AWARD NUMBER: W81XWH-22-1-0367

TITLE: Artificial Intelligence Analysis of Histopathology Slides to Develop Biomarkers of Response to Immunotherapy in Kidney Cancer

PRINCIPAL INVESTIGATOR: Anupama Reddy

CONTRACTING ORGANIZATION: Vindhya Data Science Inc., Morrisville, NC

REPORT DATE: October 2023

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
October 2023	Annual	30Sep2022-29Sep2023

4. TITLE AND SUBTITLE

Artificial Intelligence Analysis of Histopathology Slides to Develop Biomarkers of Response to Immunotherapy in Kidney Cancer

5a. CONTRACT NUMBER
W81XWH-22-1-0367

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

ANUPAMA REDDY

E-Mail:

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

VINDHYA DATA SCIENCE INC.
120 BAYLESS RIDGE COURT
MORRISVILLE NC 27560

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

: Immunotherapy has emerged as a promising new therapy for kidney cancer with durable clinical responses in a subset of the patients. However, discovery of biomarkers that predict patient response to immunotherapy has thus far been unsuccessful. Diverse sets of biomarkers have been proposed (e.g., PDL1 immunohistochemistry, tumor mutation burden, gene expression signatures), but have failed to validate in clinical studies. There is an urgent need to identify predictive biomarkers for selecting kidney cancer patients most likely to respond to immunotherapy. Histology slides, which are utilized primarily for cancer diagnosis, have been shown to contain a wealth of information using artificial intelligence (AI). While recent advances in AI can accurately predict kidney cancer subtypes, to the best of our knowledge, models to predict response to therapy have not been explored.
Focus Area: Our project will focus on clear cell renal cell carcinoma (CCRCC) subtype of kidney cancer
Hypothesis: We hypothesize that histological features related to immune cell types and their spatial patterns in relation to the tumor are predictive of response to immunotherapy

15. SUBJECT TERMS
None listed.

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

TABLE OF CONTENTS

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Our goal is to develop biomarkers of immunotherapy response using AI models from H&E-stained histology images. We will first train our model to identify immune cell types on histology images, and then utilize the 2-D spatial patterns of these immune cells as features to predict response to immunotherapy. For the first part of our model, we will generate training labels for classifying the immune cells using multiplex immunofluorescence (mIF). Our two-staged model will then be applied to histological images with H&E stains to predict responders to immunotherapy.

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

Image analysis, AI models, biomarkers of immunotherapy, kidney cancer

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

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Three major tasks in this project were:

1. Identify samples for analysis and run multiplex immunofluorescence on a subset of 10 samples.
2. Modeling and prediction of immune cell types.
3. Developing AI models for predicting response to immunotherapy.

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Major Task 1: Collect samples for analysis and run mIF on a subset of 10 samples.

1. Related to this task, we first submitted all required documents for HRPO approval. HRPO approval was obtained.
2. Collection of Cohort1 (180 sample TMA) and cohort2 (70 sample TMA) along with clinical data and genomics was performed by Dr. Scott Haake and Dr. Katy Beckermann. In addition, we also collected a third cohort of 100 PRCC patients (Cohort3). This last cohort was not mentioned in the grant, but due to availability we also utilized this dataset in the current project.
3. We performed spatial transcriptomics platform on Cohort 2 (Nanostring CosMx for 1000 genes) and Cohort 1 (Vizgen Merscope for 500 genes). The rationale to perform spatial transcriptomics for the first two cohorts, compared to our proposal of mIF, was that it was a newer and promising assay that was introduced in May 2022 (after acceptance of our proposal) and instead of 30 markers, we would now profile substantially higher number of genes (500-1000), which would enable us to identify immune cell types more accurately. Both cohorts were assayed by the companies that build the assay (i.e. Nanostring, and Vizgen) as part of their pilot programs. We analyzed these datasets extensively and consulted with the assay companies and found that there was a very low yield in terms of the number of transcripts detected per cell. Figure 1A shows the data for cohort 2, the slides along the x-axis, and the samples along the y-axis. The colors and values in the heatmap represent the average number of transcripts per cell that was detected for each of these samples. We expected >200 transcripts per cell based on He et al. to be able to detect cell types. Our data from cohort 1 shows that only 5 of the 66 samples passed QC.

Figure 1A: Heatmap showing the average number of transcripts detected in each of the samples across the 3 slides (Cohort 1) assayed using Nanostring CosMx assay. Only 5 of the 66 samples passed QC (number of transcripts per cell > 200)

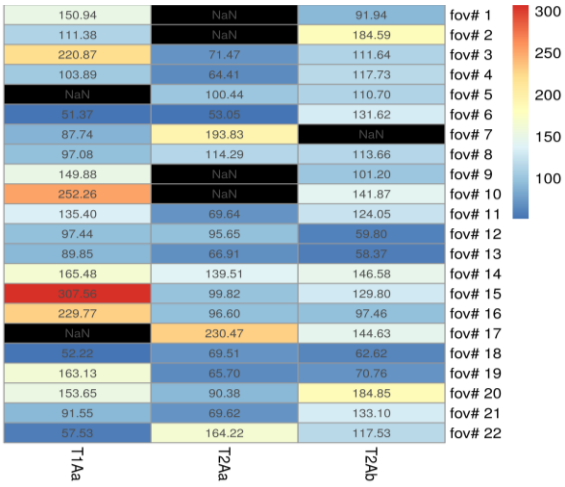
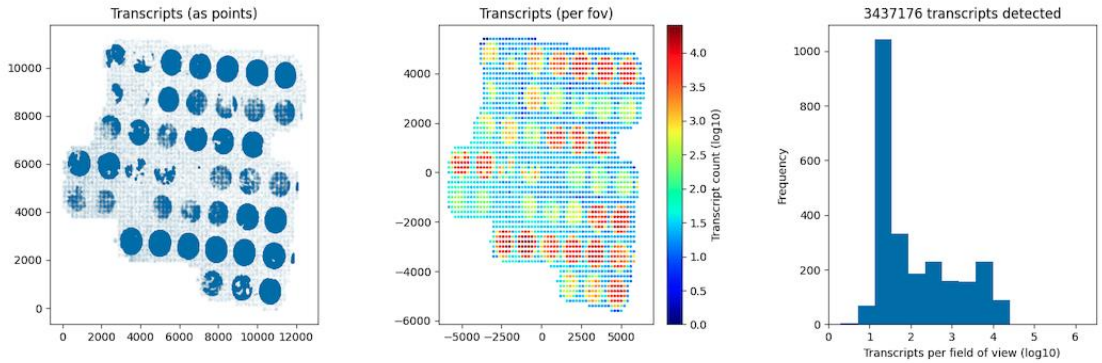


Figure 1B: Distribution showing the number of transcripts detected per cell using Cohort 1 assayed with Merscope. Majority of the cells have ~50 transcripts per cell which again resulted in failed QC for this assay.



4. We performed spatial proteomics on Cohort 3 using 31 markers and a multiplex immunofluorescence assay (Akoya Biosciences). This assay passed QC and we analyzed this data in step 2 below. The data was downloaded an organized on the google cloud platform.

Milestone for Major task 1 was achieved by May 2023.

Major Task 2: Modeling and prediction of immune cell types

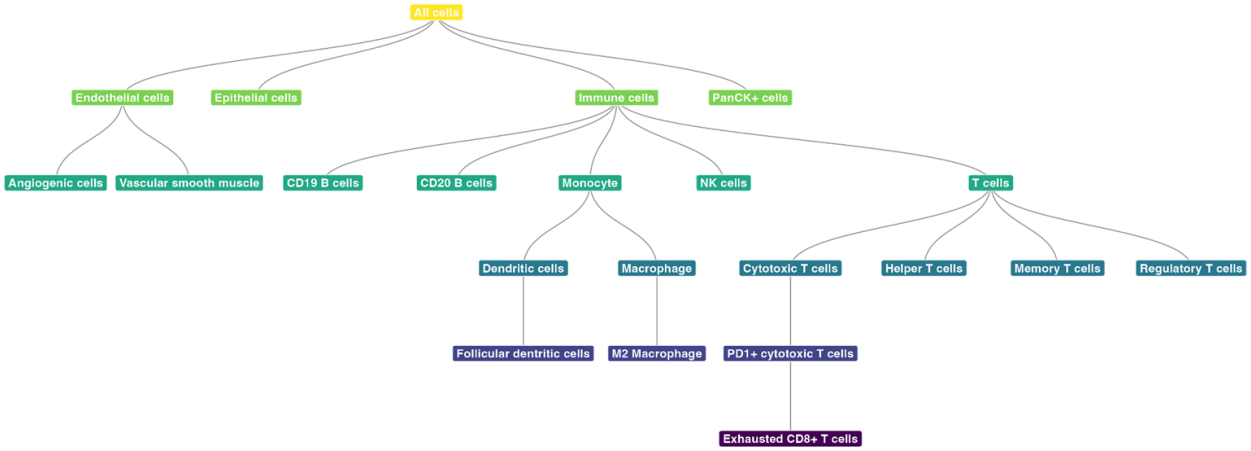
The mIF data from Cohort 3 passed QC and analyzed using the following steps. The mIF data from Cohort 3 passed collection QC and analyzed using the following steps. The TMA spots were identified from the larger whole-slide image with ~150 spots per slide and stored as individual 31-channel images; these were then used for cell segmentation using Cellpose. Cellpose is a neural network-based segmentation algorithm trained on diverse histological images that can efficiently identify cells using both nuclear and membrane masks. These segmentation images were then converted into ImageJ ROIs and each cell was measured for the following metrics: cellular area, mean fluorescence intensity, maximum fluorescence intensity, and XY coordinates. These were exported into tabular format and subsequent processing was performed in R.

These raw pixel intensity values were then transformed by square root normalization to compress them into a usable space and reduce variation. Due to the batch differences between the TMAs, we had to systematically select cutoff values for each slide that captured the bulk of the visually observable fluorescence and allow for the determination of individual cell types. These types were called based on a decision tree method, which allows for the *a priori* introduction of existing biological knowledge into the assignment of hierarchical cell types. For instance, immune cells are the known superset of T cells, B cells, myeloid lineages, etc. and by introducing a systematic calling procedure that integrates both the most specific and upstream markers, we can work to account for the inherent noise in these images.

For the assignment of individual cell types, we developed a novel algorithm to assign each individual cell a range of probabilities that it is a member of each individual class. This allowed us to both study the cellular neighborhoods based on the strict typing assigned to each cell as well as their potential identity as other cell types. This also works to counteract overconfidence in cell type assignment, which may mask rarer or less optimized calls of specific cell populations; for instance the high perceived expression of certain markers (like CDH1 or VIM) may lead to erroneous calls that may or may not be true representations of the underlying cellular identity; future optimization in image collection by our scientific partners and others in the community may help to minimize these challenges.

We then use both the strict and probabilistic type assignments to determine the cells in close physical proximity to each individual cell within each sample. This is a highly configurable function that also captures the neighborhood on the basis of individual markers, allowing for the discovery of further novel biology through the detection of marker expression that are either present in multiple cell types or are known to be non-specific or representative or more subtle cellular states (exhaustion, activation) rather than cell types *per se*. Using these three metrics (strict typing, probabilistic typing, and marker expression), we can define spatial neighborhoods for each cell, allowing us to conduct further spatial analysis, including clustering each cell type based on their spatial relationships.

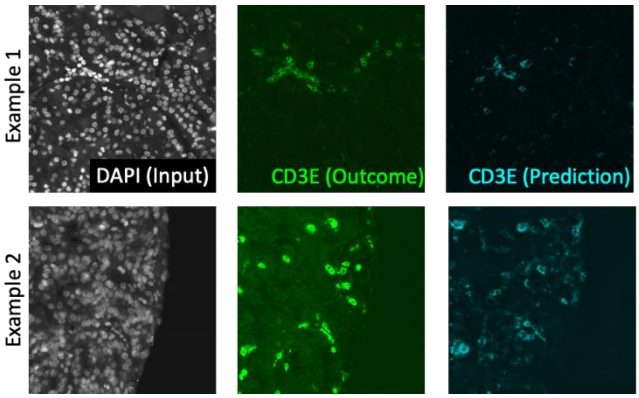
Our primary output from this analysis is the assignment of each cell to a cluster within its own type based on the cellular or marker composition of its neighborhood. Given the high dimensionality of these data (>2.5 million cells with >20 measures per cell), certain measures exceed sensible computational capacity (distance, correlation, covariance), requiring the application of other methods. Kmeans clustering is one widely used option that scales well with increasing dimensionality and is the basis of our ongoing developments. Our preliminary findings have uncovered a spatial link between $CD163^{+}$ macrophages and $CD4^{+}$ T cells and their association with poor prognosis.



Milestone for major task 2: 100% Achieved by August 2023

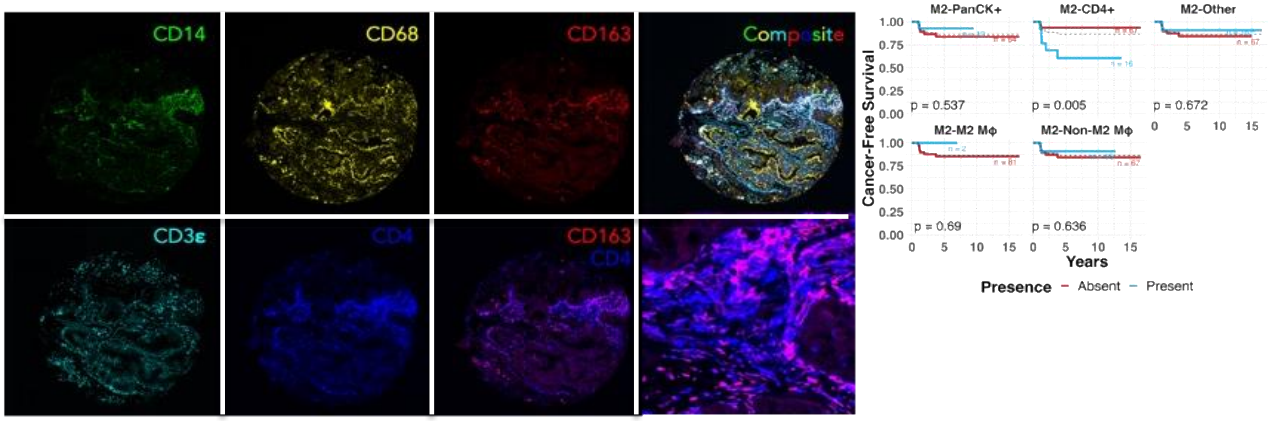
Major Task 3: Developing AI models for predicting response to immunotherapy.

For this task, we are currently developing AI models from the 100 patients in Cohort 3 to predict response to clinical variables, including recurrence and progression-free survival. We have developed an AI model that is able to detect features present in the nuclear stained images from cohort 3 to predict the distribution of various immune cells in tumor microarrays. This model builds on the widely accepted pix2pix architecture, where a generator produces "fake" images and a discriminator is trained to distinguish them from real images. Notably, the generator never receives the output image, instead learning entirely from the discriminator's classifications. To evaluate our model's performance, we will use quantitative metrics to compare the model's output to ground truth images. One such metric, known as the masked intensity ratio (MIR), compares the pixel intensity within a masked region outlining cell boundaries to the pixel intensity outside this mask.



In our proposal, we had planned to use H&E images for training the models. However, we ran into issues with co-registration of the H&E images assayed on serial sections with the mIF images. As a result, we have used the ubiquitous DAPI channel for this purpose. As a result of this challenge, we are validating our methods on 5 whole slide images, instead of publicly available data (TCGA, CPTAP).

Using these spatial clusters that we identified from Major tasks 2 and 3, we have identified biomarkers associated with various clinical phenotypes. We identified an interesting and previously undescribed association between CD163+ macrophages (nominally "M2" macrophages in the Mills classification scheme[13]) and CD4+ T cells as well as other myeloid populations expressing CD4. While CD4+ macrophages have been known for decades given their important role in HIV latency and therapeutic intractability, their role in kidney cancer remains largely unknown. Such associations between the expression of atypical cellular markers and existing cell populations are entirely unable to be identified based on bulk sequencing approaches and are likely to be discarded. We are currently validating this spatial biomarker in a set of additional whole slide images.



Milestone for Major Task 3: 80% complete. No Cost Extension filed till Jan 31, 2024.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to Report.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

The results of the initial image analysis using the Akoya Bioscience Codex platform were disseminated in conferences as:

1. Poster presentation at Annual American Association for Cancer Research Conference in April 2023
2. Invited talk at the Kidney Cancer Research Summit in July 2023
3. Invited talk at the North Carolina Next Generation Sequencing Intellectual Exchange Group (NGS-IEG) (October 2023)
4. The work was presented at the Quantitative Methods in Life Sciences Symposium (May 2023)
5. This work will be presented at the ASCO-GU Symposium in January 2024

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

During the next reporting period, we will prepare the manuscript for publishing our findings in the project in a peer reviewed journal.

4. **IMPACT:** D*escribe 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?
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).

We have developed a number of innovative approaches in this project:

1. Spatial proteomics analysis and calling cell types using a novel probabilistic tree-based method
2. Deep learning methods to predict immune cell types from nuclear stains in kidney cancer
3. Spatial biomarkers associated with clinical recurrence

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

The findings from our work will move the field in two main ways:

- Our novel algorithm enables the discovery of previously unknown association between certain cell populations
- The new biology will give rise to the discovery of new targets and biomarkers in kidney cancer.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

After the completion of the project, we will have developed new AI methods to predict response to therapy in kidney cancer. The methods used can be applied to any other cancers as well, thus making an impact on the entire field of cancer research. Our analysis methodology also allows the analysis of histopathology images in novel ways providing additional biological insights.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

The discovery of new associations between cell populations and patient survival will enable the discovery of biomarkers of response, thus allowing new treatment strategies based on histopathology images alone. This will have a significant impact on patient treatments in kidney cancer.

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official 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:*

We were delayed in our project due to the following reasons:

1. Due to the failure of the spatial transcriptomics assays
2. Due to problems with co-registering H&E images with the mIF images

As a result, we applied for a no cost extension of the project till Jan 31, 2024.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

We were delayed in the submission of the manuscript for the reasons mentioned above. We are currently planning to complete the manuscript and submit to a peer-reviewed journal by January 2024.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report.

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

Nothing to report.

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals

Significant changes in use of biohazards and/or select agents

Nothing to report.

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. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Conference Presentation: Beckermann K, Haake S, Nesta A, Caponegro M, Nirmala NR, Reddy A. Abstract 6622: Developing spatial molecular correlates of response to immunotherapy in kidney cancer. *Cancer Res*. 2023;83(7_Supplement):6622. doi:10.1158/1538-7445.AM2023-6622

  Peer-reviewed article: Brewer, J., Vento, J, Haake S, Reddy, A. Spatial proteomics enable identification of prognostic biomarkers in papillary RCC. *Manuscript under preparation*.

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Invited Talk: SLAS 2023 Data Sciences and AI Symposium, November 2023: Spatial Proteomics Enables Identification of Prognostic Biomarkers in Kidney Cancer – Invited talk by Anupama Reddy.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

We developed several novel approaches for the analysis of image data such as:
cell segmentation,
quality control,
data normalization,
cell typing,
spatial analysis and
unsupervised spatial clustering and deep learning methods for virtual staining of cells.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name: Anupama Reddy, PhD
Project Role: PI
Nearest Person Months worked: 1
Contribution to Project: Overall supervision and guidance on the project

Name: Jared Brewer, PhD
Project Role: Data Scientist
Nearest Person Months worked: 5
Contribution to Project: Data scientist performing the spatial proteomics analysis and deep learning

Name: Alex Nesta, PhD
Project Role: Data Scientist
Nearest Person Months worked: 2
Contribution to Project: Data scientist performing the spatial transcriptomics analysis

Name: Dr. Scott Haake
Project Role: SubAward PI
Nearest Person Months worked: 1
Contribution to Project: Provided kidney cancer TMAs and guidance on the project

Name: Dr. Katy Beckermann
Project Role: Clinician Researcher
Nearest Person Months worked: 1
Contribution to Project: Provided kidney cancer TMAs and guidance on the project

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to Report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
<u>Organization Name:</u>
<u>Location of Organization: (if foreign location list country)</u>
<u>Partner's contribution to the project</u> (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Vanderbilt University Medical Center

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) should be updated and submitted with attachments.*

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*