AWARD NUMBER: W81XWH-18- 1-0495

TITLE: Investigating the Role of Piezo1 in Pancreatic Cancer-Related Immune Suppression and Disease Progression

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CONTRACTING ORGANIZATION: New York University School of Medicine

REPORT DATE: DECEMBER 2020

TYPE OF REPORT: Final Technical

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*



| 1. REPORT DATE | 2. REPORT TYPE | 3. DATES COVERED |
|---|---|---|
| DECEMBER 2020 | FINAL | 15AUG2018 - 14AUG2020 |

**4. TITLE AND SUBTITLE**

Investigating the Role of Piezo1 in Pancreatic Cancer-Related Immune Suppression and Disease Progression

**5a. CONTRACT NUMBER**

**5b. GRANT NUMBER**
W81XWH-18-1-0495

**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**

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**5d. PROJECT NUMBER**
CA170450

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**
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**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

**10. SPONSOR/MONITOR'S ACRONYM(S)**

**11. SPONSOR/MONITOR'S REPORT NUMBER(S)**

**12. DISTRIBUTION / AVAILABILITY STATEMENT**

Approved for Public Release; Distribution Unlimited

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**

Our goal is to perform clinical trials in PDA patients testing strategies to modulate the microbiome to enhance the efficacy for immunotherapy. However, the optimal regimen has not been defined. We will perform experiments in mouse PDA models and in human organotypic systems which are designed to define the most efficacious microbiome modulatory regimens - either antibiotics or probiotics - to combine with immunotherapy. In Aim 1 we will test the immune-activating and tumor-protective effects of specific antibiotic and probiotic regimens in mouse models of PDA and an innovative microfluidic-based organotypic model derived from freshly resected human PDA. In Aim 2 we will determine the regimen that most effectively synergizes with immunotherapy. Aim 3 will encompass the first clinical trial in PDA targeting the microbiome as a strategy to enable immunotherapeutic efficacy. Collectively, these Aims will lead to a new treatment paradigm for PDA patients that targets the microbiome.

**15. SUBJECT TERMS**
Pancreatic Cancer, immune suppression, cancer.

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
|---|---|---|---|---|---|
| a. REPORT | b. ABSTRACT | c. THIS PAGE | | | USAMRMC |
| Unclassified | Unclassified | Unclassified | Unclassified | 10 | 19b. TELEPHONE NUMBER *(include area code)* |

## TABLE OF CONTENTS

1. **INTRODUCTION:** Piezo1 is a mechanosensitive ion channel that has gained recognition for its role regulating diverse physiological processes. However, the influence of Piezo1 in inflammatory disease, including infection and tumor-immunity, is unknown. We postulated that Piezo1 links physical forces to immune regulation in leukocytes.

2. **KEYWORDS:**

   PDA – Pancreatic ductal adenocarcinoma
   RB1 – retinoblastoma protein
   HDAC2 – Histone deactetylase 2
   TME – Tumor microenvironment
   PDOTS – Patient-derived organotypic tumor spheroids
   MDSC – Myeloid-derived suppressor cells

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.*

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

     *We postulated that Piezo1 activation by mechanical forces promotes immune suppression in the pancreatic tumor microenvironment by driving the tolerogenic differentiation of monocytic cells. We further hypothesized that targeting Piezo1 will enhance anti-tumor immunity. Our objective is to determine the efficacy of targeting Peizo1 as a novel immunotherapy for pancreatic ductal adenocarcinoma (PDA). The FY17 Military Relevance Focus area is "Gaps in Pancreatic Cancer Treatment".*

     **What was accomplished under these goals?**

     1. We discovered mechanical signal transduction via Piezo1 in both myeloid and T cells. Further, Piezo1 is the primary sensor of mechanical pressure in leukocytes. Global inhibition of Piezo1 was protective against cancer and resulted in a diminution in monocyte infiltration. Selective deletion of Piezo1 in myeloid cells also protected against cancer. Mechanistically, we discovered that Piezo1 promotes myeloid cell expansion by suppressing *Rb1* expression via upregulation of HDAC2. Collectively, these findings uncover Piezo1 as a targetable immune checkpoint that drives immune-suppressive myelopoiesis.

     2. *Aim 1. To determine the effects of global or compartment-specific inhibition or activation of Piezo1 on PDA progression*
        *Aim 2. To determine the mechanistic influence of Piezo1 on monocytic cellular differentiation in the PDA tumor microenvironment*
        *Aim 3. To determine whether targeting Peizo1 enables efficacy of checkpoint-based immunotherapy in PDA*

3. *To assess the influence of global Piezo1 signaling on the progression of pancreatic ductal adenocarcinoma (PDA), we utilized GsMTx4 and Yoda1, respectively, to inhibit or activate Piezo1. Inhibition of Piezo1 conferred tumor-protection in an orthotopic PDA model using tumor cells derived from $Pdx1^{Cre};Kras^{G12D};Tp53^{R172H}$ (KPC) mice (**Figure 1a**). By contrast, activation of Piezo1 accelerated tumor growth (**Figure 1b**). We then analyzed the intra-tumoral immune phenotype in PDA-bearing mice treated with GsMTx4. We discovered that the $Gr1^{+}CD11b^{+}$ myeloid derived suppressor cell (MDSC) population was sharply diminished upon Piezo1 inhibition (**Figure 1c**). By contrast, activation of Piezo1 in vivo with Yoda1 increased cellular expansion of MDSC in PDA (**Figure 1c**). To evaluate the therapeutic efficacy of Piezo1 inhibition in human PDA, we treated patient-derived organotypic tumor spheroids (PDOTS) from freshly resected human tumor specimens with GsMTx4 or vehicle using a 3-dimensional microfluidic system we recently validated. Piezo1 inhibiton resulted attenuated spheroid growth in PDOTS (**Figures 1e**). By contrast, Piezo1 activation accelareted spheroid growth in PDOTS (**Figures 1f**).*

Based on our observations that pan-inhibition or deletion of Piezo1 reduced MDSC infiltration in PDA, whereas Piezo1 activation increased MDSC infiltration (***Figure 1 a – d***), we postulated an immune-

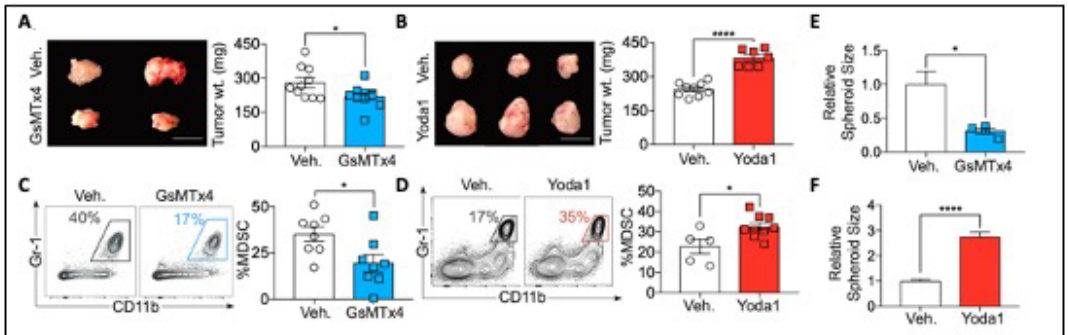


**Figure 1. Piezo1 signaling enables pancreatic cancer progression. (A)** WT mice bearing orthotopic KPC tumor were treated with GsMTx4 or vehicle and sacrificed at 3 weeks. Representative images and quantitative analysis of tumor weights are shown (scale bar = 1 cm). This experiment was repeated 4 times with similar results (n=5/group). **(B)** WT mice bearing orthotopic KPC tumor were treated with Yoda1 or vehicle and sacrificed at 3 weeks. Representative images and quantitative analysis of tumor weights are shown (scale bar = 1 cm). **(G,H)** WT mice bearing orthotopic KPC tumor were treated with (G) GsMTx4 or (H) Yoda1 and sacrificed at 3 weeks. The fraction of intra-tumoral $Gr1^{+}CD11b^{+}$ MDSC was determined by flow cytometry. (E,F)PDOTS from freshly resected human tumors (n=5) were treated with (E) GsMTx4 or (F) Yoda1 for 3 days. Spheroid sizes were measured. Data is presented as fold change compared to vehicle.

regulatory role for Piezo1 signaling in myeloid cells. To investigate the impact of Piezo1 signaling in myeloid cells, we generated $Lyz2M^{Cre};Piezo1^{fl/fl}$ mice. Next, we challenged $Lyz2M^{Cre};Piezo1^{fl/fl}$ mice and controls with orthotopic KPC tumors. $Lyz2M^{Cre};Piezo1^{fl/fl}$ mice were protected against PDA (***Figure 2a***). Tumors in $Lyz2M^{Cre};Piezo1^{fl/fl}$ mice exhibited a reduced MDSC infiltrate akin to pan-inhibition of Piezo1 (***Figure 2b***). Moreover, consistent with our previous reports that MDSC suppress adaptive immunity in PDA, we found that targeting Piezo1 in myeloid cells resulted in enhanced intra-tumoral CD4+ and CD8+ T cell immunogenicity (***Figure 2c, d***).

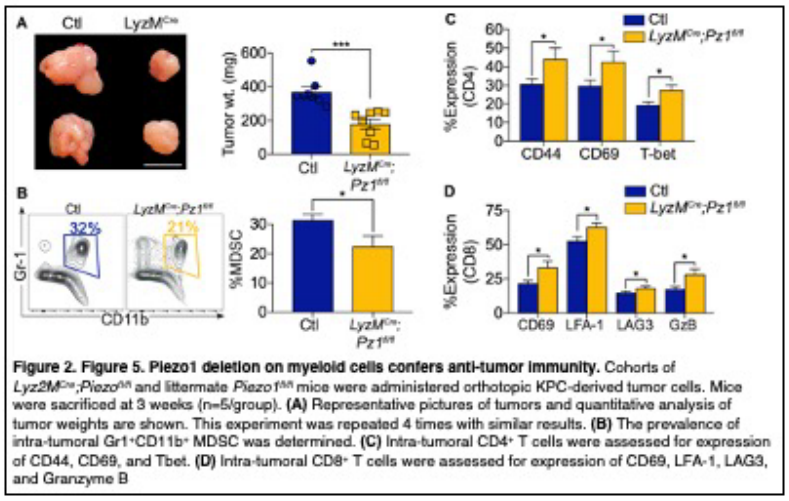


**Figure 2. Figure 5. Piezo1 deletion on myeloid cells confers anti-tumor immunity.** Cohorts of $Lyz2M^{Cre};Piezo^{fl/fl}$ and littermate $Piezo1^{fl/fl}$ mice were administered orthotopic KPC-derived tumor cells. Mice were sacrificed at 3 weeks (n=5/group). **(A)** Representative pictures of tumors and quantitative analysis of tumor weights are shown. This experiment was repeated 4 times with similar results. **(B)** The prevalence of intra-tumoral $Gr1^{+}CD11b^{+}$ MDSC was determined. **(C)** Intra-tumoral $CD4^{+}$ T cells were assessed for expression of CD44, CD69, and Tbet. **(D)** Intra-tumoral $CD8^{+}$ T cells were assessed for expression of CD69, LFA-1, LAG3, and Granzyme B

Since Rb1 can suppress MDSC expansion, we postulated that Piezo1 controls MDSC levels in disease by regulation of Rb1. Consistent with our hypothesis, we found that increasing mechanical pressure in myeloid cells lowered expression of Rb1 (***Figure 3a***). Similarly, activating Piezo1 using Yoda1 reduced Rb1 expression, indicating that Piezo1 signaling or mechanical stimulation suppress Rb1 (***Figure 3b***). Since *Rb1* expression is inhibited by histone deacetylase 2 (HDAC2), we hypothesized that Piezo1 signaling expands MDSC by suppressing *Rb1* via epigenetic silencing. Consistent with our hypothesis, we observed reduced HDAC2 expression in Lyz2M$^{Cre}$;Piezo1$^{fl/fl}$ myeloid cells (***Figure 3c***). Using ChIP-sequencing (ChIP-seq), we confirmed enhanced histone acetylation of the Rb1 promoter in bone marrow cells of Lyz2M$^{Cre}$;Piezo1$^{fl/fl}$ (***Figure 3d***). Moreover, inhibition of HDAC-2 abrogated tumor protection and differential MDSC contraction in Lyz2M$^{Cre}$;Piezo1$^{fl/fl}$ mice (***Figure 3e, f***) indicating that deletion of Piezo1 in myeloid cells protects against tumor growth in an HDAC2 dependent manner.

*Of note, we did not investigate the role of inhibition of Piezo1 concomitant to checkpoint blockade therapy.*

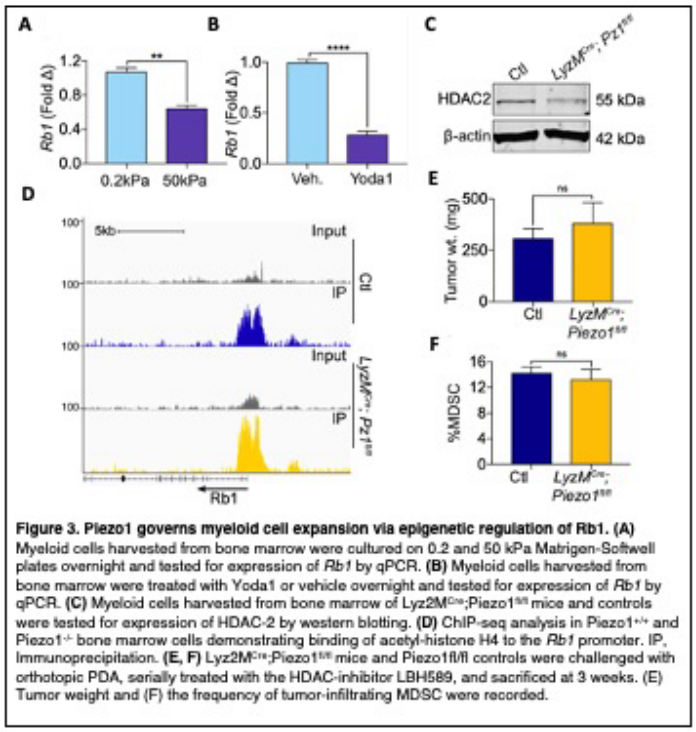


**Figure 3. Piezo1 governs myeloid cell expansion via epigenetic regulation of Rb1. (A)** Myeloid cells harvested from bone marrow were cultured on 0.2 and 50 kPa Matrigen-Softwell plates overnight and tested for expression of *Rb1* by qPCR. **(B)** Myeloid cells harvested from bone marrow were treated with Yoda1 or vehicle overnight and tested for expression of *Rb1* by qPCR. **(C)** Myeloid cells harvested from bone marrow of Lyz2M$^{Cre}$;Piezo1$^{fl/fl}$ mice and controls were tested for expression of HDAC-2 by western blotting. **(D)** ChIP-seq analysis in Piezo1$^{+/+}$ and Piezo1$^{-/-}$ bone marrow cells demonstrating binding of acetyl-histone H4 to the *Rb1* promoter. IP, Immunoprecipitation. **(E, F)** Lyz2M$^{Cre}$;Piezo1$^{fl/fl}$ mice and Piezo1fl/fl controls were challenged with orthotopic PDA, serially treated with the HDAC-inhibitor LBH589, and sacrificed at 3 weeks. (E) Tumor weight and (F) the frequency of tumor-infiltrating MDSC were recorded.

- **What opportunities for training and professional development has the project provided?**
  - *This project provided training opportunities for 1 postdoctoral fellow (Berk Aykut, MD) and 1 research technician (Ruonan Chen).*
- **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?**
  - *Nothing to Report.*

4. **IMPACT:** *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*
   - **What was the impact on the development of the principal discipline(s) of the project?**
     - *In aggregate, these data show that Piezo1 is an ion channel checkpoint which has the capacity to markedly suppress immunological responses via enhancing myeloid cell-induced tolerance. Moreover, pharmacological targeting of Piezo1 in myeloid may have therapeutic implications to cancer immunotherapy.*

- **What was the impact on other disciplines?**
    - *Nothing to Report.*
- **What was the impact on technology transfer?**
    - *Nothing to Report.*
- **What was the impact on society beyond science and technology?**
    - *Nothing to Report.*

5. **CHANGES/PROBLEMS:** *The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*
    - **Changes in approach and reasons for change**
        - *Nothing to Report.*
    - **Actual or anticipated problems or delays and actions or plans to resolve them**
        - *Nothing to Report.*
    - **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**
        - *Nothing to Report.*
    - **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:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*
    - **Publications, conference papers, and presentations**
      *Report only the major publication(s) resulting from the work under this award.*
        - **Journal publications:** Aykut B, Chen R, Kim JI, Wu D, Shadaloey SAA, Abengozar R, Preiss P, Saxena A, Pushalkar S, Leinwand J, Diskin B, Wang W, Werba G, Berman M, Ki Buom Lee S, Khodadadi-Jamayran A, Saxena D, Coetzee WA, Miller G. Targeting Piezo1 unleashes innate immunity against cancer and infectious disease. Science Immunology. 2020, Aug; 5(50): 1-12. Cited in PubMed; PMID: 32826342.
        - **Books or other non-periodical, one-time publications.** *Nothing to Report.*
        - **Other publications, conference papers, and presentations.** *Nothing to Report.*

- **Website(s) or other Internet site(s)**
  *The ChIP-seq data are deposited in the Gene Expression Omnibus database under accession number GSE155340.*

- **Technologies or techniques**
  *Nothing to Report.*

- **Inventions, patent applications, and/or licenses**
  8/2018: Targeting Piezo1 for Treatment of Cancer and Infectious Diseases (PCT/US2019/044703)

- **Other Products**
  *Nothing to Report.*

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

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

  - *Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."*

    ***Example**:*

| | |
|---|---|
| Name: | *George Miller, MD* |
| Project Role: | *PI* |
| Researcher Identifier (e.g. ORCID ID): | https://orcid.org/0000-0001-8482-4100 |
| Nearest person month worked: | 1.0 |
| Contribution to Project: | *Dr. Miller conceived all experiments, wrote the manuscript, obtained funding, and supervised the project.* |
| Funding Support: | |

| | |
|---|---|
| Name: | *Berk Aykut, MD* |
| Project Role: | *Postdoctoral fellow* |
| Researcher Identifier (e.g. ORCID ID): | https://orcid.org/0000-0001-8343-4258 |
| Nearest person month worked: | 4.0 |
| Contribution to Project: | *Dr. conceived and performed most of the experiments, analyzed and interpreted the data and performed statistical analysis.* |
| Funding Support: | |

| | |
|---|---|
| Name: | *Ruonan Chen* |
| Project Role: | Undergraduate student |
| Researcher Identifier (e.g. ORCID ID): | https://orcid.org/0000-0003-1758-6151 |
| Nearest person month worked: | 4.0 |

| | |
|---|---|
| Contribution to Project: | *Ms. Chen conceived and performed experiments, analyzed and interpreted the data, performed statistical analysis.* |
| 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?**
  - *Nothing to Report.*
- **What other organizations were involved as partners?**
  - *Nothing to Report.*

8. **SPECIAL REPORTING REQUIREMENTS**
   - **COLLABORATIVE AWARDS:**
   - **QUAD CHARTS:**
9. **APPENDICES:**