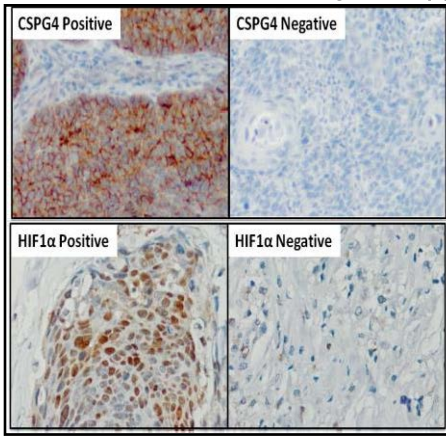


Figure 4. CSPG4 and HIF1α expression in a patient derived TNBC tumor. Immunohistochemical (IHC) staining of a formalin fixed, paraffin embedded TNBC tumor section with the CSPG4-specific mAb D2.8.5-C4B8 or with HIF1α-specific mAb. Samples were counterstained with hematoxylin. The isotype matched mAb MK2-23 was used as a control (data not shown). **Left upper panel** shows a lesion with strongly positive staining of CSPG4 and **right upper panel** shows a lesion with negative staining of CSPG4. **Left lower panel** shows a lesion with strongly positive staining of HIF1α and **right lower panel** shows a lesion with negative staining of HIF1α. Magnification (400X).

Subtask 2. Statistical analysis of the IHC results and association with clinical data. The results of the IHC for CSPG4 expression in TNBC tumors will be correlated with the histopathological and clinical characteristics of the tumors.

The cohort of 63 TNBC tumors analyzed in this study was part of a collection of 241 consecutive malignant breast tumors which included ER^+ and PR^+ breast tumors. In this group of tumors T stage, N stage and IC grade were correlated with disease free survival and overall survival, attesting to the integrity of the group of breast tumors analyzed. In contrast, in the group of 60 TNBC tumors analyzed T stage, N stage and IC grade were correlated neither with disease free survival nor with overall survival. These findings may reflect the small number of TNBC tumors analyzed.

Immunohistochemical staining of the 63 TNBC tumors with mAbs detected CSPG4 in 55 tumors; in 24 of them the staining intensity was high (score 3) and low (score 2) in the remaining 31.

CSPG4 expression was not correlated with T stage, N stage, IC grade, disease free survival and overall survival.

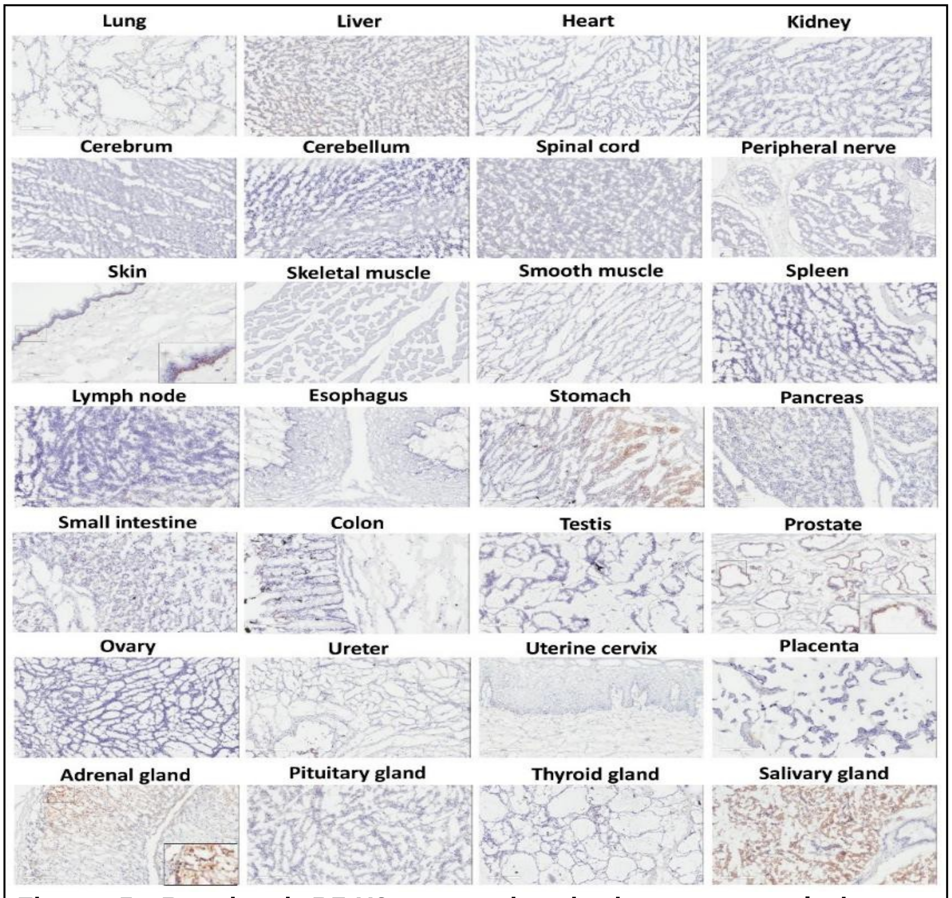


Figure 5. Restricted B7-H3 expression in human normal tissues. Expression of B7-H3 in normal human tissues (TMA) as assessed in frozen slides stained with the 376.96 mAb used at final concentration of 1 μg/mL. Slides stained only with the secondary antibody were used as negative control. Representative photomicrographs are shown. TMA photomicrographs are representative of at least three sections per tissue. Scale bars are 200 μm.

Task 2. T cells transduced with a CSPG4-specific CAR containing a PD-1 shRNA (CSPG4-specific CAR+ PD-1 shRNA-T cells) in combination with IL-2-anti-idiotypic (anti-id) mAb MK2-23 fusion protein and LDE225, an inhibitor of the SHH pathway, eradicate both differentiated TNBC cells and TNBC CICs incubated under hypoxic conditions *in vitro*.

Subtask 1. To generate CAR T cells and assess their function in normoxic (20%O_2 tension) and hypoxic (1%O_2 tension) conditions *in vitro* ***(see page 9)***

Subtask 2. To optimize the mitogenic effect of LDE225 and IL-2 anti-id mAb MK2-23 fusion protein on CSPG4-specific CAR T cells.

The possibility that the fusion protein generated with the anti-id mAb MK2-23 may inhibit the interaction of CSPG4 CAR T cells with target cells has prompted us to explore the possibility to generate a fusion protein

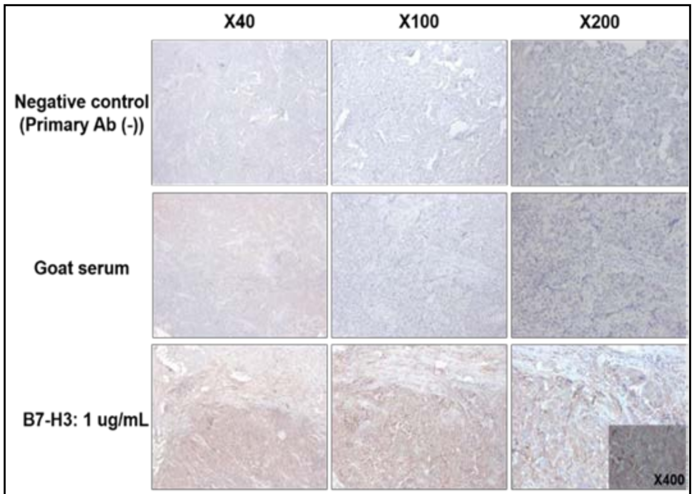


Figure 6. Specific staining of TNBC tumors by B7-H3-specific Ab. TNBC sections were IHC stained with B7-H3-specific antibody.

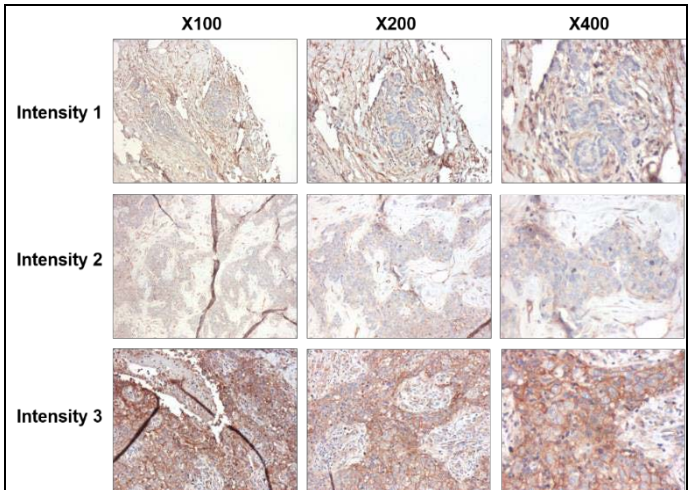
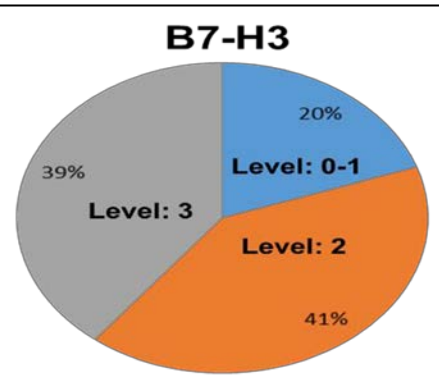


Figure 7. Representative staining pattern of TNBC tumors with B7-H3-specific goat mAb. TNBC lesions in TMA were IHC stained with B7-H3-specific antibody.



Intensity level	Patients	Proportion
0-1	13	20%
2	27	41%
3	26	39%

Figure 8. B7-H3 expression in TNBC tumors with lack or low CSPG4 expression.

with a B7-H3-specific mAb, since B7-H3 is expressed independently of CSPG4 on tumor cells. As a preliminary step to this possibility, Dr. Dotti has analyzed the B7-H3 expression on a large number of normal tissues. As shown in **Fig. 5,** B7-H3 was detected only in adrenal glands, salivary glands and some epithelial cells in stomach. In addition, we have tested 5 TNBC cell lines for expression of B7-H3 and found that all them express B7-H3. To assess the *in vivo* relevance of these results we have analyzed the expression of B7-H3 in surgically removed TNBC tumors. Representative examples of the staining of surgically removed TNBC tumors by B7-H3-specific mAb are shown in **Fig. 6** and **7**. A summary of the B7-H3 expression in TNBC tumors is shown in **Fig 8**. These results indicate that a fusion protein generated with a B7-H3-specific mAb will not react with most normal tissues but should target most TNBC tumors. Therefore a IL-2-B7-H3 fusion protein is being generated using the methodology described by Gillies (Gillies SD. A new platform for constructing antibody-cytokine fusion proteins (immunocytokines) with improved biological properties and adaptable cytokine activity. Protein engineering, design & selection. PEDS. 2013;26(10):561-9. doi: 10.1093/protein/gzt045. PubMed PMID: 24025193.)

We have also developed strategies to enhance the expression of CARs on T cells with the expectation that this enhances their antitumor activity. As a first step, we have utilized anti-id mAb recognizing the mAb 225.28 and 763.74 from which the CARs used in this study are generated, as probes to monitor the expression of CARs on transduced T cells, assessing both percentage of positive cells and expression level. Representative examples are shown in **Fig.9**. The test with anti-idiotypic monoclonal antibodies is also useful to monitor changes in the expression level of CARs by T cells. We have found that *in vitro* treatment of CSPG4 CAR T cells with the histone deacetylase (HDAC) inhibitor vorinostat up-regulates the CSPG4 CAR expression level on T cells, as indicated by the increased mean fluorescence intensity (MFI) (**Fig. 10**). On the other hand, no change is detected in the % of T cells which express CSPG4 CAR. (**Fig. 11**) The effect of the HDAC inhibitor is both dose and time dependent (**Fig.11**). Preliminary results are compatible with the possibility that vorinostat-treated CSPG4 CAR T cells have an increased anti-tumor activity (**Fig.12**). This effect on the other hand is not mediated by modulation of CSPG4 expression on TNBC cells (**Fig. 13**).

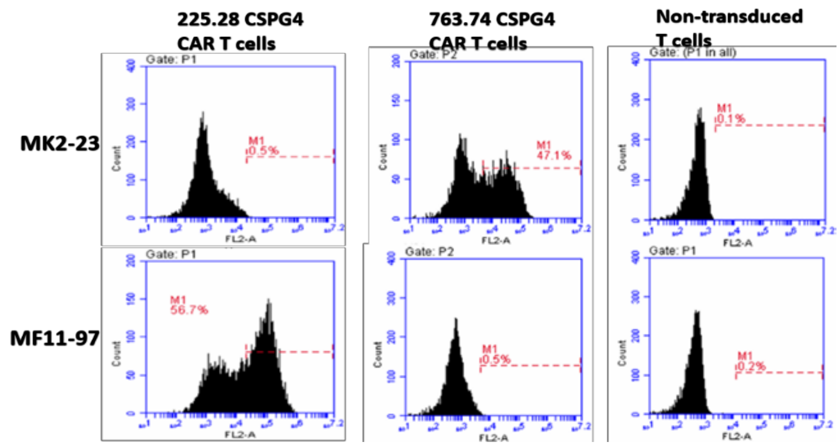


Figure 9. Transduction efficiency of T cells with CSPG4 CARs generated from the CSPG4-specific mAbs 225.28 and 763.74. T cells transduced with CARs generated from the CSPG4-specific mAb 225.28 and 763.74 were stained with the anti-idiotypic mouse mAbs MF11-97 and MK2-23. mAb MF11-97 and MK2-23 were generated with mAb 225.28 and 763.74, respectively. Then cells were stained with rabbit anti-mouse IgG-PE antibody. Non-transduced T cells were used as a control. Stained cells were subjected to flow cytometry analysis. The % of cells stained with the anti-idiotypic mAbs are shown.

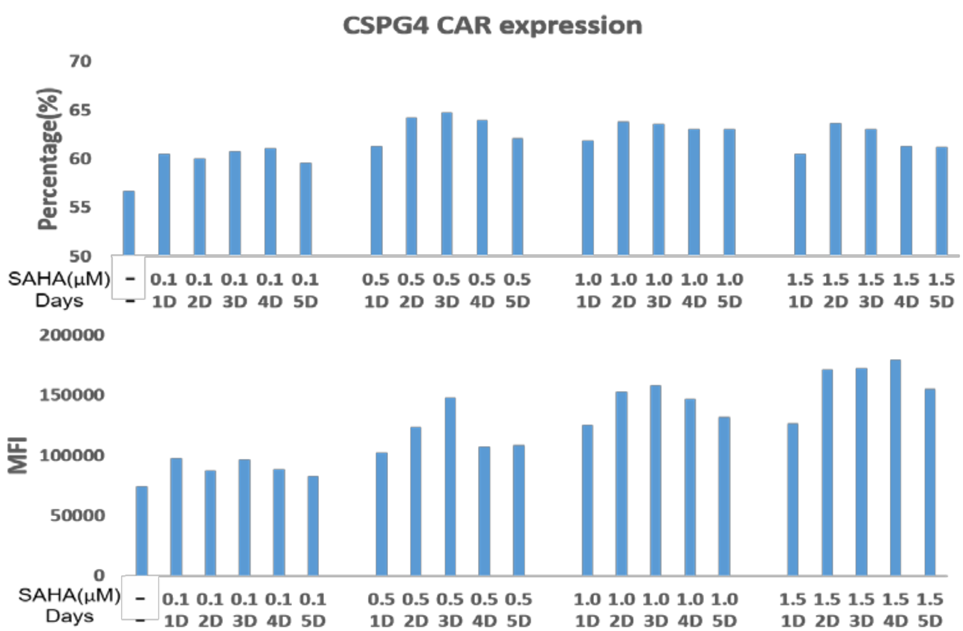


Figure 10. Up-regulation of CAR expression on CSPG4 CAR T cells by the HDAC inhibitor vorinostat.
CSPG4 CAR T cells were treated at 37 ℃ with vorinostat at 0.1, 0.5,1.0 and 1.5 μM for up to 5 days. Cells were then stained with anti-idiotypic mAb MF11-97 and rabbit anti-mouse IgG-PE antibodies. Stained cells were then analyzed by flow cytometry. mAb MK2-23 was used as an isotype control. MFIs and percentages of stained cells are shown.

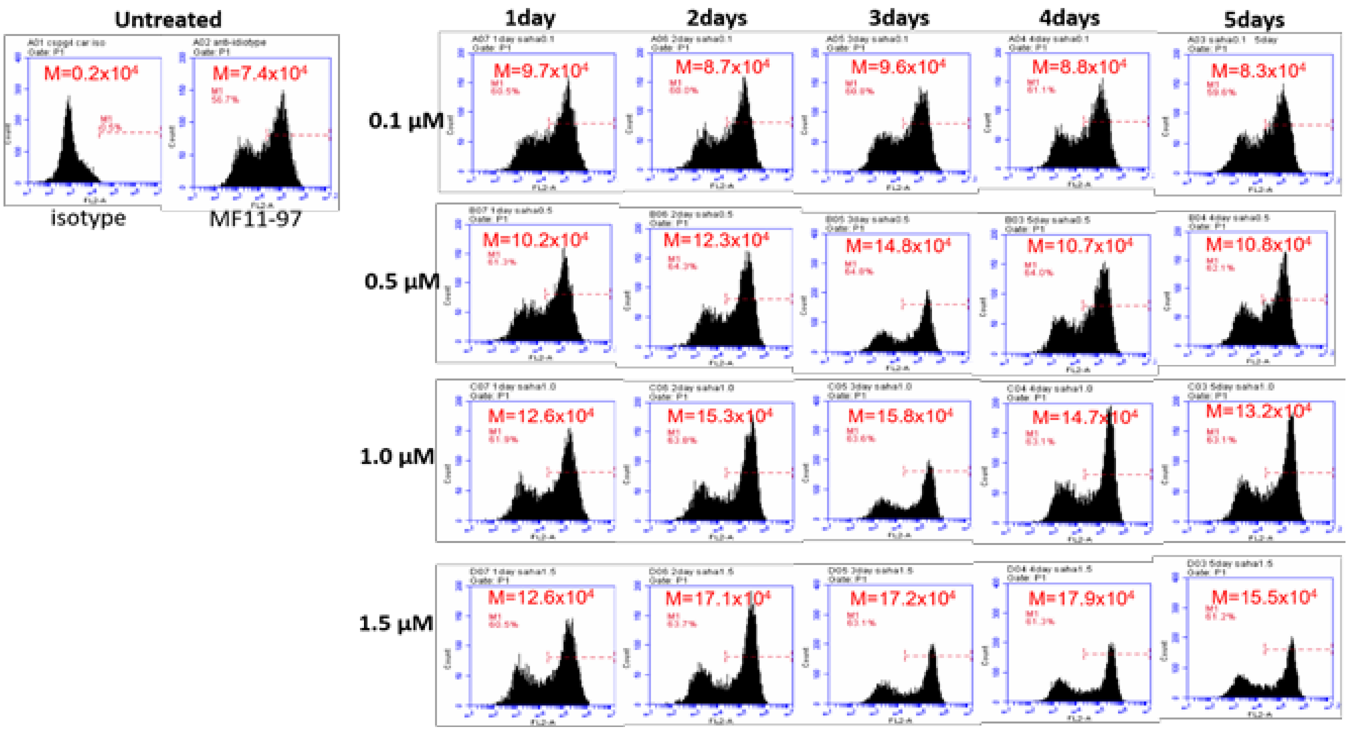


Figure 11. Up-regulation of CAR expression on CSPG4 CAR T cells by the HDAC inhibitor vorinostat. CSPG4 CAR T cells were treated at 37 ℃ with vorinostat at 0.1, 0.5, 1.0 and 1.5 µM for up to 5 days. Cells were then stained with anti-idiotypic mAb MF11-97 and rabbit anti-mouse IgG-PE antibodies. Stained cells were then analyzed by flow cytometry. mAb MK2-23 was used as an isotype control. MFIs (M) and percentages of stained cells are shown.

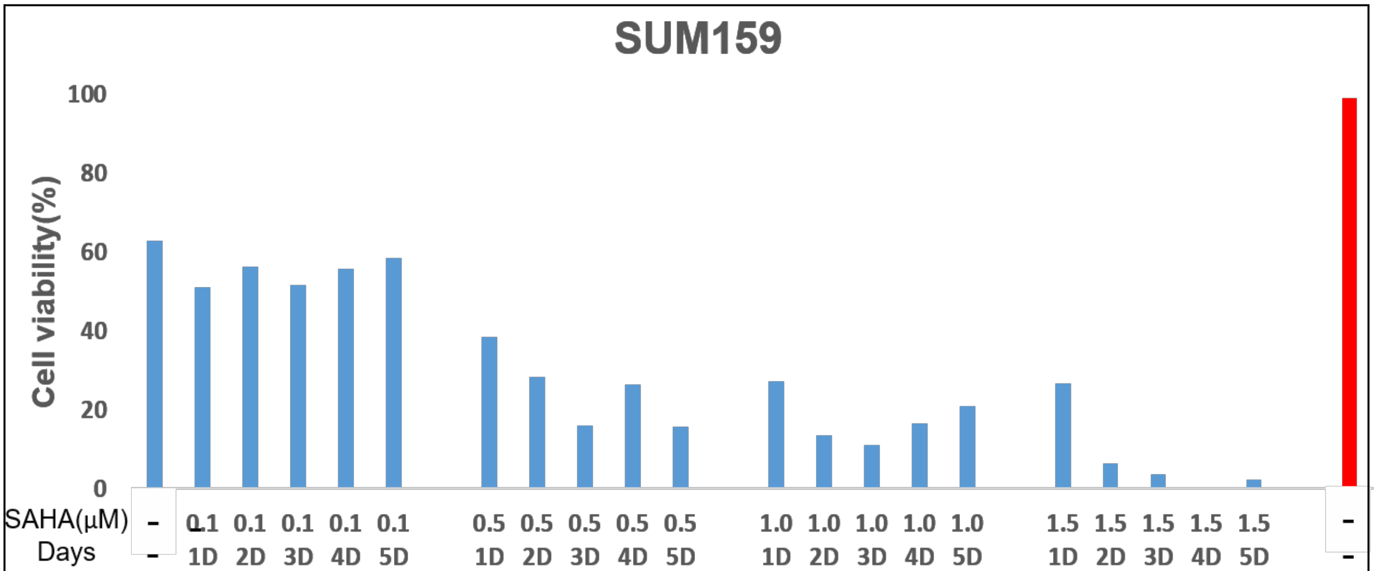
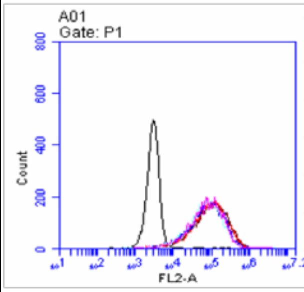


Figure 12. Enhancement by the HDAC inhibitor vorinostat of the anti-tumor activity of CSPG4 CAR T cells with TNBC cells SUM159. We treated CSPG4 CAR T cells with SAHA(at the indicated concentrations) for up to 5days. CAR T cells were then co-cultured with SUM159 cells at E:T ratio=5:1. Non-transduced T cells were used as a control. At the end of a 3days incubation at 37℃, co-cultured cells were detached, stained with trypan blue and viable cancer cells were counted. CAR T cells and non-transduced T cells were easily differentiated from tumor cells because of the markedly smaller size. Percentage of cancer cell numbers of co-cultured cells are shown.

SUM159(SAHA 0.5 μM)

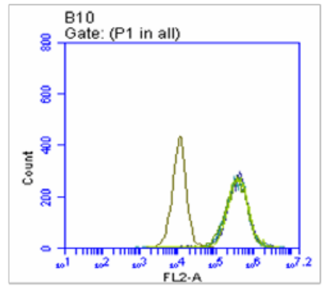
CSPG4: ISO , UNTR, 24h, 48h,72h,96h



MFIs of CSPG4	
Isotype	3245
Untreated	119962
SAHA 0.5μM 24h	115774
SAHA 0.5μM 48h	111494
SAHA 0.5μM 72h	113434
SAHA 0.5μM 96h	117120

SUM149 (SAHA 0.5 μM)

CSPG4: ISO , UNTR, 24h, 48h,72h,96h



MFIs of CSPG4	
Isotype	9222
Untreated	347451
SAHA 0.5μM 24h	334332
SAHA 0.5μM 48h	340349
SAHA 0.5μM 72h	349050
SAHA 0.5μM 96h	344597

Figure 13. CSPG4 is not up-regulated on human TNBC cell lines by HDAC inhibitor vorinostat. SUM159 and SUM149 were treated with vorinostat (0.5 μM) for up to 4days at 37 ℃. Cells were then stained with the CSPG4-specific mAb 225.28. Stained cells were analyzed by flow cytometry. mAb F3-C25 was used as an isotype control. MFIs of stained cells are shown.

Task 1. Work performed at UNC. We have generated and validated a CAR from the CSPG4-specific mAb 225.28, since this mAb crossreacts with mouse vessels, suggesting that results obtained with the CAR derived from this mAb in the proposed *in vivo* experiments will more likely mimic the clinical setting. At UNC, in the lab of Dr Dotti, the sequence of the scFv225 were optimized and CAR generated using different orientation of VH and VL, and all containing the CD28 endodomain (**Table 1**).

- Old scFv225 sequences
  - **SFG.CSPG4.CAR(scFv225[VH-VL]).CD28z = 469**
  - **SFG.CSPG4.CAR(scFv225[VL-VH]).CD28z = 470**
- New scFv225 sequences
  - **SFG.CSPG4.CAR(scFv225[VH-VL]).CD28z = 469***
  - **SFG.CSPG4.CAR(scFv225[VL-VH]).CD28z = 470***

Table 1. CAR constructs

T cells were generated from 5 different donors and transduced with replication-deficient human retrovirus encoding the scFv225 CSPG4.CAR constructs. For evaluation of transduction efficiency, samples were first incubated with the murine IgG1 anti-idiotypic mAb MF11-30 (specific for scFv225) for 30 minutes at 25ºC, washed with PBS, and subsequently stained with a rat anti-mouse IgG1 PE-conjugated antibody (clone X56, BD Biosciences) for 20 minutes at 4ºC. After a final wash with PBS, T cells were analyzed for transduction by flow cytometry (**Fig. 14 A, B**).

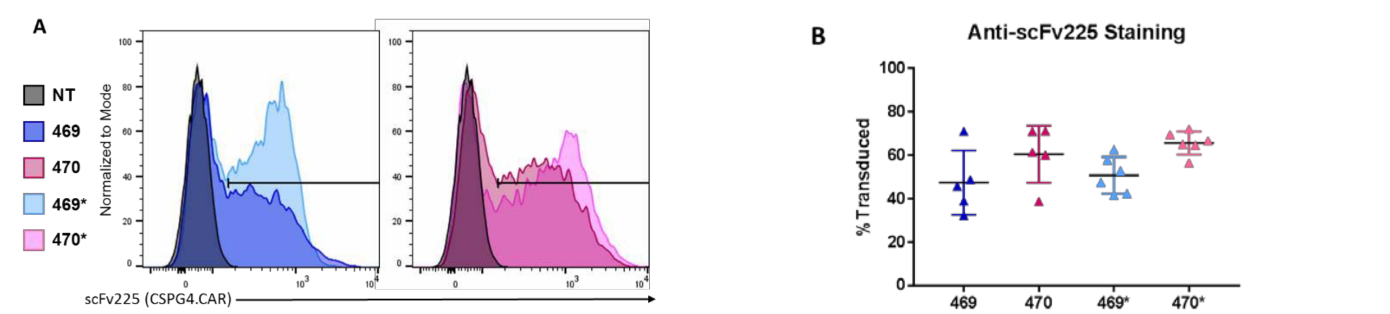


Figure 14. CAR transduction. A. representative flow plots of anti-scFv225 staining; the left panel represents old and new 469 [VH-VL] constructs, the right represents old and new 470 [VL-VH] constructs. %Transduced was determined by gating above the non-transduced (NT) cell population. **B.** Summary data from 5 T cell donors

To compare function, T cells were tested against CSPG4+ tumor cells using co-cultures assays at different Effector: target ratios. The number of T cells plated was normalized for the transduction efficiency of each construct, and NT cells were added to equalize the total number of T cells in each well. Different effector:target (E:T) ratio were used. On day 4, cocultures were collected and stained for flow cytometry with CD3-APC (to identify T cells), B7H3-BV421 (to identify tumor cells), and the viability dye ZombieAqua.

Immediately before analysis by the flow cytometer, the samples were filtered and Invitrogen CountBright beads were added to determine absolute cell counts. All CAR T cells but not NT–T cells efficiently eliminated tumor cells (**Fig. 15**).

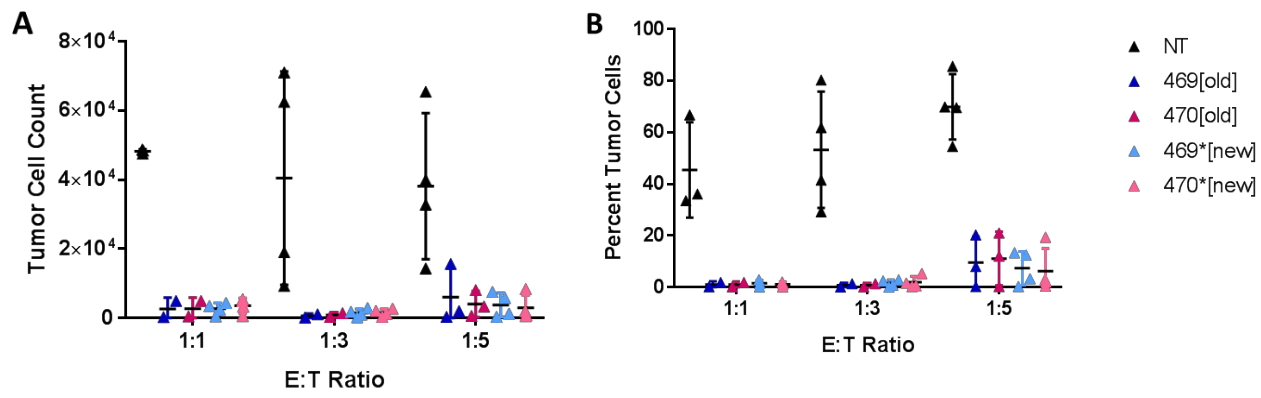


Figure 15. Antitumor activity of CSPG4+ T cells. A. Tumor cell counts at day 4 calculated using Count Bright beads. **B.** Percentages of tumor cells after gating on live cells (assessed by viability dye).

We are currently comparing these constructs *in vivo* in our NS mouse model to investigate if any of them has superior antitumor activities not revealed by *in vitro* assays.

- ☐ **What opportunities for training and professional development has the project provided?**
  Dr. Dotti, UNC group: Nothing to report
  Dr. S. Ferrone, MGH group: Nothing to report

- ☐ **How were the results disseminated to communities of interest?**
  Dr. Dotti, UNC group: Nothing to report
  Dr. S. Ferrone, MGH group: Nothing to report

- ☐ **What do you plan to do during the next reporting period to accomplish the goals?**
  Dr. Dotti, UNC group: complete subtask 1 and 4 for aim 2 and initiate work on subtask 4 for aim 3 as described in the original application.

4. IMPACT:

- ☐ **What was the impact on the development of the principal discipline(s) of the project?**
  Dr. Dotti, UNC group: Nothing to report
  Dr. S. Ferrone, MGH group: Nothing to report

- ☐ **What was the impact on other disciplines?**
  Dr. Dotti, UNC group: Nothing to report
  Dr. S. Ferrone, MGH group: Nothing to report

- ☐ **What was the impact on technology transfer?**
  Dr. Dotti, UNC group: Nothing to report
  Dr. S. Ferrone, MGH group: Nothing to report

- ☐ **What was the impact on society beyond science and technology?**
  Dr Dotti, UNC group: Nothing to Report
  Dr. S. Ferrone, MGH group: Nothing to report

5. CHANGES/PROBLEMS:

☐ **Changes in approach and reasons for change**

Dr. Ferrone is replacing the IL-2-anti-id mAb MK2-23 fusion protein with a fusion protein generated by linking IL-2 to a B7-H3-specific mAb, since there is the concern that IL-2 anti-id mAb MK2-23 may inhibit the interactions of CSPG4 CAR T cells with TNBC cells. As a first step for the preparation of IL-2-B7-H3-specific mAb fusion protein, we have tested the B7-H3 expression in normal tissues and in TNBC tumors. The results are consistent with the possibility that the administration of IL-2-B7-H3 mAb fusion protein will not cause side effects and will target IL-2 to the tumor microenvironment. Therefore, now we proceed to the preparation of the fusion protein.

In addition, we have tested the effect of epigenetic drugs on the CAR expression on transduced T cells and on their anti-tumor activity. Therefore, we will test this approach in combination with other strategies to enhance the anti-tumor activity of CSPG4 CAR T cells.

☐ **Actual or anticipated problems or delays and actions or plans to resolve them**

We have a delay in completing subtask 1 of Specific Aim 1because the HIF1alpha antibody has been discontinued. We have identified a rabbit mAb which seems to work in immunohistochemical staining. We are optimizing its use. Therefore, we expect to complete this subtask in one month.

Because of changes in strategies we have a delay in completing subtask 2 and 3 of Specific Aim 2.

☐ **Changes that had a significant impact on expenditures**

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

☐ **Significant changes in use or care of human subjects**

☐ **Significant changes in use or care of vertebrate animals.**

☐ **Significant changes in use of biohazards and/or select agents**

6. PRODUCTS:

Dr. Dotti, UNC group: Nothing to report

Dr. S. Ferrone, MGH group: Nothing to report

☐ **Publications, conference papers, and presentations**

- **Journal publications.**
- **Books or other non-periodical, one-time publications.**
- **Other publications, conference papers, and presentations.**

☐ **Website(s) or other Internet site(s)**

☐ **Technologies or techniques**

☐ **Inventions, patent applications, and/or licenses**

☐ **Other Products**

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

☐ **What individuals have worked on the project?**

Name:	*Dotti, Gianpietro*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*0.97 month*
Contribution to Project:	*Dr Dotti has supervised the Dr Sun and provided support for generation of the optimized CAR*. He has also been discussing progress and updates with Dr Ferrone at MGH
Funding Support:	

Name:	*Savoldo, Barbara*
Project Role:	*Co-investigator*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*0.59 month*
Contribution to Project:	*Dr Savoldo has supervised Dr Sun in some of the functional assays described in Fig2*
Funding Support:	

Name:	*Sun, Chuang*
Project Role:	*Research associate*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*6 months*
Contribution to Project:	*Dr Sun has performed work optimizing the CAR and on the silencing of the PD1 molecule. He has performed experiments described in fig1,2 and 3*

Name:	Ferrone, Soldano
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2.4 CM
Contribution to Project:	Dr. Ferrone has supervised the project and has participated in the planning and interpretation of the results generated by the IHC experiments. He has supervised the experiments to enhance the anti-tumor activity of CAR T cells. He has discussed progress and updated with Dr. Dotti at UNC.
Funding Support:	

Name:	Dr. Elena Brachtel
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	.24 CM
Contribution to Project:	Dr. Brachtel has generated the TNBC TMA, has read the IHC staining and has participated in the analysis and interpretation of the IHC results.

Name:	Dr. Steven Isakoff
Project Role:	Co-investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	.12 CM

Contribution to Project:	Dr. Isakoff has participated in the planning of experiments and in the interpretation of the results generated by the experiments.
Funding Support:	

Name:	Dr. Xinhui Wang
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1.2 CM
Contribution to Project:	Dr. Wang has produced and purified the mAbs used for the IHC experiments. She has revised the IACUC protocol required to produce monoclonal antibody containing ascites in mice. She has participated in the supervision of the postdoctoral fellow Dr. T. Michelakos.

Name:	Dr. Theodoros Michelakos
Project Role:	Post-Doctoral
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	12 CM
Contribution to Project:	Dr. Michelakos has performed the experiments to enhance the anti-tumor activity of CAR T cells

Name:	Teppei Yamada
Project Role:	Post-doctoral fellow

Researcher Identifier (e.g. ORCID ID):	

Nearest person month worked:	12 CM

Contribution to Project:	Dr. Yamada has participated in the IHC experiments to analyze CSPG4 and B7-H3 expression in TNBC tumors.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Dr. Dotti, UNC group: Nothing to report
Dr. Ferrone, MGH group: Nothing to report

☐ **What other organizations were involved as partners?**

None

8. SPECIAL REPORTING REQUIREMENTS

☐ **COLLABORATIVE AWARDS:**

☐ **QUAD CHARTS:**

FEDERAL FINANCIAL REPORT

(Follow form instructions)

1. Federal Agency and Organizational Element to Which Report is Submitted DOD DA Army Medical Research Acquisition	2. Federal Grant or Other Identifying Number Assigned by Federal Agency (To report multiple grants, use FFR Attachment) W81XWH-16-1-0500	Page 1	of 1 pages

3. Recipient Organization (Name and complete address including Zip code)
Office of Sponsored Research, UNC-Chapel Hill
CB#1350, 104 Airport Drive
Chapel Hill, NC 27599-1350

4a. DUNS Number	4b. EIN	5. Recipient Account Number or Identifying Number (To report multiple grants, use FFR Attachment)	6. Report Type	7. Basis of Accounting
608195277	56-6001393	5105511	☐ Quarterly ☐ Semi-Annual ☑ Annual ☐ Final	☑ Cash ☐ Accrual

8. Project/Grant Period From: (Month, Day, Year)	To: (Month, Day, Year)	9. Reporting Period End Date (Month, Day, Year)
1/1/2017	12/31/2017	12/31/2017

10. **Transactions**	Cumulative
(Use lines a-c for single or multiple grant reporting)	
Federal Cash (To report multiple grants, also use FFR Attachment):	
a. Cash Receipts	$146,885.83
b. Cash Disbursements	$239,986.36
c. Cash on Hand (line a minus b)	($93,100.53)
(Use lines d-o for single grant reporting)	
Federal Expenditures and Unobligated Balance:	
d. Total Federal funds authorized	$607,500.00
e. Federal share of expenditures	$239,986.36
f. Federal share of unliquidated obligations	$0.00
g. Total Federal share (sum of lines e and f)	$239,986.36
h. Unobligated balance of Federal funds (line d minus g)	$367,513.64
Recipient Share:	
i. Total recipient share required	$0.00
j. Recipient share of expenditures	$0.00
k. Remaining recipient share to be provided (line i minus j)	$0.00
Program Income:	
l. Total Federal program income earned	$0.00
m. Program income expended in accordance with the deduction alternative	$0.00
n. Program income expended in accordance with the addition alternative	$0.00
o. Unexpended program income (line l minus line m or line n)	$0.00

11. Indirect Expense	a. Type	b. Rate	c. Period From	Period To	d. Base	e. Amount Charged	f. Federal Share
	Predetermined	52.0%	1/1/2017	12/31/2017	$ 157,886.09	$ 82,100.27	$ 82,100.27
						$ -	$ -
				g. Totals:	$ 157,886.09	$ 82,100.27	$ 82,100.27

12. *Remarks: Attach any explanations deemed necessary or information required by Federal sponsoring agency in compliance with governing legislation:*

13. Certification: By signing this report, I certify that it is true, complete, and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent information may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

a. Typed or Printed Name and Title of Authorized Certifying Official **Bettina Lampkin** **Sponsored Projects Accounting Manager**	c. Telephone (Area code, number and extension) (919) 962-4691 d. Email address OSRbilling@unc.edu
b. Signature of Authorized Certifying Official	e. Date Report Submitted (Month, Day, Year) 3/14/2018 **RW**
	14. Agency use only:

Standard Form 425
OMB Approval Number: 0348-0061
Expiration Date: 10/31/2011

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