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

PRINCIPAL INVESTIGATOR: Timothy McGraw, PhD

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

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Timothy McGraw, PhD & Brendon Stiles, MD

E-Mail: temcgraw@med.cornell.edu; brstiles@montefiore.org

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Joan & Sanford I. Weill Medical
College of Cornell University
1300 York Avenue, Box 89
New York, NY 10065

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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:**

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:**

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?

During the NCE year we:

1. Established the anti-tumor effect of anti-ART antibody (22C12) on other cancers using mouse models. Anti-Art1 antibody significantly reduced the growth of subcutaneous B16 mouse melanoma cell tumors compared to an isotype control antibody (Figure 1). Intriguingly, shRNA knockout of ART1 in B16 melanoma cells profoundly reduces tumor growth, establishing a role for ART1 in tumors formed by these melanoma cells.

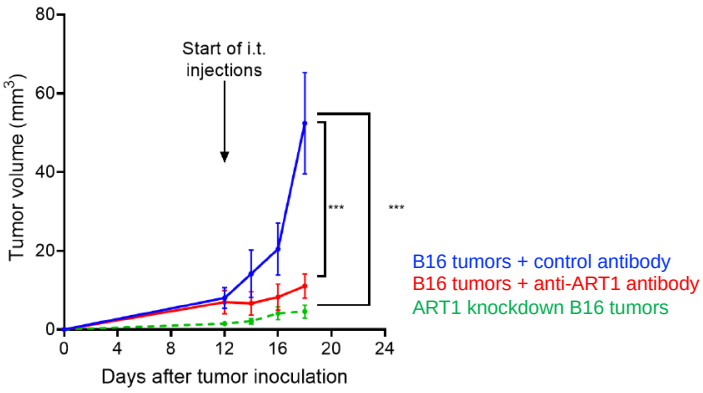

Figure 1. Effect of anti-ART1 antibody on B16 melanoma cell tumorigenesis. See text for details.

These findings suggest that in other tumor types that express high levels of ART1, including colorectal and glioblastomas, anti-ART antibody might be an effective anti-tumor agent.

2. We continued to work with Tri-I TDI Antibody Core for antibody optimizing, including head-to-head testing of other clones.

3. Using preclinical models, we continued work on optimizing protocols to begin clinical trials of an anti-ART1 antibody for ART1 overexpressing tumors.

4. We engaged with Dr. Moghanaki's new VA facility (VA of Greater Los Angeles) to determine feasibility of adding VA human studies in Aim 3 to confirm our murine model data. We have been approved for a second NCE period 09/01/2022 – 08/31/2023 where we will obtain the human samples and perform IF analyses.

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

Nothing to report

How were the results disseminated to communities of interest?

Nothing to report

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

We will analyze human samples from a VA cohort and a combinatorial clinical trial to determine ART1 expression and its relationship to immune phenotype.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Over the past three years, 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 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?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

The PI Dr. Brendon Stiles moved from Weill Cornell Medicine to Albert Einstein College of Medicine/Montefiore in February 2021. As a result, the co-investigator Dr. Timothy McGraw at Weill Cornell Medicine was approved to take over the PI role and continue the work. Also, the Co-Investigator Dr. Drew Moghanaki moved from the Atlanta VA to the VA of Greater Los Angeles. The transition period has delayed certain experiments and the securing of human samples, which was expected. We hope the addition of another NCE period will allow the completion of the work.

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

Because of pandemic related delays and the transition of key personnel to new institutes, we have had some delay in procuring the appropriate source of human samples for ART1 analyses, and thus the IRB approval process has been delayed (although human studies were previously approved on our institutional biobanking IRB). Local IRB approval has now been obtained and once the VA sample source has been confirmed, the HRPO paperwork will be submitted. We envision completing the work in the NCE period.

Changes that had a significant impact on expenditures

Nothing to report

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, but is now approved locally. HRPO paperwork is being prepared once the VA samples are confirmed.

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. Wennerberg E, Mukherjee S, Spada S, Hung C, Agrusa CJ, Chen C, Valeta-Magara A, Rudqvist NP, Van Nest SJ, Kamel MK, Nasar A, Narula N, Mittal V, Markowitz GJ, Zhou XK, Adusumilli PS, Borczuk AC, White TE, Khan AG, Balderes PJ, Lorenz IC, Altorki N, Demaria S, McGraw TE, Stiles BM. Expression of the mono-ADP-ribosyltransferase ART1 by tumor cells mediates immune resistance in non-small cell lung cancer. Sci Transl Med. 2022 Mar 16;14(636):eabe8195. doi: 10.1126/scitranslmed.abe8195. Epub 2022 Mar 16. PMID: 35294260; PMCID: PMC9256502.

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

Nothing to report

Other publications, conference papers and presentations.

Previously reported presentations include:

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]

New presentations were not made in 2022.

- **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: Timothy McGraw, PhD

Project Role: Co-Investigator
Months worked: 1.2
Contribution to project: Dr. McGraw took over as PI in February 2021 upon the departure of PI Dr. Stiles from Weill Cornell. Dr. McGraw collaborates with Dr. Stiles on the mechanistic aspects of the proposal.

Name: Brendon Stiles, MD
Project Role: Co-Investigator
Months worked: 1.2
Contribution to project: Dr. Stiles relinquished his PD/PI role to Dr. Timothy McGraw upon Dr. Stiles' departure from Weill Cornell to Einstein/Montefiore in February 2021. He continues to work on the analysis of data.

Name: Nasser Altorki, MD
Project Role: Co-Investigator
Months worked: 0.12
Contribution to project: Dr. Altorki collaborates with Dr. McGraw to ensure the in vitro and in vivo modes are consistent with clinical findings

Name: Sandra Demaria, MD
Project Role: Co-Investigator
Months worked: 0.12
Contribution to project: Dr. Demaria collaborates with Dr. McGraw 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: 1.2
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: Jennifer Wen, BS
Project Role: Technician
Months worked: 1.2
Contribution to project: Ms. Wen works closely with the post-doc Dr. Mukherjee on all 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. Altorki and Dr. McGraw received an NCI U54 grant entitled, “CAP-IT Center for LNP RNA Immunoprevention.” Dates are 09/01/2022 – 08/31/2027. The investigators are spending 10% effort (1.2 calendar months) each.

What other organizations were involved as partners?

Organization Name: VA Greater Los Angeles Healthcare System (Atlanta VA)
Location of Organization: Los Angeles, CA

Partner's contribution to the project: The subaward with the Atlanta VA was put on hold as Co-Investigator Dr. Drew Moghanaki moved from the Atlanta VA to the VA of Greater of Los Angeles. Dr. Moghanaki 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 VA of Greater Los Angeles 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).

Organization Name: Albert Einstein College of Medicine/Montefiore (Bronx, NY)
Location of Organization: New York, NY
Partner's contribution to the project: Dr. Stiles moved to Albert Einstein from Weill Cornell in February 2021. As a result, a subaward was issued to the site for Dr. Stiles' effort spent in collaboration with the new PI Dr. Timothy McGraw here at Weill Cornell.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

Nothing to report

9. APPENDICES:

Nothing to report