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TITLE: Quantifying In Situ Adaptive Immunity in Human Tubulointerstitial Lupus Nep

PRINCIPAL INVESTIGATOR: Marcus Clark

CONTRACTING ORGANIZATION: The University of Chicago

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Fort Detrick, Maryland 21702-5012

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Distribution Unlimited

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**6. AUTHOR(S)**

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**5d. PROJECT NUMBER**

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

The University of Chicago
5801 S Ellis Ave
Chicago, IL 60637

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U.S. Army Medical Research and Development Command
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**12. DISTRIBUTION / AVAILABILITY STATEMENT**

Approved for Public Release; Distribution Unlimited

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**

The specific goals of this grant are to use high-dimensional (16 primary antibodies or more) multicolor confocal microscopy to describe the frequency and spatial distributions of the different types of immune cells infiltrating the lupus kidney (Aim 1). We will then use machine learning approaches, including CDM3, to quantify the true complexity of inflammation and identify functional relationships between different T cell populations and the cells that are presenting antigen, and therefore activating, pathogenic T cells (Aim 2). By understanding which cells, functional relationships and mechanisms are associated with progression to renal failure, we will both identify new therapeutic targets and the patients for which such therapies are likely to be efficacious.

**15. SUBJECT TERMS**

Lupus, lupus nephritis, tubulointerstitial inflammation, adaptive immunity, B cells, human, confocal microscopy, deep machine learning

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**TABLE OF CONTENTS**

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

   From original grant: The specific goals of this grant are to use high-dimensional (16 primary antibodies or more) multicolor confocal microscopy to describe the frequency and spatial distributions of the different types of immune cells infiltrating the lupus kidney (Aim 1). We will then use machine learning approaches, including CDM3, to quantify the true complexity of inflammation and identify functional relationships between different T cell populations and the cells that are presenting antigen, and therefore activating, pathogenic T cells (Aim 2). By understanding which cells, functional relationships and mechanisms are associated with progression to renal failure, we will both identify new therapeutic targets and the patients for which such therapies are likely to be efficacious.

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

   Lupus, lupus nephritis, tubulointerstitial inflammation, adaptive immunity, B cells, human, confocal microscopy, deep machine learning

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.*

   **Aim 1: We hypothesize that the relative frequencies and distributions of the main cellular components of immunity (ex., pDCs, T cell subsets, NK cells) in TII will define different mechanistic subsets.** We postulate that the cellular mechanisms driving TII will vary between patients for a given degree of TII.
   **Aim 2: We hypothesize that different adaptive cell networks will characterize different degrees of lupus TII.** We postulate that severe TII will be associated with additional and more complex adaptive cell mechanisms.

   **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.*

**Aim 1.** With the pandemic, we focused our efforts on improving the computational pipeline to provide accurate segmentation of different cell classes. One of the major technical insights that we had this year was that using separate Mask R-CNNs, in series and in parallel was the preferred way to construct an analytical pipeline. This approach was optimal for several reasons including being able to use optimal training sets for each specific task. In order to refine our analytical pipeline, we concentrated on two datasets, including a six color confocal microscopy dataset from 55 patients in which renal outcome was known. Below is a description of the initial analysis of the longitudinal cohort. In addition, we have done extensive analysis of high-dimensional images from a cross-sectional cohort of 18 patients. These studies, which were within the scope of the present DOD grant, are reported in bioRxiv ( https://biorxiv.org/cgi/content/short/2021.09.03.458909v1). However, as the longitudinal data demonstrates the potential impact of $CDM_4$, and where the project will go next, these data are provided in detail below.

Lupus nephritis is often characterized by chronic and intense inflammation in which it is difficult to accurately identify and segment specific cells using standard approaches due to the density of cells and the high levels of structured background signal. Therefore, we trained deep convolutional neural networks (CNNs) to perform automatic cell detection, classification, and segmentation (collectively known as instance segmentation) on the HR dataset. To achieve optimal performance across all imaged cell classes, we split the five-class cell detection into two tasks: instance segmentation of lymphocytes and instance segmentation of DCs (**Fig 1A**). For each task, a separate instance of a region-based CNN architecture, Mask R-CNN, was independently trained (**Fig 1B**). Each Mask R-CNN was trained on 246 manually segmented images with a validation set of 65 manually segmented images used for hyperparameter tuning. On a test set of 30 images from patients unique to the training and validation data, the lymphocyte detection network had an F1-score of 0.75 and the DC detection network of 0.57 while the overall F1-score for detection of all 5 cell classes was 0.74. Visual comparison of manual truth and predictions in individual ROIs revealed excellent concordance (**Fig. 1C**). By implementing deep CNNs, we achieve rapid and accurate whole-cell segmentation and classification.

Automatic cell segmentations were first used to describe and quantify the spatial distribution all five cell classes in the image dataset. Comparison of overall cell densities (total cells/ROI) in ESRD- patients and ESRD+ patients revealed no significant differences (**Fig 2A**). However, the total cell count per sample was higher in the ESRD+ cohort, reflecting larger overall areas of inflammation (**Fig 2B**). In contrast to overall cell density, there were apparent differences in the cellular constituents of inflammation between the two patient cohorts (**Fig 2C-G**). Surprisingly, ROIs from ESRD- patients had higher densities of B cells relative to ROIs from ESRD+ patients ($p=1.23x10^{-7}$, **Fig 2C**). In contrast, ROIs from ESRD+ patients had increased densities of CD4- T cells ($p=3.40x10^{-15}$, **Fig 2D**). There were no significant differences in the densities of CD4+ T cells, pDCs, or mDCs between patient cohorts (**Fig 2E-G**).

Although there were fewer ESRD+ patients, they had more ROIs captured per biopsy. To mitigate any effect from these cohort and individual class imbalances, we performed a bootstrapping analysis. The pools of ESRD+ and ESRD- ROIs were iteratively sampled with replacement 1000 times to produce samples of 200 ROIs from each group (ESRD+ and ESRD-). The distribution of mean cell densities between ESRD+ and ESRD+ patients revealed distinct, non-overlapping peaks for both B cells and CD4- T cells (**Fig. 2H-I**). In contrast, there was substantial overlap in the cell

densities between ESRD+ and ESRD- patients for CD4+ T cells, pDCs and mDCs (**Fig. 2J-L**). The 95% confidence intervals of the difference in means between ESRD+ and ESRD- patients revealed for both B cells and CD4- T cells did not cross zero (data not shown). In contrast, the difference in means for the other cell types did cross zero (data not shown). These data indicate that the observed differences in B cell and CD4- T cell densities between ESRD+ and ESRD- patients are robust. From these data we conclude that high B cell densities are associated with a good prognosis while high densities of CD4- T cells are associated with progression to renal failure.

When we examine these densities on the patient level, we observe that patients with high CD4- T cell densities, B cell densities tend to be low (**Fig 2M**). As indicated by point size, these tended to be ESRD+ patients with higher TI chronicity scores. The converse appeared true, as patients with higher B cell densities tended to have low TI chronicity scores and be ESRD-. These data suggest that lupus TII is associated with two or more distinct inflammatory states, each associated with a different prognosis.

By applying our analytical pipeline (Cell Distance Mapping, version 4, $CDM_4$) to a longitudinal cohort, we have moved beyond the original aims of this grant to demonstrate the validity of our approach in stratifying patients based on outcome and to identify underlying associated pathogenic mechanisms. These and other results are contained in a paper that has been submitted to BioRxiv (bioRxiv:https://biorxiv.org/cgi/content/short/2021.09.03.458909v1).

**Aim 2.** Aim 2 is focused on understanding how the organization of immune cells in the kidney defines different pathogenic states. We have now conducted relevant initial analyses. Furthermore, by putting these findings in the context of prognosis, we have moved beyond the original grant to define clinically relevant immune cell architectures.

We next explored the relative *in situ* spatial relationships between the different immune cell classes. First, for every cell in the dataset, we identified the nearest neighbor using centroid to centroid distances. CD4+ and CD4- T cells were significantly more likely to have a B cell as their nearest neighbor in ESRD- biopsies ($p=1.00x10^{-36}$ and $p=6.23x10^{-19}$, respectively) (**Fig 3A**). In contrast, CD4+ T cells and B cells were significantly more likely to have a CD4- T cell nearest neighbor in ESRD+ biopsies ($p=7.53x10^{-29}$ and $p=6.35x10^{-9}$, respectively) (**Fig 3B**). Additionally, both B cells and CD4- T cells showed a strong propensity for co-localization with cells of the same type ($p=1.09x10^{-8}$ and $p=1.75x10^{-9}$, respectively).

Higher order local cellular organization was then probed by grouping cells into spatially discrete neighborhoods. DBSCAN, a density-based clustering algorithm, was implemented to group cells into discrete neighborhoods defined by a maximum intercellular centroid-centroid distance. Variation in this maximum distance between 50 and 150 pixels resulted in a range of neighborhood sizes varying between those that contained just a few cells (50 pixels) to those that encompassed large areas of inflammation (150 pixels) (**Fig 3C,** data not shown). A maximum distance of 100 pixels (~10.6 µm) was selected, as this distance approximates a cell body and appeared to resolve observable regional behavior across the dataset.

Using this 100-pixel cutoff and a minimum neighborhood size of two, DBSCAN detected 4022 cell neighborhoods in the dataset. Each neighborhood was quantitatively characterized by a set of 24

features including cell type frequency, cell type proportion, ratios of cell types, average cell shape features, and the overall area of the neighborhood (data not shown). K-means clustering was then applied to define classes of neighborhoods. The neighborhoods were split into six classes, as determined ideal by bootstrapping cluster descriptors including the within cluster sum of squares (WCSS) and the delta WCSS (data not shown). The test score from a leave-one-out t-test approach was used to determine which features or combination of features best distinguished the six neighborhood groups (**Fig 3D**). The most distinctive feature(s) for each group was used to describe the cell neighborhoods as follows: 1) B cell enriched cluster, 2) CD4- T cell enriched cluster, 3) Large, lymphocyte enriched cluster, 4) CD4+ T cell enriched cluster, 5) mDC enriched cluster, and 6) pDC enriched cluster (**Fig 3E**).

Tertiary lymphoid structures (TLSs) have been previously identified in the context of lupus nephritis. Although we cannot explicitly define TLSs in this dataset, we hypothesized that the large, lymphocyte enriched neighborhoods might approximate TLSs. For example, we noted that within this group, 28.6% of the cells were B cells and 48.3% were CD4+ T cells. Furthermore, 96.1% of these neighborhoods met the following criteria: 1) contained at least 20 cells, 2) both B cells and CD4+ T cells were represented in the neighborhood and 3) at least 50% of all cells were B cells and/or CD4+ T cells. Therefore, the vast majority of large, lymphocyte enriched neighborhoods, have features consistent with TLSs.

We then examined how these six classes of neighborhoods were distributed between the ESRD- and ESRD+ patients. After normalizing for the number of ROIs captured for each patient, ESRD- and ESRD+ patients had no difference in their total neighborhood count per ROI (**Fig 3F**). However, ESRD+ patients had significantly higher prevalence of CD4- T cell enriched neighborhoods relative to the ESRD- patients ($p = 0.043$) (**Fig 3G**). The per ROI prevalence of the other classes of neighborhoods did not correlate with renal outcome (data not shown**)**. We next examined if the CD4- neighborhoods differed between the ESRD-, ESRD+ and ESRD current patient groups (**Fig 3H**). ESRD+ and ESRD current patients had a statistically higher prevalence of neighborhoods from the CD4- cluster than ESRD- patients ($p=0.03$, $p=0.009$, respectively). These data demonstrate that, on a per patient basis, the prevalence of small, CD4- T cell enriched neighborhoods demonstrates the strongest association with progressive renal disease.

These and other results are contained in a paper that has been submitted to BioRxiv (bioRxiv:https://biorxiv.org/cgi/content/short/2021.09.03.458909v1).

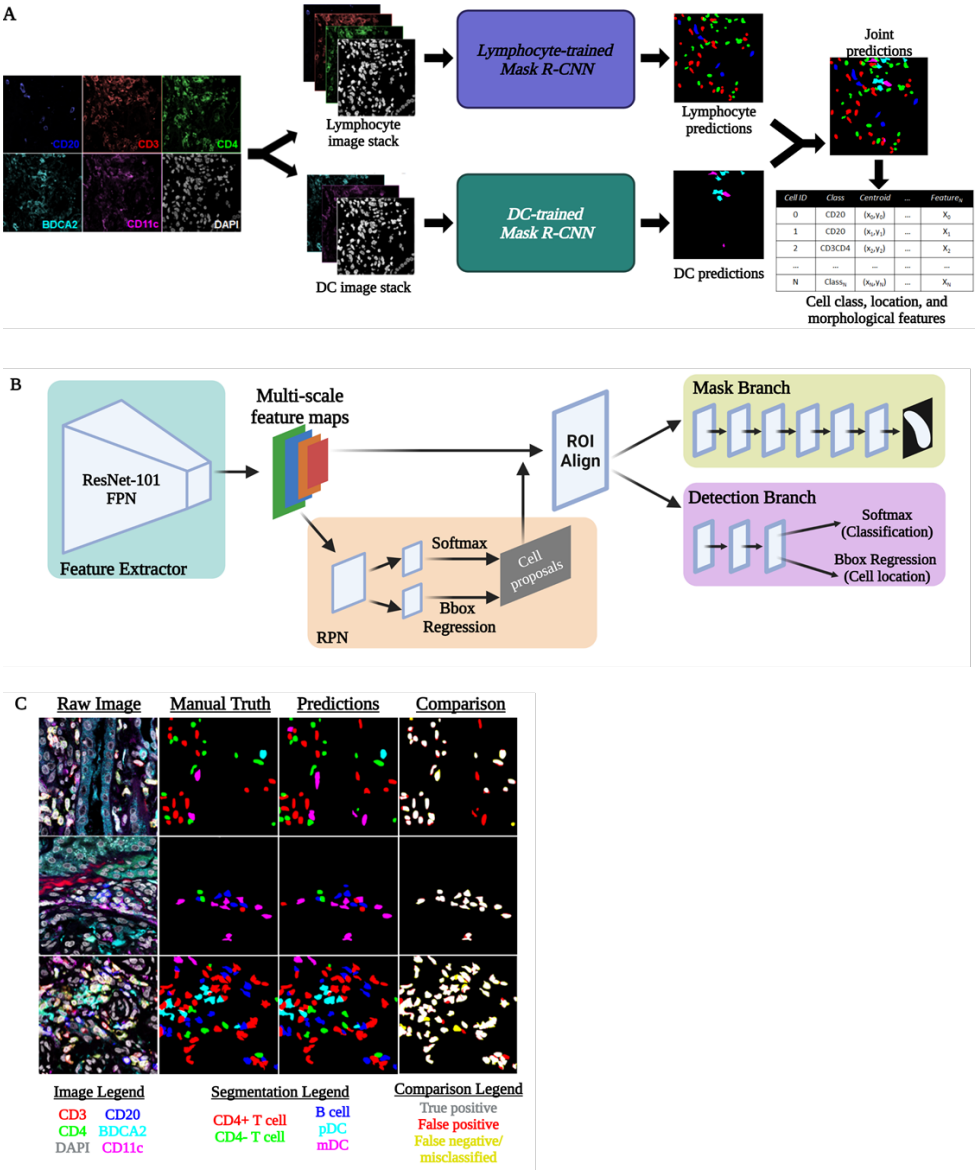



**Figure 1. Instance segmentation of immune cells in high-resolution fluorescence microscopy images of LN kidney biopsies.** A) Automatic instance segmentation of five immune cell classes was performed by combining predictions from two instances of Mask R-CNN: one trained to segment CD20+, CD3+CD4-, and CD3+CD4+ lymphocytes and one trained to segment pDCs and mDCs. Cell location, class, and morphological features were calculated from joint predictions. B) The Mask R-CNN architecture is comprised of a ResNet Feature Pyramid Network (FPN) backbone used for feature extraction, a region proposal network (RPN) used to generate cell proposals, and two parallel branches used for 1) semantic segmentation (mask branch), and 2) classification (softmax layer) and localization (bounding box (Bbox) regression) of cell proposals. C) Representative segmentations produced by the multi-network pipeline showed strong agreement with the expert-defined manual segmentations.

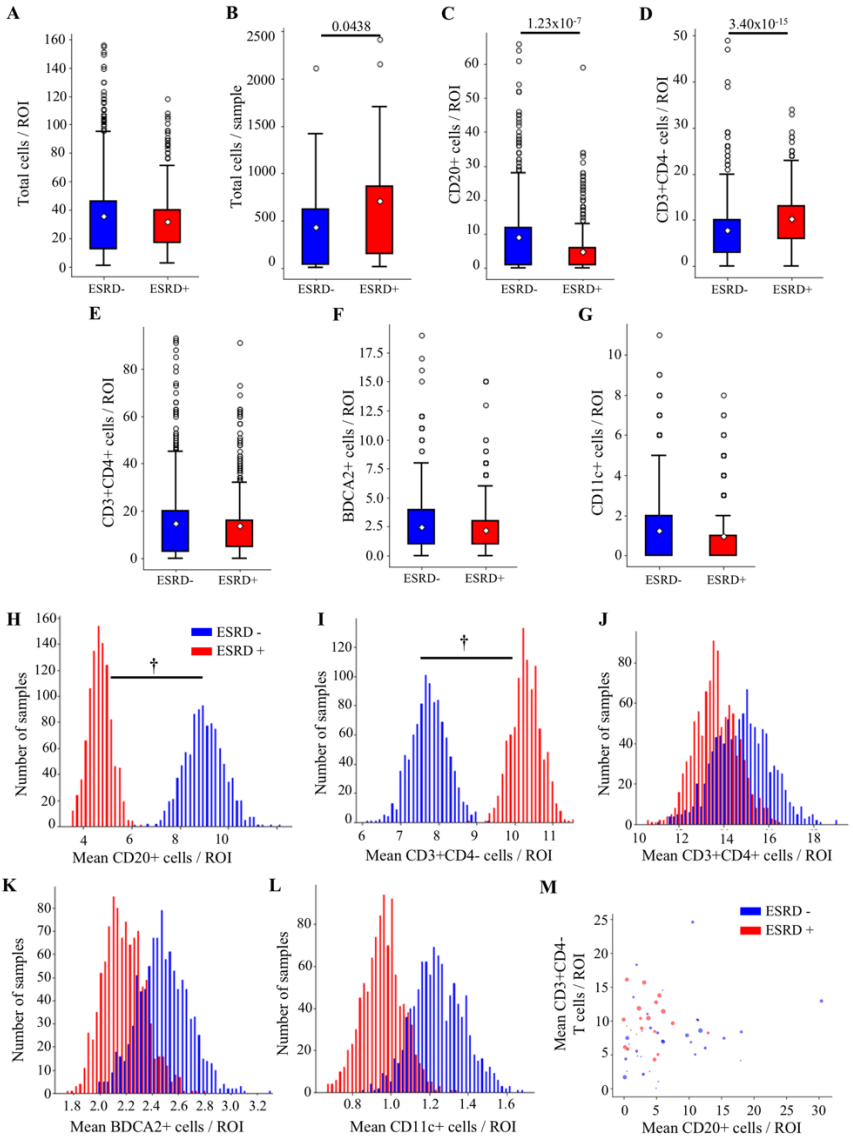



**Figure 2. Higher CD4- T cell density and lower B cell density associated with progression to ESRD.** Difference in the number of indicated cell classes per ROI between patients who progressed to ESRD (ESRD+, n=428 ROIs) and those who do not (ESRD-, n=437 ROIs) for A) Total cells per ROI across the HR dataset (p=0.318), B) Total cells per patient sample, C) CD20+ cells per ROI, D) CD3+CD4- cells per ROI, E) CD3+CD4+ cells per ROI, F) BDCA2+ cells per ROI, G) CD11c+ cells per ROI. All cell density comparisons were done with a Mann-Whitney U Test with a Bonferroni correction for multiple comparisons. Significant p values noted in the plots. Bootstrapped sample means of ESRD+ (red) and ESRD-(blue) for H) CD20+ cells per ROI, I) CD3+CD4- cells per ROI, J) CD3+CD4+ cells per ROI, K) BDCA2+ cells per ROI, and L) CD11c+ cells per ROI. Average B cell and CD4- T cell count per ROI for each patient biopsy is shown in (M). Point size is weighted by the TI chronicity score for each patient. († 95% confidence interval does not overlap with 0)

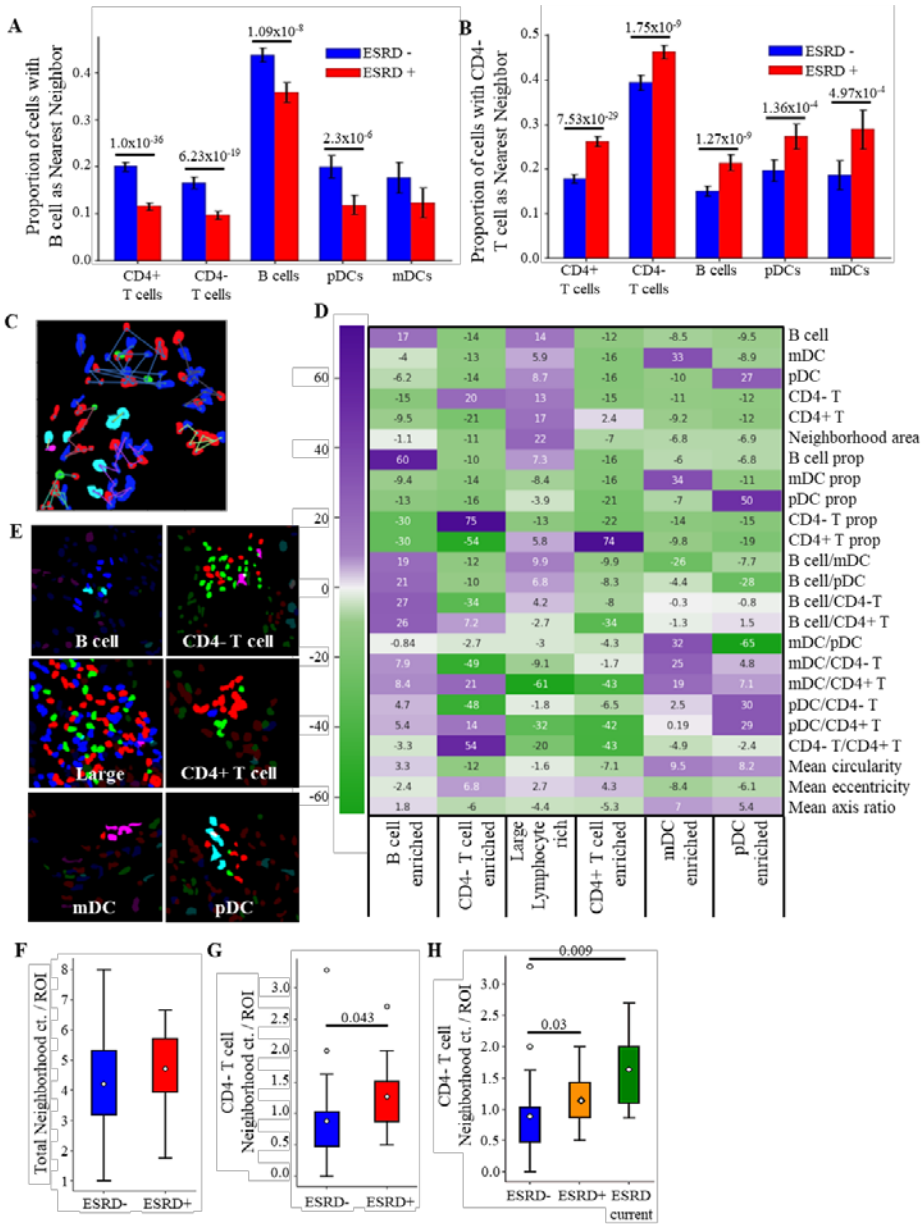



**Figure 3. Specific cellular neighborhoods associated with renal failure.** Proportions of cells that have A) CD3+CD4- T cells and B) CD20+ B cells as nearest neighbors in ESRD+ and ESRD- patients (chi-squared test for independence with Bonferroni correction for multiple comparisons, significant p values noted in the plots). C) Representative neighborhoods detected by the DBSCAN with a distance metric of $\leq$ 100 pixels. D) Heatmap showing test statistics for each features from leave-one-out t-tests used to define six types of cell neighborhoods. E) Representative neighborhoods of each cluster. The abundance of neighborhoods between the patient cohorts, normalized by the number of ROIs per patient, is compared by Mann-Whitney U Test, with a Bonferroni correction for F) all cell neighborhoods, G) CD4- T cell neighborhoods; A 3-group comparison for CD4- neighborhoods, splitting the ESRD+ population into ESRD+ and ESRD current patients is shown in (H). Significant p values noted in the plots.

**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.*

With the pandemic, our ability to participate in conferences and workshops has been limited. 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.*

Nothing to Report.

**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."*

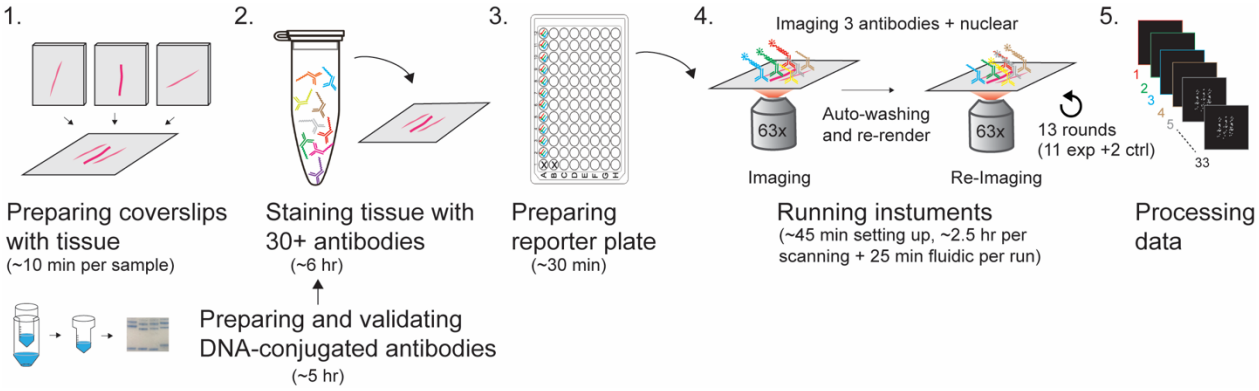
*Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.*

As demonstrated in this report, we have developed a robust pipeline ($CDM_4$), capable of identifying probably pathogenic and prognostic markers in relatively small patient cohorts. In this coming year, we plan to adapt $CDM_4$ to high dimensional confocal micrographs. We have already begun this process by validating that transfer learning can be done in which trained data sets obtained from conventional confocal microscopy are applied to high dimensional datasets.

In contrast to our initial set of hypotheses, which focused on antigen presentation to CD4+ T cells, we found that CD8+ T cells and other related T cell populations were likely to be pathogenic.

Therefore, we have shifted the emphasis of our work away from antigen presentation and towards characterizing these non-CD4+ T cell populations.

The second goal is to develop more high-throughput methods for staining renal tissue in high-dimension. Our initial experiments were done on a CaliberID, using manual stripping and reprobing. The resulting data set was biologically illuminating and critical for the development of our current analytic suite, $CDM_4$. However, this imaging approach is too low-throughput. Therefore, we have switched to the CODEX platform. The optical stage of the CODEX package is not of high quality and is not suitable for the high resolution imaging needed to capture functional adaptive cell networks. Therefore we have purchased and installed a custom set up which consists of a Leica DMi8 microscope, Dragonfly 202 spinning disk confocal with ZYLA camera and five lasers, and the CODEX microfluidic system. Over this next year, we plan to validate a panel of approximately 32 antibodies that can be used to probe renal tissue and that are compatible in the CODEX system. We are particularly interested in further characterizing the CD4- T cell population described above and determine the mechanisms by which they potentially are damaging the kidney in lupus nephritis. Provided below is a schematic of the CODEX workflow.




***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).*

In lupus nephritis, moderate or severe inflammation is a strong predictor of who will ultimately fail conventional therapy and progress to renal failure. However, within this group, some people have progressive disease and some do not. This suggests that renal inflammation is heterogeneous and that some inflammatory states are worse than others. In recent work, we have demonstrated at least two different inflammatory states can exist in the lupus kidney. In those patients with progressive disease, their intrarenal inflammation is enriched for small clusters of CD4- T cells that include

CD8+ T cells, γδ T cells and what have been referred to as double negative T cells. In contrast, in those patients who do well they have a relative enrichment in B cells. These data provide a new way to stratify lupus nephritis patients and point to new therapeutic opportunities. In particular, our data suggest that therapies targeting T cells might be efficacious in some patients.

**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.*

Our results demonstrate that in our model human disease, lupus nephritis, a quantitative understanding of in situ inflammation reveals novel pathogenic mechanisms not previously appreciated in studies of blood. Furthermore, we provide a new computational pipeline that, for the first time, can accurately segment and identify immune cells in complex inflammatory environments. Our data provide an approach that likely can be applied to the study of other autoimmune diseases.

**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.*

Nothing to Report.

**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.*

Nothing to Report.

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:*

**Changes in approach and reasons for change**
*Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.*

Nothing to Report.

**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.*

The pandemic forced us to focus on the computational aspects of the project. That said, we were able to make excellent progress.

**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**
*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**

N/A

**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).*

Artificial Intelligence and Cellular Segmentation in Tissue Microscopy Images. Durkee MS, Abraham R, **Clark MR**, Giger ML. Am J Pathol. 2021 Jun 12:S0002-9440(21)00261-3. doi: 10.1016/j.ajpath.2021.05.022. Online ahead of print. Yes, federal support

Quantifying the effects of biopsy fixation and staining panel design on automatic instance segmentation of immune cells in human lupus nephritis.
Durkee MS, Abraham R, Ai J, Veselits M, **Clark MR**, Giger ML. J Biomed Opt. 2021 Jan;26(2):022910. doi: 10.1117/1.JBO.26.2.022910. PMID: 33420765 Free PMC article.
Yes federal support

Cellular aspects of the pathogenesis of lupus nephritis. Chang A, **Clark MR**, Ko K. Curr Opin Rheumatol. 2021 Mar 1;33(2):197-204. doi: 10.1097/BOR.0000000000000777. PMID: 33394604. Yes, federal support

Machine Learning to Quantify *In Situ* Humoral Selection in Human Lupus Tubulointerstitial Inflammation. Kinloch AJ, Asano Y, Mohsin A, Henry C, Abraham R, Chang A, Labno C, Wilson PC, **Clark MR.** Front Immunol. 2020 Nov 27;11:593177. doi: 10.3389/fimmu.2020.593177. eCollection 2020. PMID: 33329582. Yes, federal support.

In lupus nephritis, specific in situ inflammatory states are associated with refractory disease and progression to renal failure. Abraham R, Durkee M, Ai J, Veselits M, Casella G, Asano Y, Chang

A, Ko K, Oshinsky C, Peninger E, Giger M, Clark MR.
bioRxiv: https://biorxiv.org/cgi/content/short/2021.09.03.458909v1. Yes, federal support.

**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.*

Nothing to Report.

- **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.*

As described below, over the last year we have developed a novel computational pipeline which has been reported in submitted manuscripts.

- **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.*

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| As described above, we have developed a novel computational pipeline, $CDM_4$, which has been reported in submitted papers. |

## 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".*

*<u>Example:</u>*

| | |
|---|---|
| *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.)*

No change.

**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.*

Relevant to this grant is the following new application:

Alliance for Lupus Research (Clark) 12/1/20-11/30/23
*In situ* adaptive immunity in Lupus tubulointerstitial inflammation

This grant focuses on using CDM for identifying prognostic states and potential therapeutic targets in longitudinal lupus nephritis cohorts. It constitutes a logical extension of the present DOD grant which was focused on initial development of computational tools and application to cross-sectional cohorts. There is no budget overlap.

**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:*
*Organization Name:*
*Location of Organization: (if foreign location list country)*
*Partner's contribution to the project (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.*

| |
|---|
| Nothing to report. |

**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://ers.amedd.army.mil*** *for each unique award.*

**QUAD CHARTS:** *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) 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.*