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TITLE: Targeting ART1, a Novel Immune Checkpoint, for the Treatment of Lung Cancer

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CONTRACTING ORGANIZATION: Joan & Sanford I. Weill Medical College of Cornell University**,** New York, NY

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14. ABSTRACT

We have found ART1 expressed in multiple human non-small cell lung cancer (NSCLC) cell lines and in mouse and human NSCLC tumors. ART1, an ARTC family mono-ADP-ribosyltransferase, functions extracellularly to target arginine-rich cell surface proteins on neighboring cells or target soluble proteins in the local tumor microenvironment. **Following ART1 knockdown in murine immune competent lung cancer models, we note a highly significant increase in cytotoxic tumor infiltrating CD8 T cells and a subsequent decrease in tumor burden.** Mono-ADP-ribosylation of the P2X7 receptor on subsets of CD8+ T cells may induce receptor activation and apoptosis, a process described as NAD-induced cell death (NICD). ART1-induced mono-ADP-ribosylation of P2X7R could therefore allow cancer cells overexpressing ART1 to blunt the T cell immune response against them by inducing T cell apoptosis. Although little is known about ART1 in human cancer, its expression may be upregulated by ER stress and its enzymatic activity increased by release of NAD+ into the local microenvironment, both of which occur following cytotoxic cancer therapy, particularly radiation therapy. In the current application *addressing the LCRP Area of Emphasis "Identify innovative strategies for prevention and treatment of lung cancer",* we therefore propose to evaluate the therapeutic efficacy of anti-ART1 antibodies which we have already developed and functionally optimized and to determine whether ART1 blockade has additive anti-tumor effects to immune checkpoint blockade and radiation therapy. **We hypothesize that therapeutic inhibition of ART1 with an anti-ART1 monoclonal antibody will promote immune mediated rejection of lung cancer. Moreover, we hypothesize that ART1 inhibition combined with radiation therapy will demonstrate synergistic anti-tumor effects.** We therefore propose the following:

Specific Aims:

Aim 1. *To test the therapeutic efficacy and mechanisms of action of anti-ART1 monoclonal antibodies in murine immune-competent lung cancer models.* Efficacy of monoclonal antibodies that bind human/mouse ART1 with functional enzymatic inhibition will be evaluated in murine tail vein and flank tumor models with and without concomitant immune checkpoint blockade (ICB). Mechanistic aspects will be examined by flow cytometry and IF of immune cell populations, with a focus on P2X7R expressing T cells.

Aim 2. *To test the therapeutic efficacy and mechanisms of action of anti-ART1 therapy combined with radiation therapy in murine immune-competent lung cancer models.* Lung and flank tumors will be treated with varying doses of radiation therapy prior to or during ART1 knockdown or treatment with anti-ART1 antibodies. Changes in tumor ART1 expression induced by radiation will be quantified. Systemic immune response will be assessed and tumors and lungs evaluated for differences in tumor burden and changes in immune infiltration and abscopal effects, again with and without ICB.

Aim 3. *To characterize ART1 expression and immune phenotype in lung cancers from VA patients and to evaluate the ART1/P2X7R axis in human lung tumors treated with preoperative immunotherapy and radiation therapy.* We will use multiplex immunofluorescence to compare ART1, P2X7R, and other markers of immune phenotype in biopsied or resected specimens following no treatment (VA patients), treatment with ICB alone, or ICB + radiation therapy and compare to clinical/pathologic features and treatment outcomes.

Innovation: ART1 has only recently been described to play a role in cancer progression. Our proposal describing ART1 as a "checkpoint" limiting immune mediated cell death through NICD is entirely novel, as is the combination of ICB or radiation therapy with ART1 blockade.

Impact: We anticipate that the dramatic anti-tumor phenotype seen with ART1 knockdown will be reproduced with a clinical grade therapeutic antibody. The most efficacious therapeutic inhibitor from these preclinical studies will be optimized for clinical use. We envision using targeted inhibition of mono-ADP-ribosylation in the large proportion of ART1 over-expressing lung tumors to facilitate immune-mediated destruction of established cancers or of micrometastatic disease. It is likely that additive effects with ICB and radiation therapy will be apparent and clinically meaningful. **Results from these studies will provide the rationale for future clinical trials testing ART1 blockade with radiotherapy and immunotherapy.** *By evaluating ART1 expression and immune phenotype in tumor samples from VA patients, the study will have direct relevance to veterans which will be particularly realized if a therapeutic antibody can be developed for clinical use*.

15. SUBJECT TERMS lung cancer, ART1, immunotherapy, immune response, T-cell, radiation therapy, combination therapy, NSCLC					
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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

We propose to study the role of a protein called ART1 in lung cancer. ART1 has not been previously described in lung tumors, but we have found several clues to suggest that it might play a role in lung cancer progression. In our proposal, we plan to test a therapeutic antibody that blocks the actions of ART1. We anticipate that this antibody will decrease tumor growth and metastasis by blocking the ability of ART1 to kill T cells. Such treatment strategies have already shown great success in lung cancer by using antibody-based drugs that block checkpoint inhibitors such as PD1 or PD-L1. In our application, we propose to 1) test anti-ART1 antibodies against lung cancer in mouse models with normal immune systems, 2) test combination therapy of anti-ART1 antibodies with immune therapy and radiation therapy, and 3) evaluate ART1 expression and its relationship to immune cells, particularly T cells expressing P2X7R, in a variety of human lung tumors and try to understand the role of ART1 expression in patients treated with radiation therapy or immunotherapy. We anticipate that an anti-ART1 therapeutic antibody will have a tremendous impact on lung cancer patients, offering another type of immunotherapy which helps to release the brakes on T cells and unleash the patient's own immune system on his or her cancer.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

lung cancer, ART1, immunotherapy, immune response, T-cell, radiation therapy, combination therapy, NSCLC

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

Aim 1: To test the therapeutic efficacy and mechanisms of action of anti-ART1 monoclonal antibodies in murine immune-competent lung cancer models (1-24 months)

Task 1: To evaluate the ability of anti-ART1 therapeutics to inhibit tumor formation in orthotopic lung and flank tumor models (4-18 months)

For tail vein injection, KP1 cells will be injected into the tail vein of immunocompetent C57BL/6 mice (4-6 week old, both male and female). (n=15 x 4 lines (KP1 parent, KP1doxshART1, KP1luc, KP1ART1OE, KP1Ova) x 3 doses = Total mice = 180 mice

For the flank model KP1 cells are injected subcutaneously into the lower flank of the mice. (n=15 x 4 lines (KP1 parent, KP1doxshART1, KP1luc, KP1ART1OE, KP1Ova) x 3 doses = Total mice = 180 mice

Task 2: To evaluate the effects of ART1 inhibition on tumor infiltrating and systemic immune cell populations. (6-18 months)

Tumor bearing lung tissue for flow cytometry analyses will be freshly harvested from mice, and single-cell suspensions prepared and stained. T cell subsets will be characterized by mutli-color flow cytometry.

Task 3: Characterization of P2X7R+ CD8 T cells. (6-18 months)

We will further characterize the P2X7R+ CD8 T cell populations with established flow cytometry panels. Furthermore, P2X7R+ CD8 T cells created from single cell suspensions of KP1 tumors will be sorted via flow cytometry. P2X7R KO mice will be used to replicate key experiments proposed in Aim 1.1 to determine whether the effect of ART1 inhibition is driven solely by interactions with P2X7R positive cells. T-cells harvested from these mice will be used in vitro experiments to further determine the role of P2X7R in NICD. C57BL/6 mice (n=15) x 4 lines x 3 doses = Total mice = 120 mice + 60 P2X7R KO mice

Aim 1 Milestone(s) Achieved: The role of P2X7R+ T cells will be determined.

Local IRB/IACUC Approval (1 months)

Milestone Achieved: HRPO/ACURO Approval (4 months)

Aim 2: To test the therapeutic efficacy and mechanisms of action of anti-ART1 therapy combined with radiation therapy in murine immune-competent lung cancer models. (4-24 months)

Task 1: To evaluate the effects of radiation therapy on ART1 expression in murine tumors. (4-18 months)

Confirm that ART-1 expression is enhanced in KP1 tumors after in vivo treatment with RT. C57BL/6 mice (n=15) x 3 doses x 2 models (orthotopic + flank) = Total mice = 90 mice

Task 2: To evaluate primary tumor and abscopal effects of combining radiation therapy with anti-ART1 therapy. (6-24 months)

Tumor-targeted RT will be used to treat subcutaneous tumors obtained by injection of KP1doxshART1 cells and mice treated with or without doxycycline to knock down ART1. C57BL/6 mice (n=15) x 3 doses x 2 models (flank + opposite flank) = Total mice = 90 mice

Task 3: To evaluate the effects of radiation therapy and ART1 blockade in an orthotopic lung tumor model. Irradiate KP1 tumor bearing lungs. Total mice = 45 mice (12-24 months)

AIM 3: To characterize ART1 expression and immune phenotype in lung cancers from VA patients and to evaluate the ART1/P2X7R axis in human lung tumors treated with preoperative immunotherapy and radiation therapy. (4-24 months)

Task 1: To determine ART1 expression and its relationship to immune phenotype, specifically to abundance of P2X7R+ TILs in human lung cancers (4-24 months)

Novel multiplex IF analysis (MultiOmyxTM platform, NeoGenomics Labs), of 35 human tumors from core biopsies or surgically resected specimens from NSCLC patients without previous treatment.

Task 2: To determine changes of ART1 expression in response to immunotherapy ± radiation therapy and to explore predictive associations of ART1 expression to treatment outcomes. (4-24 months)

Novel multiplex IF analysis (MultiOmyxTM platform, NeoGenomics Labs), of 25 human tumors from core biopsies or surgically resected specimens from NSCLC patients enrolled in an ongoing investigator-initiated neoadjuvant clinical trial in which early stage lung cancer patients either receive anti-PD-L1 blockade (Durvalumab, AstraZeneca) alone or together with non-ablative stereotactic radiation prior to surgical resection

Milestone(s) Achieved: Quantify and phenotype immune cells with multiple IF markers in order to better understand their role in the tumor microenvironment. Relate the quantification of these cells to ART1 expression.

What was accomplished under these goals?

We have accomplished a great deal in the past year. Our first manuscript regarding the role of ART1 in lung cancer is completed and being reviewed internally prior to submission to a high impact journal.

Regarding Aim 1, we spent a great deal of time on Task 3, characterizing the presence and function of P2RX7+ CD8 T cells in our tumor models. We have now worked out quite a bit regarding the biology of these cells which we believe are critical in regulating the immune response to cancer. We first sought to assess whether gene expression of P2rx7 and Cd38 in CD8 T cells is altered during lung tumor progression. We performed RNA sequencing analysis of CD8 T cells isolated from lungs and spleens from mice orthotopically inoculated with wild type KP1 lung tumors. We found that that P2rx7 expression was moderately increased in CD8 T cells isolated from mouse lungs with KP1 tumor burden at day 7 after KP1 injection and highly increased at day 17 compared with CD8 T cells isolated from lungs of naïve mice. CD8 T cells isolated from tumor bearing lungs , also showed increased expression of Ifng and Prf1 as well as Pdcd1, Ctla4, Havcr2, Lag3, and Tigit compared with naïve control CD8 T cells. The observed changes in P2rx7 and Cd38 expression appeared to be confined to the tissue resident CD8 cells, as we did not observe similar expression changes in spleen-derived CD8 T cells. Increased surface expression of P2X7R in populations of CD8 T cells from KP1-tumor bearing mice was confirmed by flow cytometry.
We hypothesized that among tumor-infiltrating CD8 T cells, the $P2X7R^+$ subset would be susceptible to NICD in ART1-expressing tumors. Indeed, infiltration of $P2X7R^+$ CD8 T cells was reduced in KP1-ART^{OE} flank tumors compared to in tumors with ART1 knockdown. In order to determine whether the $P2X7R^+$ CD8 T cells are particularly susceptible to ART1-mediated NICD and whether co-expression of CD38 could play a protective role by catabolizing free NAD^+ from the immediate micromilieu, we established an in vitro T cell co-culture assay with HEK293 cells engineered to overexpress ART1 (HEK-$ART1^{OE}$). The HEK-$ART1^{OE}$ cells maintain robust cell surface expression of enzymatically active ART1 and were shown to ADP-ribosylate tumor cell surface targets in the presence of free NAD^+ as measured by MAR/PAR immunofluorescence staining. In the presence of etheno-tagged NAD^+ (e-NAD), ADP-ribosylation of cell surface targets was assessed by flow cytometry using an antibody detecting e-NAD, while propidium iodide (PI) was used to measure cell death. CD8 T cells from the lungs of KP1 tumor-bearing lungs were sorted and incubated for 2 hours with eNAD in the presence or absence of HEK-$ART1^{OE}$ cells. ADP-ribosylation was detected only in the $P2X7R^+$ subset, and was significantly enhanced in the presence of HEK-$ART1^{OE}$ cells in $CD38^-$ but not $CD38^+$ T cells. Loss of membrane integrity measured by PI staining paralleled eNAD positivity and was highest in $P2X7R^+$ $CD38^-$ CD8 T cells, suggesting that the ability to catabolize NAD^+ partially protects $CD38^+$ cells from NICD. In contrast, CD4 T cells (gated as $CD3^+$ $CD8^-$) were resistant to ADP-ribosylation regardless of their

expression of P2X7R and CD38, suggesting that alternative mechanisms may prevent ADP-ribosylation or NICD in these cells.

To further test our hypothesis that $P2X7R^+$ CD8 T cells are uniquely sensitive to ART1-mediated ADP-ribosylation and that CD38 expression is protective, CD8 T cells were sorted from the lungs of KP1 tumor-bearing wild type mice and P2X7R-deficient ($P2X7R^{-/-}$) mice, and incubated in the presence of e-NAD with or without recombinant murine ART1 and/or anti-CD38 antibody. The enzymatic activity of murine ART1 was confirmed by NAD-Glo assay. The baseline staining for e-NAD was increased in the presence of ART1 in wild type but not P2X7R-deficient CD8 T cells, and it was further enhanced in the presence of anti-CD38 antibody. These results confirm that P2X7R is a main target of ART1-mediated ADP-ribosylation of CD8 T cells, and suggest that expression of CD38 by CD8 T cells attenuates ART1-mediated ADP-ribosylation. We are currently working to further functionally characterize P2X7R+ CD8 T cells. We believe that they represent a unique population of tissue resident memory cells which contribute to tumor immunity.

Regarding Aim 1, Tasks 1 and 2, we have also made nice progress. We explored the possibility of targeting surface ART1 therapeutically with a novel monoclonal antibody targeting ART1. Therapeutic antibody candidates were initially developed through immunization of AlivaMab transgenic mice with a human immunoglobulin repertoire using human ART1. Candidate antibodies that bound to both human and mouse ART1 and inhibited mono-ADP-ribosylation were further developed. One monoclonal antibody, 22C12, which potently inhibited ART1 enzymatic activity in the primary screening assay, was further tested in vitro and in vivo. Half-maximum inhibition of ADP-ribosylation (IC_{50}), as determined by cell surface ADP-ribosylation of HEK-$ART1^{OE}$ cells, was achieved at 14 nM antibody concentration. The ability of 22C12 Ab to block cancer cell induced mono-ADP-ribosylation was confirmed in KP1-$ART1^{OE}$ cells co-cultured with NAD^+ and binding of 22C12 to KP1-$ART1^{OE}$ cells was assessed by flow cytometry staining. We performed intratumoral injections of the 22C12 antibody (5 mg/kg), or the equivalent dose of isotype control antibody, into subcutaneously implanted KP1-$ART1^{OE}$ flank tumors starting on day 11 when tumors had become palpable. The injections were repeated every three days until day 23. On day 25, mice were sacrificed, and tumors harvested, weighed and analyzed by flow cytometry for tumor infiltration of CD8 T cells and assessing their expression of P2X7R and CD38. ART1 blockade resulted in a significantly delayed tumor growth compared to tumors treated with isotype control antibody, with average tumor weight at day 25 significantly lower in the mice treated with 22C12 compared with isotype control antibody. Flow cytometry analysis showed that tumors treated with 22C12 antibody had a higher percentage of $P2X7R^+$ CD8 T cells with low or negative CD38 expression compared with control tumors while there was no significant difference in frequencies of $P2X7R^-$ CD8 T cells or in $P2X7R^+$ CD8 T cells with high expression of CD38, indicating that the ART1 blockade may preferentially rescue the $P2X7R^+$ CD8 T cells that lack high CD38 expression from ART1-mediated NICD. We next designed an orthotopic model induced by tail vein injection of KP1-$ART1^{OE}$ cells, in which mice were treated intraperitoneally with 22C12 antibody (25 mg/kg) or the equivalent dose of isotype subclass-matched control antibody from the day of tumor injection until day 19. Mice treated with 22C12 antibody had significantly reduced lung weight compared to control mice. Mouse lungs were fixed and stained with H&E to assess lung tumor burden, which showed fewer and significantly smaller lung tumor nodules in mice treated with 22C12 antibody compared with control treated mice. Flow cytometry analysis of revealed, similarly to in the flank tumor model, an enrichment of $P2X7R^+CD38^{low/-}$ CD8 T cells in mice treated with

22C12 antibody. These studies indicate that therapeutic targeting of ART1 inhibits tumor growth and promotes tumor infiltration of $P2X7R^{+}CD8$ T cells and that systemic ART1 blockade can reduce lung tumor growth and dissemination.
We have been unable to evaluate other antibody candidates (14G01 and the non-enzyme blocking 09B07) due to production delays in our antibody facility. We are currently scaling up production of those therapeutic antibodies and expect to perform experiments with those in the next several months.

Regarding Aim 2, much of this has been on hold as we accumulated and validated data from Aim 1. We have further characterized the in vitro response of ART1 expression to radiation in cancer cell lines and have modeled the radiation plans with the Demaria lab. Now that we have selected 22C12 as the appropriate therapeutic to move forward we will launch combination therapies with 22C12 and radiation therapy using several murine cell lines.

For Aim 3, while we are still awaiting approval of the CDMRP specific IRB we have continued to collect specimens under our institutional biobanking IRB and in the context of our clinical trial NCT02904954. The trial, utilizing a combination of anti-PD-L1 antibody and nonablative radiation has completed enrollment. We are prepared to analyze the ART1/P2X7R/CD38 axis in correlative work from this trial.

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

Postdoctorates working on the project presented work and attended several national meetings for professional development. One abstract was selected for the Presidential Session at SITC2019 by Dr. Erik Wennerberg. Additionally abstracts were presented at AACR/IASLC by another postdoctorate, Sumit Mukherjee, at the AACR annual meeting by both postdoctorates, and were accepted for presentation at the CSHL PARP meeting.

Dr. Stiles attended the AACR/IASLC meeting and discussed the project with several investigators and will attend the CSHL PARP meeting. Additionally, Dr. Stiles traveled to meet with the development team at Ribon Therapeutics where he presented and discussed aspects of the project.

Furthermore, Dr. Stiles has had ongoing meetings and discussions with the Tri-I TDI regarding development and optimization of the therapeutic antibody candidates.

How were the results disseminated to communities of interest?

In addition to conference presentations, Dr. Stiles is active on twitter (@BrendonStilesMD) and has actively promoted the ART1 work presented at conferences to his followers, many of whom are students, patients, or advocates.

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

We have been accepted into the second round of development by the Tri-I TDI Antibody Core. We are optimizing our lead antibody candidate, 22C12, for further combination studies and for humanization as a pathway to clinical trials. We don't expect antibody quantities to be a limitation moving forward. During the next year, using preclinical models, we will work out the optimal situation to begin clinical trials of an anti-ART1 antibody for ART1 overexpressing tumors. Additionally, we will leverage translational data from the human studies in Aim 3 to confirm our murine model data.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Over the past year, there has been renewed interest in targeting mono-ADP-ribosyltransferases as a therapeutic strategy for cancer. We believe that ART1 is one such bona fide cancer immunotherapy target. In addition to our studies in lung cancer, we have seen a similar effect of decreased tumorigenesis with ART1 knockout or blockade in melanoma models and expect to see it in other tumor models as well. We believe that our manuscript, when published, will generate significant interest in ART1 and in the ART/P2X7R/CD38 axis based upon a number of recent high impact publications on the role of P2X7R in development of tissue resident memory CD8 T cells and on the role of CD38 in immunotherapy resistance. The relationship of ART1 to cancer progression is still relatively novel, but we believe that the connection will open many investigative pathways.

What was the impact on other disciplines?

Interestingly, we believe that the protective effects of ART1 are evolutionarily ancient and that the axis we describe most likely has a basic role in infection and immunity. We think that our findings will help guide basic thinking in the field of immunology.

What was the impact on technology transfer?

We are currently filing a patent to protect the intellectual property. We expect to develop a fully humanized therapeutic antibody against ART1. Through our relationship with the TDI, the pharmaceutical company Takeda has an option to further develop the drug clinically. We also have an application into Takeda for more funding for drug development. Additionally, we have discussed potential partnerships with a company, Ribon Therapeutics, focused on developing therapeutics targeting mono-ADP-ribosylation.

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

A proposed alternative was to evaluate small molecule inhibitors of ART1. However, we were unable to demonstrate reliable inhibition of ART1 induced mono-ADP-ribosylation in several in vitro studies despite previous reports in the literature. Reassuringly, when we met with Ribon Therapeutics they also suggested that compounds like Novobiocin, MIBG, and a handful of PARPs

were not optimal inhibitors in their hands either. We have therefore turned our focus solely on the therapeutic antibody candidates.

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

We have had some delay in the approval process of our CDMRP specific IRB (although human studies were previously approved on our institutional biobanking IRB). We expect that approval to be completed soon. Additionally, our institution was particularly hit hard by the COVID-19 pandemic. The PI, Dr. Stiles, was actively involved in the care of COVID-19 patients from March through June, as the leader of the inpatient tracheostomy service. The laboratory was closed during that time period, which significantly slowed down the work we were able to complete on the proposed Aims. We are now back up and running with some restrictions, but envision completing the majority of the work on time.

Changes that had a significant impact on expenditures

The COVID-19 pandemic slowed down lab expenditures, although we continued to provide full salary support for all lab members. It did slow down our ability to hire a new technician. One has just recently been hired and is due to start this month.

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

IRB process was delayed. The expected approval date is November 2020.

Significant changes in use or care of vertebrate animals

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS:

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

  Journal publications.

  Nothing to report

Books or other non-periodical, one-time publications.

Nothing to report

Other publications, conference papers and presentations.

SITC 2019, Presidential session: Expression of ART1, an extracellular mono ADP-ribosylase, promotes lung cancer growth and dissemination by limiting tumor infiltration of P2X7R+ CD8+ T cells and CD103+ dendritic cell. Erik Wennerberg, PhD; Clarey Hung; Amanda Valeta; Timothy E. McGraw; Sandra Demaria, MD; Brendon Stiles, MD

AACR/IASLC 2020: A05 ART1, a Mono-ADP-Ribosyltransferase, Regulates Tumor-Infiltrating CD8+ T Cells and Is Highly Expressed in EGFR Mutated Lung Cancers. Sumit Mukherjee, Erik Wennenberg, Clarey Hung, et al. J Thorac Oncol 2020;15(2):S13.

AACR 2020 Annual Meeting: Abstract 1820: Art1, an extracellular mono-ADP-ribosyltransferase, is upregulated in response to cellular stress and promotes lung cancer growth. Sumit Mukherjee, Erik Wennerberg, Clarey Hung, Najla Saadallah, Shashi Kariyawasam, Christopher Agrusa, Amanda Valeta, Nasser Altorki, Timothy McGraw, Sandra Demaria and Brendon Stiles. DOI: 10.1158/1538-7445.AM2020-1820 Published August 2020

CSHL 2020 PARP Family and ADP-ribosylation Meeting: ART1 tumor expression mediates immune resistance in non-small cell lung cancer by elimination of P2RX7+ CD8 tissue resident memory T cells and conventional type I dendritic cells. Erik Wennerbeg[1], Sumit Mukherjee[2], Clarey Hung[2], Timothy McGraw[3], Sandra Demaria[1], Brendon Stiles[2]

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

  Nothing to report

- **Technologies or techniques**

  Nothing to report

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

  Nothing to report

- **Other Products**

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Brendon Stiles, MD
Project Role: PI
Months worked: 1.2
Contribution to project: Dr. Stiles oversees all aspects of the proposal as PD/PI.

Name: Nasser Altorki, MD
Project Role: Co-Investigator
Months worked: 0.12

Contribution to project: Dr. Altorki collaborators with Dr. Stiles to ensure the in vitro and in vivo modes are consistent with clinical findings.

Name: Timothy McGraw, PhD
Project Role: Co-Investigator
Months worked: 0.12
Contribution to project: Dr. McGraw collaborates with Dr. Stiles on the mechanistic aspects of the proposal.

Name: Sandra Demaria, MD
Project Role: Co-Investigator
Months worked: 0.12
Contribution to project: Dr. Demaria collaborates with Dr. Stiles on the radiation aspects of the experiments.

Name: Drew Moghanaki, MD (Atlanta VA)
Project Role: Co-Investigator
Months worked: 0.36
Contribution to project: Expert guidance on lung cancer models involving radiotherapy and ensures that the models represent the patient condition.

Name: Sumit Mukherjee, PhD
Project Role: Post-Doc
Months worked: 8.4
Contribution to project: Dr. Mukherjee leads all the experiments and troubleshoots all aspects of the proposal. He is responsible for data analysis alongside Dr. Stiles. He meets with Dr. Stiles on a weekly basis.

Name: Najla Saadallah, BS
Project Role: Technician
Months worked: 6
Contribution to project: Ms. Saadallah works closely with the post-doc Dr. Mukherjee on animal studies and in vitro experiments.

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. Stiles completed the Metastasis Research Grant from the Free to Breathe Foundation (LCRF) on which he was a PI, entitled "ART1: a novel therapeutic target for the prevention of metastases from non-small cell lung cancer." Dates were 07/02/2015 – 12/31/2019

Dr. Altorki and Dr. McGraw received a two-year research grant UG3CA244697 from the NIH NCI entitled, "Intercepting progression from pre-invasive to invasive lung adenocarcinoma." Dates are from 09/25/2019 – 08/31/2021. Each PI is spending 10% effort (1.2 calendar months).

What other organizations were involved as partners?

Organization Name: Foundation for Atlanta Veterans Education and Research (Atlanta VA)
Location of Organization: Decatur, Georgia
Partner's contribution to the project: The subaward with the Atlanta VA is to provide financial support for the effort of Co-Investigator Dr. Drew Moghanaki who is a radiation oncologist who provides expert guidance on lung cancer models involving radiotherapy and ensures that the models represent the patient condition. Once IRB/HRPO approval is obtained, Dr. Moghanaki will also provide TMAs and core biopsies from NSCLC patients in the veteran population at the Atlanta VA Medical Center for novel multiplex IF analysis. This is part of Aim 3 (to characterize ART1 expression and immune phenotype in lung cancers from VA patients and to evaluate the

ART1/P2X7R axis in human lung tumors treated with preoperative immunotherapy and radiation therapy), Task 1 (To determine ART1 expression and its relationship to immune phenotype, specifically to abundance of P2X7R+ TILs in human lung cancers).

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

Nothing to report

9. APPENDICES:

Nothing to report