

AWARD NUMBER: W81XWH-16-1-0500

TITLE: Strategies to Counteract Resistance Mechanisms in CAR+ T-Cell-Based Immunotherapy for Triple-Negative Breast Cancer

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REPORT DATE: September 2019

TYPE OF REPORT: ANNUAL

PREPARED FOR: U.S Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release; Distribution Unlimited

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|--|---------------------------------|---|
| <b>1. REPORT DATE</b><br>September 2019  | <b>2. REPORT TYPE</b><br>Annual | <b>3. DATES COVERED</b><br>1 Sep 2018 - 31 AUG 2019 |
| <b>4. TITLE AND SUBTITLE</b><br>Strategies to Counteract Resistance Mechanisms in CAR+ T-Cell-Based Immunotherapy for Triple-Negative Breast Cancer          |                                 | <b>5a. CONTRACT NUMBER</b>                          |
|  |                                 | <b>5b. GRANT NUMBER</b><br>W81XWH-16-1-0500         |
|  |                                 | <b>5c. PROGRAM ELEMENT NUMBER</b>                   |
| <b>6. AUTHOR(S)</b><br>Dr. Soldano Ferrone<br><br>E-Mail:<br>sferrone@mgh.harvard.edu  |                                 | <b>5d. PROJECT NUMBER</b>                           |
|  |                                 | <b>5e. TASK NUMBER</b>                              |
|  |                                 | <b>5f. WORK UNIT NUMBER</b>                         |
| <b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b><br>Massachusetts General Hospital<br>55 Fruit Street, Boston, MA 02114<br><b>AND ADDRESS(ES)</b>   |                                 | <b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>     |
| <b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b><br><br>U.S. Army Medical Research and Materiel Command<br>Fort Detrick, Maryland 21702-5012 |                                 | <b>10. SPONSOR/MONITOR'S ACRONYM(S)</b><br><br>DOD  |
|  |                                 | <b>11. SPONSOR/MONITOR'S REPORT NUMBER(S)</b>       |
| <b>12. DISTRIBUTION / AVAILABILITY STATEMENT</b><br>Approved for Public Release; Distribution Unlimited  |                                 |   |
| <b>13. SUPPLEMENTARY NOTES</b>   |                                 |   |

**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 non malignant 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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|--|--------------------|---------------------|-----------------------------------|----------------------------|--|
| <b>16. SECURITY CLASSIFICATION OF:</b> |                    |                     | <b>17. LIMITATION OF ABSTRACT</b> | <b>18. NUMBER OF PAGES</b> | <b>19a. NAME OF RESPONSIBLE PERSON</b>           |
| <b>a. REPORT</b>                       | <b>b. ABSTRACT</b> | <b>c. THIS PAGE</b> |                                   |                            | USAMRMC  |
| Unclassified                           | Unclassified       | Unclassified        | Unclassified                      | 16                         | <b>19b. TELEPHONE NUMBER</b> (include area code) |

## Table of Contents

|   | <u>Page</u>  |
|---|--------------|
| <b>Introduction</b>   | <b>4</b>     |
| <b>Keywords</b>   | <b>4</b>     |
| <b>Key Research Accomplishments</b>                         | <b>4-11</b>  |
| <b>Impact</b>   | <b>11-12</b> |
| <b>Changes/Problems</b>                                     | <b>12</b>    |
| <b>Products/Publications</b>                                | <b>12-13</b> |
| <b>Participants &amp; other collaborating organizations</b> | <b>13-14</b> |
| <b>Special reporting requirements</b>                       | <b>14</b>    |
| <b>Appendices</b>   | <b>15</b>    |

**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  
PD-1  
Chimeric antigen receptor T cell immunotherapy

**3. ACCOMPLISHMENTS:**

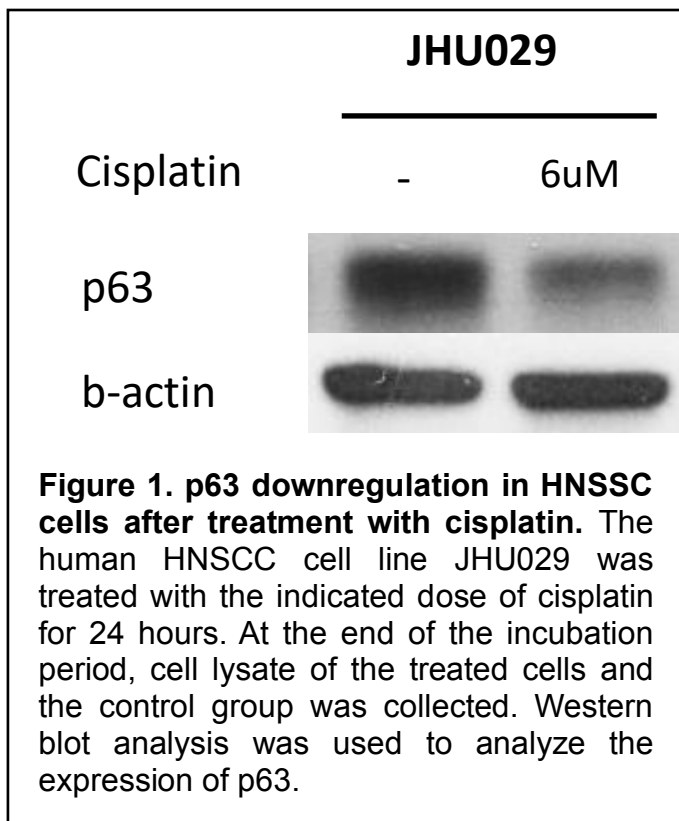
• **What were the major goals of the project?**

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

**Summary of 2018 Progress report**

As described in our previous Progress Report, CSPG4 represents an attractive target for antibody-based immunotherapy against Triple Negative Breast Cancer (TNBC) since it is highly expressed on TNBC tumors surgically resected from patients, and has restricted distribution in normal tissues. Our immunohistochemical studies utilizing the CSPG4-specific monoclonal antibodies (mAbs) D2.8, 763.74 and TP41.2 revealed that 53/63 (87%) of TNBC tumors express high levels of CSPG4. These findings prompted us to develop CSPG4-specific chimeric antigen receptors (CARs) and generate CSPG4-specific CAR T cells. The initial studies demonstrated that CSPG4-specific CAR T cells transduced with the 763.74 or 225.28 CAR could specifically recognize *in vitro* TNBC cells and had potent anti-tumor activity against them. However, their *in vivo* anti-tumor activity was limited when tested in NSG mice orthotopically grafted with human TNBC tumor cells. Therefore, we sought for strategies that could enhance the *in vivo* activity of CSPG4 CAR T cells by upregulating tumor antigen expression on TNBC cells and downregulating anti-apoptotic molecules on target cells as well. The first drug that we tested with these properties was the histone deacetylase (HDAC) inhibitor vorinostat (SAHA). In parallel studies, given the concerns regarding the patients' potential immune response to a human CSPG4-specific mouse scFv used to generate CSPG4 CAR T cells, we characterized a CSPG4-specific human scFv, SK5, and used it to generate CSPG4-specific CAR T cells. T cells transduced with this CAR specifically recognized and eliminated *in vitro* CSPG4(+) malignant cells, as tested by IFN $\gamma$  release and by the assessment of the elimination of cancer cells in co-culture experiments. However, CSPG4-specific CAR T cells generated with the SK5 scFv had no detectable

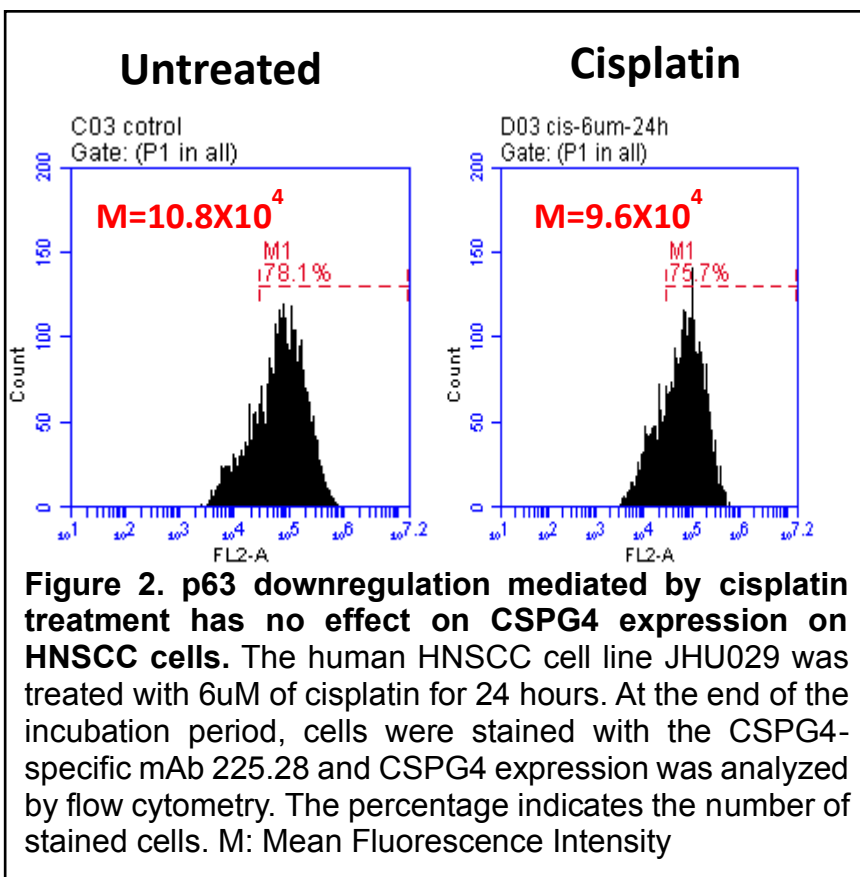


effect on the growth of human CSPG4(+) tumor cells grafted in NSG mice (data not presented in the previous Progress Report).

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

**Subtask 1: Immunohistochemical (IHC) staining of formalin fixed paraffin embedded TNBC tumors for CSPG4**

As described in the previous Progress Report, our efforts have focused on the analysis of the mechanisms underlying CSPG4 regulation in TNBC cells incubated under hypoxic conditions at 37°C. We have found that p63, STA3 and STAT5 might be involved in the regulation of CSPG4, since they act as transcription factors that bind to the promoter region of CSPG4. To further analyze the potential role of these molecules, we performed the experiments described below.

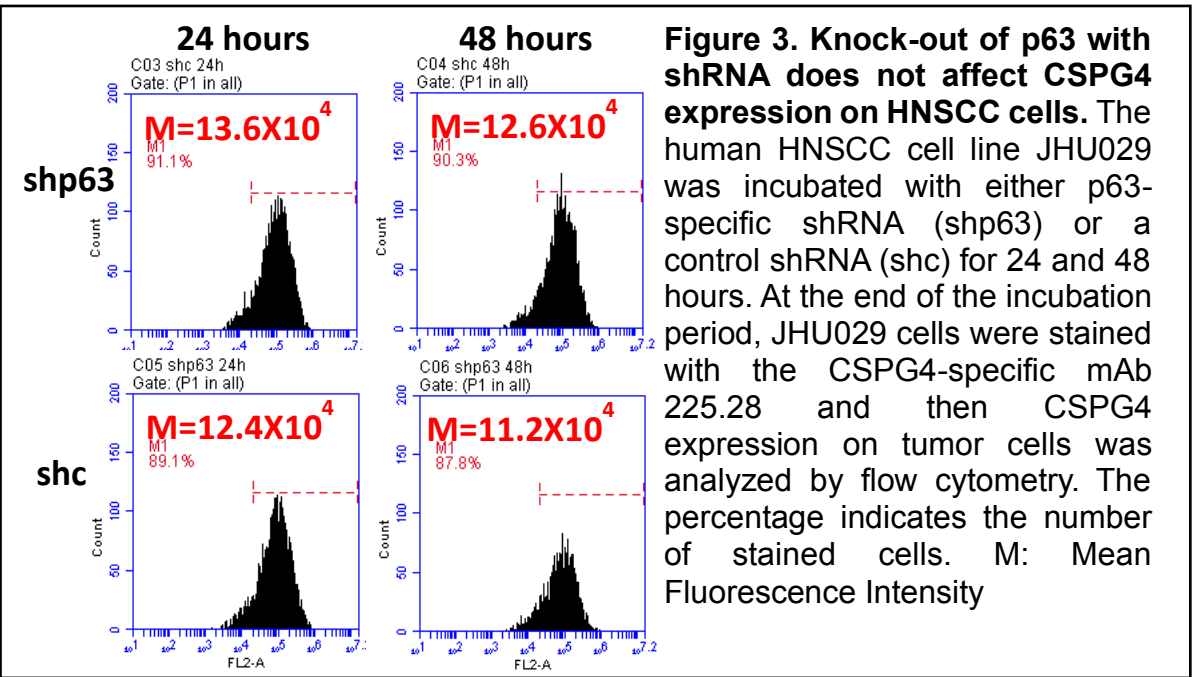


**CSPG4 regulation by p63.** To evaluate the potential role of p63 in CSPG4 expression by malignant cells, we investigated the effect of p63 degradation in tumor cells on CSPG4 expression. Based on the information in the literature, we used cisplatin, a platinum-based chemotherapeutic drug, to degrade p63. Initially, we performed our studies with head and neck squamous cell carcinoma (HNSCC) cell lines, since we had titrated cisplatin on those cells. We performed Western blot analysis of cell lysates isolated from HNSCC cells treated with cisplatin at the indicated doses to confirm p63 downregulation in treated cells. As shown in **Fig. 1**, cisplatin was able to decrease the expression level of p63; however, it did not lead to complete degradation of the molecule. Thereafter, we assessed CSPG4 expression in HNSCC tumor cells treated with cisplatin to evaluate the relationship between p63 downregulation and CSPG4 expression. Flow cytometric analysis of cisplatin-

treated cells with CSPG4-specific mAbs demonstrated that CSPG4 expression did not change (**Fig. 2**), even though p63 was downregulated. It was our working hypothesis that the downregulation of p63 expression mediated by cisplatin treatment was not sufficient to have an effect on CSPG4 expression. Therefore, we decided to completely knock-out p63 with shRNA. shRNA completely knocked-out p63 in HNSCC cells. Then, HNSCC

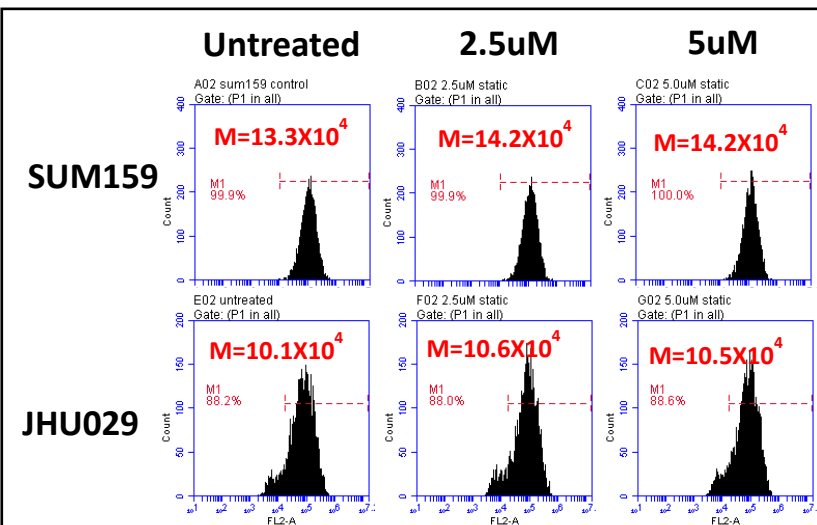
cells were stained with CSPG4-specific mAbs and analyzed by flow cytometry. CSPG4 was still expressed on these cells, despite having completely knocked-out p63 with shRNA (Fig. 3). On the basis of these results, we decided to focus our studies on STAT3 and STAT5 and assess their role in the regulation of CSPG4.

**CSPG4 regulation by STAT3.** As a next step, we tried to analyze the role of STAT3 in the regulation of CSPG4. The STAT3 inhibitor Static was first titrated on TNBC and HNSCC cells. Then, TNBC and HNSCC cells were



**Figure 3. Knock-out of p63 with shRNA does not affect CSPG4 expression on HNSCC cells.** The human HNSCC cell line JHU029 was incubated with either p63-specific shRNA (shp63) or a control shRNA (shc) for 24 and 48 hours. At the end of the incubation period, JHU029 cells were stained with the CSPG4-specific mAb 225.28 and then CSPG4 expression on tumor cells was analyzed by flow cytometry. The percentage indicates the number of stained cells. M: Mean Fluorescence Intensity

treated with the indicated doses of the drug and CSPG4 expression was analyzed by flow cytometry after staining the cells with CSPG4-specific mAbs. Expression level of CSPG4 did not change in the treated groups compared to the control group (Fig. 4). To confirm that the STAT3 inhibitor



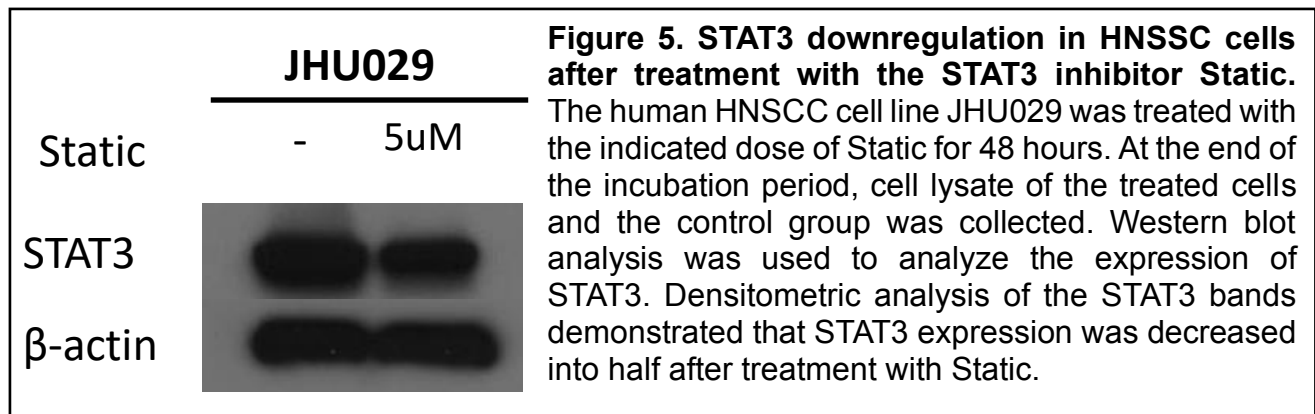
**Figure 4. Lack of effect of the STAT3 inhibitor Static on CSPG4 expression on TNBC and HNSCC cells.** The human TNBC cell line SUM159 and the human HNSCC cell line JHU029 were treated with Static at the indicated doses for 48 hours at 37°C. At the end of the incubation period, SUM159 and JHU029 cells were stained with the CSPG4-specific mAb 225.28 and then CSPG4 expression on tumor cells was analyzed by flow cytometry. The percentage indicates the number of stained cells. M: Mean Fluorescence Intensity

was active, we treated HNSCC cells with it and then isolated the cell lysate to analyze it for total STAT3 expression. As shown in Fig. 5, expression level of STAT3 decreased into half after treatment with Static.

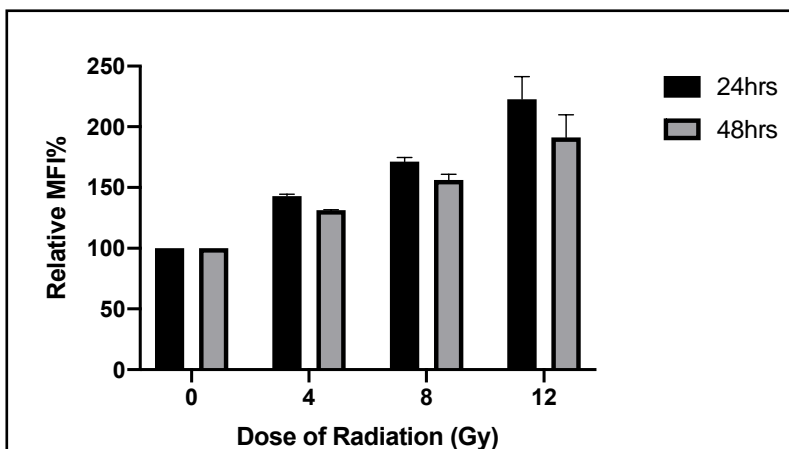
Our ongoing studies are trying to evaluate the potential role of STAT5 in the regulation of CSPG4.

**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*.**

**Subtask 3: To test whether the LDE225 and IL-2-anti-id mAb MK2-23 fusion protein enhances the ability of CSPG4 CAR T cells to eliminate both TNBC differentiated cells and TNBC CICs *in vitro*.**



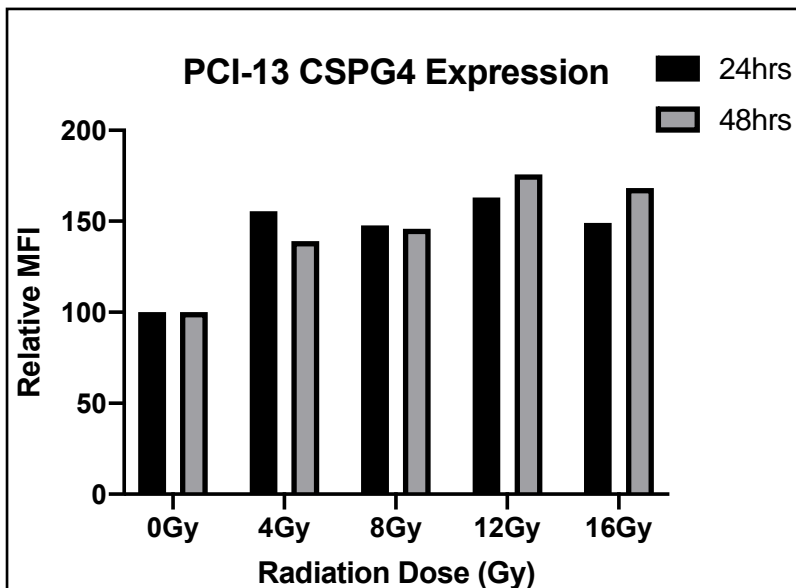
*Generation of IL-2-anti-id mAb MK2-23 fusion protein.* We have faced significant difficulties with the generation of IL-2-anti-id mAb MK2-23 fusion protein. The clones that we used for the production of the fusion protein did not yield the expected fusion protein. After extensive efforts we were able to isolate a clone of cells that seems to be able to produce the fusion protein. Cells were injected into the abdominal cavity of mice to generate ascites with the expectation to isolate the fusion protein from the ascitic fluid. Currently we are purifying the fusion protein from the ascitic fluid. If we are successful and we produce the necessary amounts to perform the studies described in the original grant application, then we will assess whether the fusion protein can enhance the activity of CSPG4 CAR T cells with TNBC cells.



**Figure 6. Radiation-induced CSPG4 upregulation on the human TNBC cell line SUM149.** CSPG4 expression was assessed by flow cytometry on human TNBC SUM149 cells following radiation exposure. Cells were exposed to radiation doses between 0-12 Gy with CSPG4 expression assessed using mAb 225.28 up to 48 hours post exposure. Expression increased in a dose and time dependent manner.

*Radiation as a means to enhance TNBC cells' susceptibility to CSPG4-specific CAR T cell mediated lysis.* Radiation therapy is a longstanding treatment modality for TNBC to achieve local control of the disease. However, there is now increasing recognition of the complex interplay between radiation therapy and the immune system, with a greater appreciation of the ability of radiation therapy to influence systemic tumor responses. It can sensitize tumor cells to immunotherapy by promoting the expression of HLA class I molecules and other apoptosis-mediated proteins not only in the irradiated site but also in distant non-irradiated metastatic spots (abscopal effect). Moreover, to the best of our knowledge, we have shown for the first time that radiation therapy can also upregulate the expression of various tumor antigens including, but not limited to, CSPG4 on TNBC cells. We performed serial titration experiments to identify the optimal dose of radiation that has minimal cytotoxic effect on TNBC cells and at the same time induces CSPG4

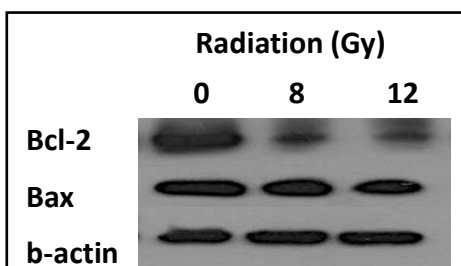
upregulation on tumor cells. As shown in **Fig. 6**, the effect of radiation is both time- and dose- dependent. Both 8 and 12Gy can significantly upregulate CSPG4 expression on TNBC cells and the effect peaks 48 hours post-radiation. This effect is not unique of TNBC, since similar results have been obtained with HNSCC cells lines (**Fig. 7**). In addition, we have assessed the effect of radiation on pro- and anti- apoptotic molecules in TNBC cells. It is our working hypothesis, that radiation can induce an imbalance between pro- and anti-apoptotic molecules, in favor of pro-apoptotic molecules, rendering TNBC cells more susceptible to CAR T cell mediated lysis. TNBC cells were treated with either 8 or 12 Gy and then were incubated for 48 hours at 37 ° C. At the end of the incubation period, cell lysate from TNBC cells was collected and Western blot analysis was performed to assess



**Figure 7. Radiation-induced CSPG4 upregulation on the human SCCHN cell line PCI-13.** CSPG4 expression was assessed by flow cytometry on human SCCHN PCI-13 cells following LDR exposure. Cells were exposed to radiation doses between 0-12 Gy with CSPG4 expression assessed using mAb 225.28 up to 48 hours post exposure. Expression increased in a dose and time dependent manner.

the expression level of pro- and anti-apoptotic molecules. As shown in **Fig. 8**, radiation can decrease the level of the major anti-apoptotic molecule, bcl-2, in a dose-dependent manner. Bax, a pro-apoptotic molecule, remains stable after radiation. Therefore, this imbalance between pro- and anti-apoptotic molecules induced by radiation can potentially increase TNBC cells' susceptibility to killing by CSPG4-specific CAR T cells.

*Enhancement by radiation of the ability of CSPG4-specific CAR T cells to eliminate in vitro differentiated TNBC cells.* To assess the significance of the results obtained in the experiments described in the previous section and to evaluate whether radiation can truly make TNBC cells more sensitive to CAR T cells, we performed a co-culture experiment after pre-treating TNBC cells with radiation. The human TNBC cell line SUM159 was treated with 8Gy and then incubated for 48 hours at 37 °C. At the end of the incubation period, SUM159 cells were collected, and only viable cells were seeded in 96-well plates. Then, CSPG4-specific CAR T



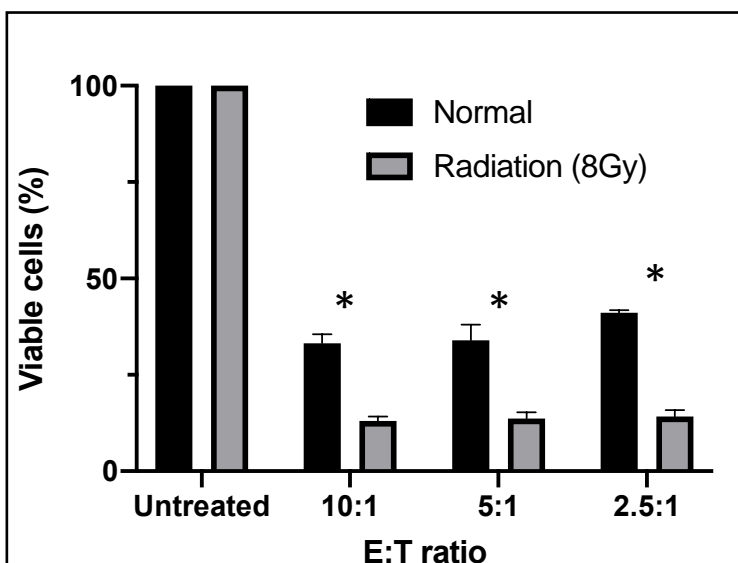
**Figure 8. Dose-dependent radiation-induced Bcl-2 downregulation.** The human TNBC cell line SUM159 cells was grown at 37°C in RPMI 1640 + 10% FCS and then subjected to low dose radiation exposure. SUM159 cells were kept in culture for an additional 72 hours. At the end of the incubation period, cell lysate was collected and Bcl-2 (anti-apoptotic) and Bax (pro-apoptotic) expression was assessed by Western blot.

cells were added at different E:T ratios ranging from 10:1 to 1:1. SUM159 cells that were not pre-treated with radiation were used as a control. CSPG4-specific CAR T cells and SUM159 were co-cultured for 48 hours and at the end of the incubation period, viability of tumor cells was assessed with a MTT assay. The results are summarized in **Fig. 9**. Pre-treatment of SUM159 cells with radiation enhanced the *in vitro* anti-tumor activity of CSPG4-specific CAR T cells against them. Thereby, this strategy can be potentially used in an *in vivo* setting, in order to enhance the efficacy of CSPG4-specific CAR T cells by administering local radiation to 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.**

*Subtask 1: CSPG4-targeted CAR T cells-based immunotherapy in combination with IL-2-anti-id mAb MK2-23 fusion protein and LDE225 is effective in suppressing metastases and recurrence in NSG mice following surgical removal of MDA-MB-231-Luc-derived xenografts.*

As already mentioned in the previous Progress Report, LDE225 has accelerated disease progression in patients with pancreatic ductal adenocarcinoma. These unexpected clinical



**Figure 9. Radiation therapy increases the *in vitro* susceptibility of TNBC cells to CSPG4 CAR T cell lysis.** The human TNBC cell line SUM159 was either pre-treated with low dose radiation (8Gy) or not, and then tumor cells were kept in culture for 72 hours at 37°C. Then, viable SUM159 cells were collected and seeded in a 96-well plate. CSPG4 CAR T cells were added at the indicated Effector : Target ratio. At the end of a 72-hour incubation period the viability of SUM159 cells was assessed with an MTT assay. \*  $p < 0.001$

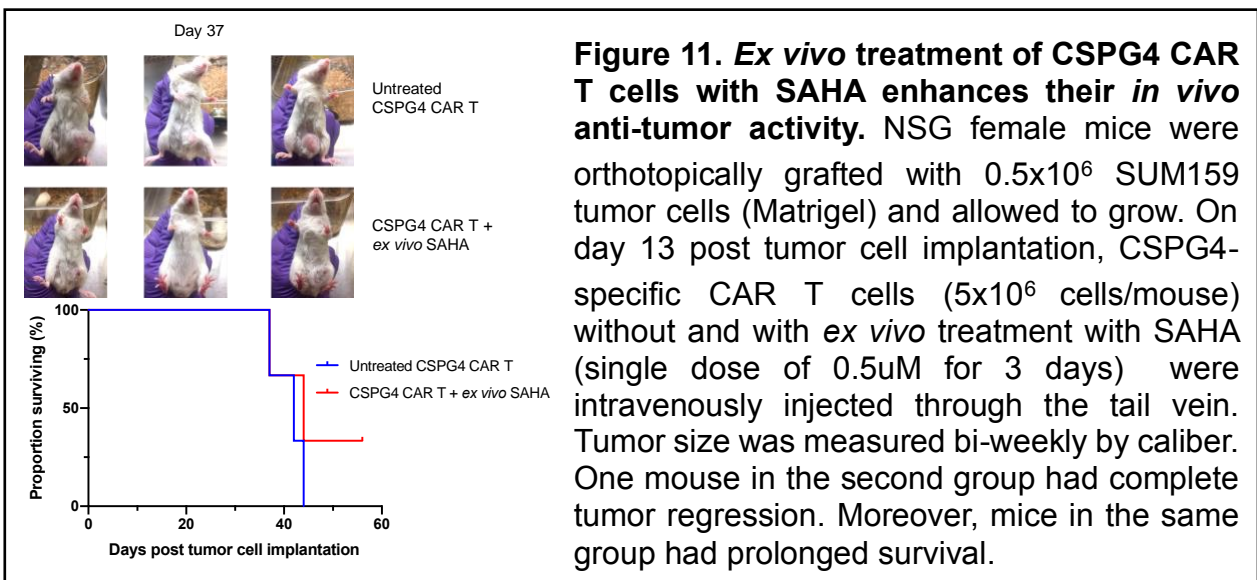
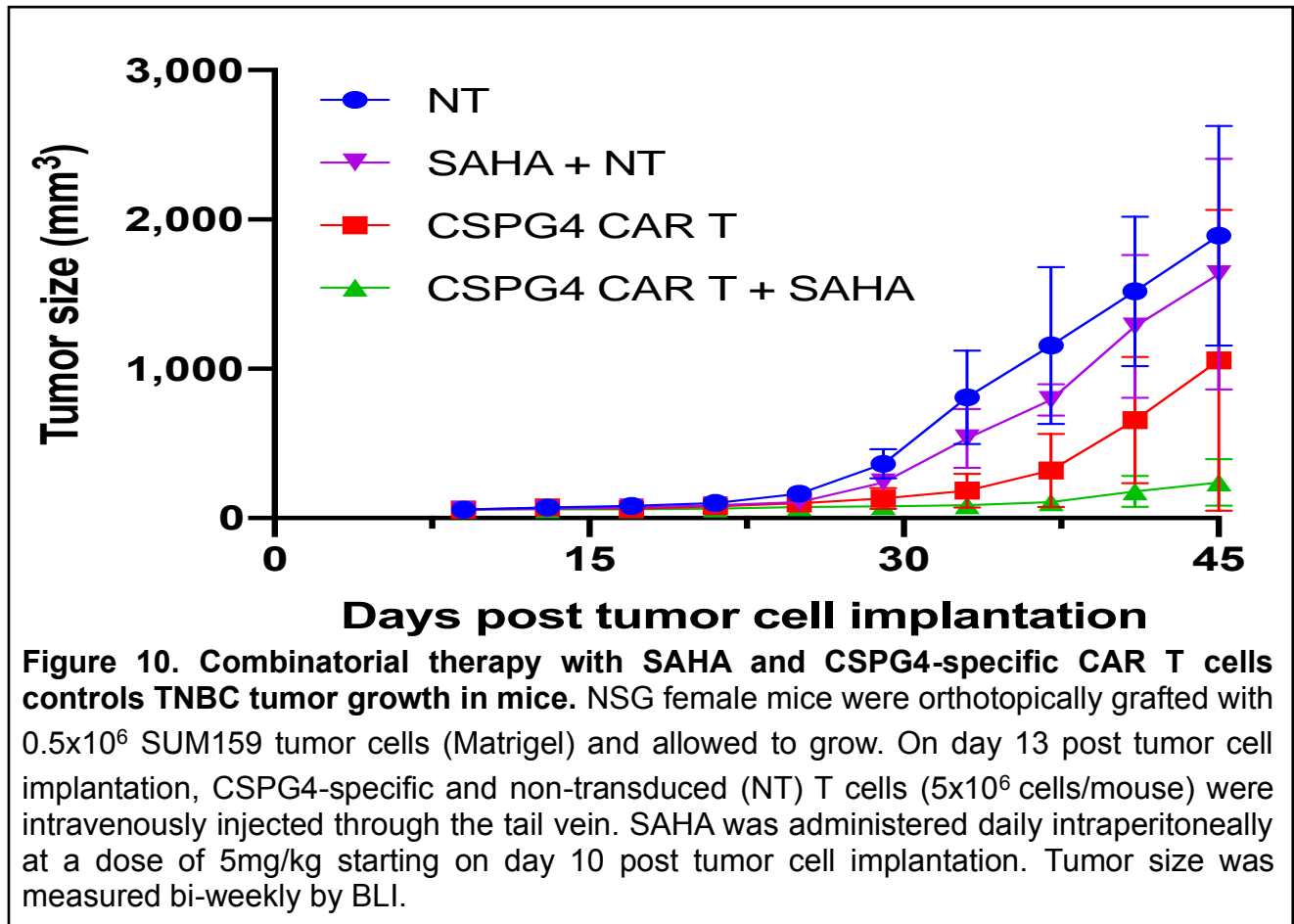
results have caused some apprehension among clinicians to use this small molecule in patients with malignant disease. These concerns have prompted us to seek for alternative strategies. As a result, we investigated whether the HDAC inhibitor vorinostat (SAHA) can enhance the antitumor activity of CSPG4 CAR T cells in a mouse model system.

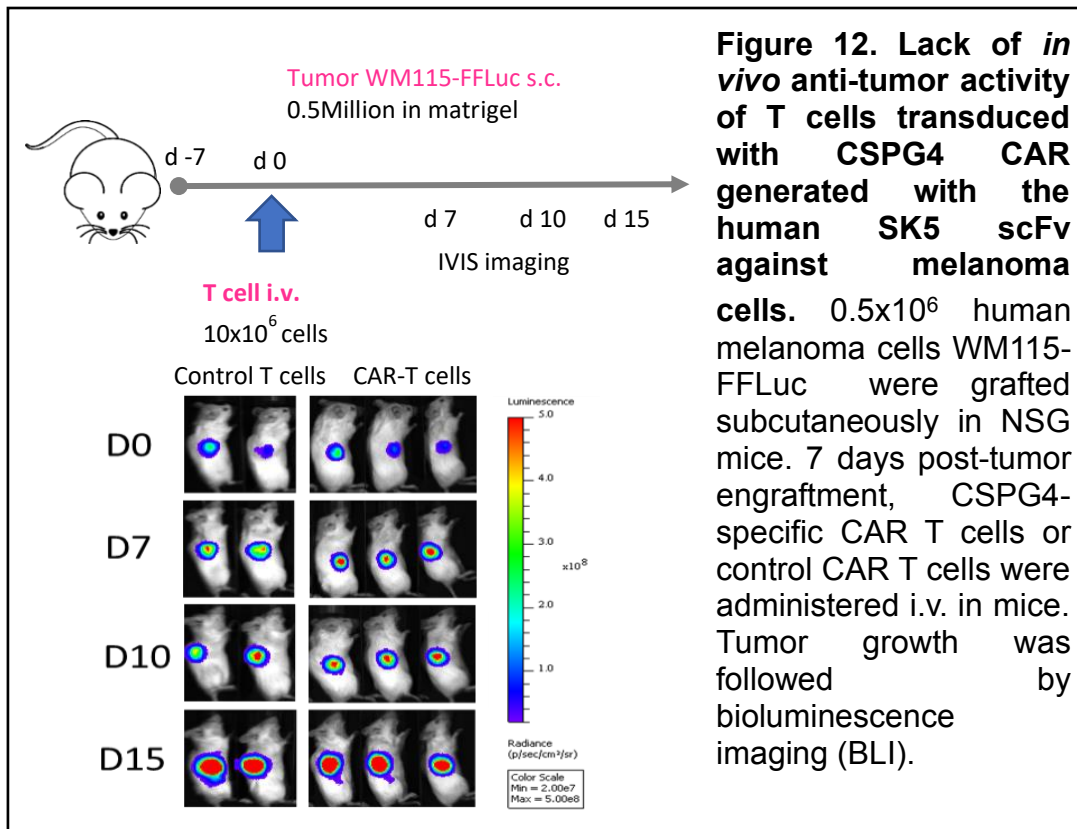
*Combinatorial treatment with CSPG4-specific CAR T cells and vorinostat.* As described in detail in the previous Progress Report, the HDAC inhibitor vorinostat can enhance the *in vitro* anti-tumor activity of CSPG4 CAR T cells against TNBC cells. This effect is not unique against TNBC cells, since similar results have been obtained by our group with HNSCC cell lines (Data presented in the previous progress report). The potential underlying mechanism of action is through upregulation of the tumor antigen. The effect seems to be both time- and dose- dependent with the maximum effect observed with 0.5 $\mu$ M for 72 hours. On the basis of these results, we performed an *in vivo* mouse study to assess the effect of the combinatorial therapy with vorinostat and CSPG4-specific CAR T cells. Luciferase labeled TNBC tumor cells were orthotopically grafted in NSG mice on Day 0. Tumor growth was monitored by bioluminescence imaging (BLI) bi-weekly. Once tumor size reached a diameter of 0.5cm, mice were randomized into 4

groups and received the following treatment: Group 1: Non-transduced (NT) T cells, Group 2: CSPG4-specific CAR T cells, Group 3: CSPG4-specific CAR T cells and vorinostat and Group 4: vorinostat and NT T cells. CSPG4-specific CAR T cells were generated with the mAb 225.28 and were administered as a single dose (5x10<sup>6</sup> cells/mouse) 3 days after mice were randomized into groups. Vorinostat was administered daily at a dose of 5mg/kg starting on the day when mice were randomized into the respective groups. As shown in **Fig. 10**, the group treated with vorinostat and CSPG4-specific CAR T cell had a significantly better control of tumor growth compared to all the other control groups. We plan to validate these results by repeating the same experiment with a higher number of mice per group. In addition, we are currently investigating whether *ex vivo* treatment of CSPG4-specific CAR T cells with vorinostat can enhance their *in vivo* anti-tumor activity. The results of a preliminary *in vivo* mouse experiment are summarized in **Fig. 11**. Briefly, human TNBC cells were orthotopically grafted into the mammary fat pad of NSG mice. Mice were then randomized into 2 groups, once the tumor had reached a size of 0.5cm. Group 1 was treated with CSPG4-specific CAR T cells (5x10<sup>6</sup> cells/mouse), and Group 2 received CSPG4-specific CAR T cells (5x10<sup>6</sup> cells/mouse) that had been *ex vivo* treated with a single dose of SAHA (0.5 $\mu$ M) 3 days before administration into mice. One mouse had a complete tumor regression as shown in **Fig 11**. Moreover, that group demonstrated prolonged survival compared to the control group.

*In vivo antitumor activity of T cells transduced with a CSPG4 CAR generated with the human SK5 scFv.* The use of a human CSPG4-specific mouse scFv to generate CSPG4 CAR T cells has generated concerns, since the immune response elicited in treated patients by mouse scFv may interfere with the persistence of CSPG4 CAR T cells in the treated patients. To overcome this potential limitation, we have developed and characterized a CSPG4-specific human scFv, as described in detail in the previous Progress Report. The newly generated CSPG4-specific CAR T cells had a prominent *in vitro* cytotoxic effect against TNBC cells. However, when tested *in vivo*, their anti-tumor activity against TNBC cells orthotopically grafted in mice was very poor (**Fig. 11**). To determine

whether the lack of *in vivo* activity of SK5 CAR T cells was unique of TNBC cells or was a general phenomenon, we tested the antitumor activity of SK5 CAR T cells with human melanoma cells grafted subcutaneously in NSG mice. As shown in **Fig. 12**, as observed with TNBC cells, T cells transduced with the SK5 CAR were not able to control the growth of human melanoma cells grafted subcutaneously in NSG mice.





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

Dr. Dotti, UNC group: Nothing to report

Dr. Ferrone, MGH group, has trained one graduate student and one post-doctoral research fellow.

- **How were the results disseminated to communities of interest?**

Dr. Dotti, UNC group: Nothing to report.

Dr. Ferrone, MGH group, has presented the data to patient advocate group at Dana Farber Cancer Center.

- **What do you plan to do during the next reporting period to accomplish the goals?**

Dr. Dotti, UNC group: Complete subtask 2 and 4 for Aim 2 and initiate work on Aim 4 as described in the original application.

Dr. Ferrone, MGH group, will focus on the work described in subtask 2 for Aim 2, subtasks 3 and 4 for Aim 3 and initiate work on Aim 4.

#### 4. IMPACT:

- **What was the impact on the development of the principal discipline(s) of the project?**
- **What was the impact on other disciplines?**

Dr. Dotti, UNC group: Nothing to Report

Dr. Ferrone, MGH group, is developing a strategy to improve the anti-tumor activity of tumor antigen-

specific CAR T cells.

- **What was the impact on the technology transfer?**

Dr. Dotti, UNC group, and Dr. Ferrone, MGH group, have submitted a joint patent application.

- **What was the impact on society beyond science and technology?**

Dr. Dotti, UNC group: Nothing to report

Dr. Ferrone, MGH group: Nothing to report

## 5. **CHANGES/PROBLEMS:**

Dr. Dotti, UNC group: Nothing to report.

Dr. Ferrone, MGH group:

- **Changes in approach and reasons for change:** Dr. Ferrone, MGH group has decided to replace sonidegib with a compound which downregulates anti-apoptotic molecules, since sonidegib has been recently shown to accelerate disease progression in some types of cancer. Therefore, it would be difficult to use in clinical trials in patients with TNBC.
- **Actual or anticipated problems or delays and actions or plans to resolve them**
- **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. Ferrone, MGH group: Nothing to report

- **Publications, conference papers, and presentations**

- **Journal publications.**

1. Piras LA, Riccardo F, Issuch S, Maniscalco L, Gattino F, Martano M, Morello E, Lorda Mayayo S, Rolih V, Garavaglia F, De Maria R, Lardone E, Collivignarelli F, Mignacca D, Giacobino D, **Ferrone S**, Cavallo F, Buracco P. Prolongation of survival of dogs with oral malignant melanoma treated by en bloc surgical resection and adjuvant CSPG4-antigen electrovaccination. *Vet Comp Oncol.* 15: 996-1013, 2017 PMID:

27146852

2. Pellegatta S, Savoldo B, Di Ianni N, Corbetta C, Chen Y, Patané M, Sun C, Pollo B, **Ferrone S**, DiMeco F, Finocchiaro G, Dotti G. Constitutive and TNF $\alpha$ -inducible expression of chondroitin sulfate proteoglycan 4 in glioblastoma and neurospheres: Implications for CAR-T cell therapy. *Sci Transl Med.* 10(430), 2018. PMID: 29491184
3. Pilla L, **Ferrone S**, Maccalli C. Methods for improving the immunogenicity and efficacy of cancer vaccines. *Expert Opin Biol Ther.* 17:1-20, 2018. PMID: 29874943
4. Eng MS, Kaur J, Prasmickaite L, Engesæter BØ, Weyergang A, Skarpen E, Berg K, Rosenblum MG, Mælandsmo GM, Høgst A, **Ferrone S**, Selbo PK. Enhanced targeting of triple-negative breast carcinoma and malignant melanoma by photochemical internalization of CSPG4-targeting immunotoxins. *Photochem Photobiol Sci.* 17(5):539-551, 2018. PMID: 29565434
5. Kasten BB, Oliver PG, Kim H, Fan J, **Ferrone S**, Zinn KR, Buchsbaum DJ. 212Pb-Labeled Antibody 225.28 Targeted to Chondroitin Sulfate Proteoglycan 4 for Triple-Negative Breast Cancer Therapy in Mouse Models. *Int J Mol Sci.* 19(4), 2018. PMID: 29561763

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

|  |  |
|--|--|
|  |  |
|--|--|

Has supervised the postdoctoral fellow:

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

Dr. Ferrone has supervised the collaborator

|  |  |
|--|--|
| Name:                                  | <i>Xinhui, Wang, MD, PhD</i>   |
| Project Role:                          | <i>Assistant Professor</i>   |
| Researcher Identifier (e.g. ORCID ID): |  |
| Nearest person month worked:           | <i>1.2 months</i>  |
| Contribution to Project:               | <i>Dr. Wang has purified the IL2 fusion protein and has participated in the design of the experiments described in the progress report as well as in the interpretation of the results generated by the described experiments.</i> |
| Funding Support:                       |  |

- **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: N/A**
- **QUAD CHARTS: N/A**

**9. APPENDICES: N/A**