

AWARD NUMBER: W81XWH-22-1-0112

TITLE: REGALS: Regulatory T Cells in Amyotrophic Lateral Sclerosis

PRINCIPAL INVESTIGATOR: Dr. Neil Shneider, MD, PhD

CONTRACTING ORGANIZATION: Columbia University Medical Center, New York, NY

REPORT DATE: OCTOBER 2023

TYPE OF REPORT: ANNUAL

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

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

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1. REPORT DATE OCTOBER 2023		2. REPORT TYPE ANNUAL		3. DATES COVERED 30SEPT2022 - 29SEPT2023	
4. TITLE AND SUBTITLE REGALS: Regulatory T Cells in Amyotrophic Lateral Sclerosis				5a. CONTRACT NUMBER W81XWH-22-1-0112	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Dr. Neil Shneider, MD, PhD E-Mail: ns327@cumc.columbia.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Columbia University Medical Center 630 W 168TH ST FL 4 NEW YORK NY 10032-3725				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)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
13. SUPPLEMENTARY NOTES					
14. ABSTRACT: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease affecting motor neurons (MNs) with the typical time from symptom onset to death of 2-3 years. The majority of ALS cases are sporadic. ALS, once considered to involve MNs exclusively, is now known as a multi-cellular and multi-systemic disorder in which MN degeneration is driven not only by cell autonomous mechanisms, but also by glial cell pathology and a confluence of other pathogenic mechanisms such as inflammation. Pathological studies have found immune abnormalities in the central nervous system (CNS) and also in the blood and cerebrospinal fluid (CSF) of subjects with ALS. Additionally, inflammation and immune abnormalities are also found in animal models of ALS. However, how the immune system is activated and what are the targets of these cytotoxic immune cells are not clear. Currently there are no meaningful treatments for ALS. In an effort to treat this aggressive form of ALS, we have organized an expanded access, compassionate use program on four sporadic ALS patients at the Eleanor and Lou Gehrig ALS Center at Columbia University in collaboration with Cellenkos Inc. This pilot clinical trial leverages cord blood-derived (CB) regulatory CD4+ T cell (Treg) therapeutic approach designed to halt ALS progression. Preliminary studies in this pilot clinical trial reported a slowing of the ALSFR-S slope decline rate from 3.0 per month to 0.7 per month associated with a decrease in inflammatory cytokines in the CSF.					
15. SUBJECT TERMS NONE LISTED					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 16	19a. NAME OF RESPONSIBLE PERSON USAMRDC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

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- 1. 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?

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.

	Timeline	
Specific Aim 1: A phase I single arm, open label safety study of CK0803 in patients with sporadic ALS		
Major Task 1: Regulatory approval	Months	
Subtask 1: HRPO approval	-6 to 0	complete (OHRO approval 6/5/2023)
Subtask 2: Consent and enrollment ALS subjects	1-24	Ongoing
Subtask 3: Determine the safety, tolerability and treatment limiting toxicities (TLTs) of CK0803	1-36	Ongoing
Specific Aim 2: Longitudinal analysis of the transcriptome and clonality of the immune response in the paired CSF and the blood before and after CK0803 therapy.		
Major Task 2: Analyze CSF and blood samples by single-cell RNA/TCR-seq	Months	
Subtask 1: Longitudinally collect CSF and blood before and after treatment	1-36	Ongoing
Subtask 2: Process samples using 10x Genomics kit	1-36	
Subtask 3: Submit samples to the core for sequencing	1-36	
Submit amendments, adverse events and protocol deviations as needed	As Needed	
Coordinate with Sites for annual IRB report for continuing review	Annually	
Coordinate with Sites for job descriptions design	1-4	
Subtask 4: Analyze computationally RNA and TCR sequences	1-48	
Subtask 5: Coordinate with Sites for flow chart for all study steps, web data collection and database requirements	4-8	
Subtask 6: Associate transcriptomic data with clinical disease	36-48	
Subtask 7: Public deposition of omics data	48	
Local IRB Approval	1	complete (05/23/2023)

Specific Aim 3: Longitudinal Biomarker analysis of paired CSF and the blood before and after CK0803 therapy.		
Major Task 3: Analysis of CSF and serum by Luminex and Quanterix	Months	
Subtask 1: Collect and cryopreserve CSF and serum	1-36	
Subtask 2: Identify healthy control samples from our biobank	3-4	
Subtask 3: Analyze samples by Luminex	36-48	
Subtask 4: Analyze samples by Quanterix	36-48	
Subtask 5: Correlate Luminex data with clinical data	36-48	
Coordinate with Sites for flow chart for all study steps, web data collection and database requirements	4-8	

What was accomplished under these goals?

For this quarterly reporting period only 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.

- The first subject was screened on August 3rd, 2023 and has received five doses of CK0803 as of October 12, 2023.
- The second subject was screened on October 10, 2023, and will receive their first dose of CK0803 on October 26, 2023.
- No serious adverse events or treatment-limiting toxicities have been identified to date.
- CSF and PBMC cells from enrolled ALS patients were collected and submitted for single-cell RNA and T cell receptor sequencing. Remaining PBMCs were cryopreserved.
- Biofluid samples (serum and CSF) were collected and frozen for future biomarker analysis.

Describe the Regulatory Protocol and Activity Status (if applicable).

Describe the Protocol and Activity Status for sections a-c, as applicable, using the format described for each section. If there is nothing significant to report during this reporting period, state "Nothing to Report."

(a) Human Use Regulatory Protocols

TOTAL PROTOCOLS:

1 human subject research protocol will be required to complete the Statement of Work

PROTOCOL(S): List the identifier and title for all human use protocols needed to complete the project. Include information about the approved target number for clinical significance, type of submission, type of approval with associated dates, and performance status.

The following format shall be used:

Protocol (of total):

Protocol [HRPO Assigned Number]:

Title:

Target required for clinical significance:

Target approved for clinical significance:

Submitted to and Approved by:

Provide bullet point list of protocol development, submission, amendments, and approvals (include IRB in addition to HRPO).

Status:

Report (i) progress on subject recruitment, screening, enrollment, completion, and numbers of each compared to original planned target(s), e.g., number of subjects enrolled versus total number proposed; (ii) amendments submitted to the IRB and USAMRMC HRPO for review; and (iii) any adverse event/unanticipated problems involving risks to subjects or others and actions or plans for mitigation.

TOTAL PROTOCOLS: 1

PROTOCOL (1 of 1 total):

Protocol [HRPO Assigned Number]: AAAU3358

Title: REGALS: Regulatory T Cells in Amyotrophic Lateral Sclerosis

Target required for clinical significance: 66

Target approved for clinical significance: 66

SUBMITTED TO AND APPROVED BY:

- Columbia University Medical Center Institutional Review Board/Human Research Protection Office

STATUS:

- (i) Number of subjects recruited/original planned target: 2/12
Number of subjects screened/original planned target: 2/12
Number of patients enrolled/original planned target: 2/12
Number of patients completed/original planned target: 0/12

- (ii) Report amendments submitted to the IRB and USAMRMC HRPO for review:
None since local IRB approval received on 5/23/2023

- (iii) Adverse event/unanticipated problems involving risks to subjects or others and actions or plans for mitigation:
None

(b) Use of Human Cadavers for Research Development Test & Evaluation (RDT&E), Education or Training

TOTAL ACTIVITIES:

No RDT&E, education or training activities involving human cadavers will be performed to complete the Statement of Work (SOW).

ACTIVITIES: *Provide the following information in a bulleted list for all RDT&E, education or training activities involving human cadavers conducted or supported during the quarter:*

- *Title of the RDT&E, education or training activity*
- *SOW task/aim associated with the activity*
- *Date the activity was conducted*
- *Identification of the organization's responsible individual (e.g., PI or individual primarily responsible for the activity's conduct)*
- *Brief description of the use(s) of cadavers in the activity and the total number of cadavers used during the reporting period*
- *Brief description of the Department of Army organization's involvement in the activity*
- *Status of document submission and approvals*
- *Problems encountered in the procurement, inventory, use, storage, transfer, transportation and disposition of cadavers used for RDT&E, education or training. Examples of problems include but are not limited to: loss of confidentiality of cadaveric donors, breach of security, significant deviation from the approved protocol, failure to comply with state laws and/or institutional policies and public relations issues.*

TOTAL ACTIVITIES: 0

ACTIVITIES: N/A

(c) Animal Use Regulatory Protocols

TOTAL PROTOCOL(S): 0

No animal use research will be performed to complete the Statement of Work.

PROTOCOL(S):

List the identifier and title for all animal use protocols needed to complete the project. Include information about the approved target number for statistical significance, type of submission, type of approval with associated dates, and performance status.

The following format shall be used:

Protocol (of total):

Protocol [ACURO Assigned Number]:

Title:

Target required for statistical significance:

Target approved for statistical significance:

Submitted to and Approved by:

Provide bullet point list of protocol development, submission, amendments, and approvals (include IACUC in addition to ACURO).

Status:

Provide bullet point list of performance and/or progress status relating to the above protocol and discuss any administrative, technical, or logistical issues that may impact performance or progress of the study (e.g. animal use protocol needs revision to minimize animal suffering, animal protocol modification to include additional staff) for the above ACURO approved protocol.

TOTAL PROTOCOL(S): 0

PROTOCOL (of total): N/A

Protocol [ACURO Assigned Number]:

Title:

Target required for statistical significance:

Target approved for statistical significance:

SUBMITTED TO AND APPROVED BY:

STATUS:

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

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

2. Products: List any products resulting from the project during the reporting period. If there are no products to report for the current quarter, state "Nothing to report."

Examples of products include:

- *publications, conference papers, and presentations;*
- *website(s) or other Internet site(s);*
- *technologies or techniques;*
- *inventions, patent applications, and/or licenses; and*

- *other products, such as data or databases, biospecimen collections, germplasm, audio or video products, software, models, educational aids or curricula, instruments or equipment, data and research material, clinical or educational interventions, or new business creation.*

Nothing to report

3. Participants & Other Collaborating Organizations

What individuals have worked on the project?

Provide the following information for: (1) Project Directors (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).

Provide the name and identify the role the person played in the project. Indicate the nearest whole person month (Calendar, Academic, Summer) that the individual worked on the project. Show the most senior role in which the person worked on the project for any significant length of time. For example, if an undergraduate student graduated, entered graduate school, and continued to work on the project, show that person as a graduate student, preferably explaining the change in involvement.

Describe how this person contributed to the project. If information is unchanged from a previous submission, provide the name only and indicate “no change.”

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.*

*Name: Neil Shneider MD, PhD
Project Role: PI
Researcher Identifier (e.g. ORCID ID): ORCID ID 0000-0002-3223-7336
Nearest person month worked: 2
Contribution to Project: Dr. Shneider has performed work in the areas of study start-up, logistical planning, and recruitment, and has provided medical oversight of all study activities.*

*Name: Wassim Elyaman, PhD
Project Role: Sub-Investigator
Researcher Identifier (e.g. ORCID ID): ORCID ID 0000-0001-7238-374X
Nearest person month worked: 2
Contribution to Project: Dr. Elyaman has performed work in the areas of preparation for sample collection and analysis by single-cell RNA/TCR-seq, Luminex and Quanterix*

*Name: Julia Yasek, NP
Project Role: Sub-Investigator
Researcher Identifier (e.g. ORCID ID): n/a
Nearest person month worked: 3
Contribution to Project: Ms. Yasek has performed work in the area of subject recruitment, and providing oversight of study visits.*

Name: Jess Singleton
Project Role: Research Manager
Researcher Identifier (e.g. ORCID ID): n/a
Nearest person month worked: 1
Contribution to Project: Ms. Singleton has performed work in the areas of regulatory management, study start-up, and logistical planning, as well as study coordination and management.

Name: Angelica Ortiz
Project Role: Study Coordinator
Researcher Identifier (e.g. ORCID ID): n/a
Nearest person month worked: 3
Contribution to Project: Ms. Ortiz has performed work in the areas of study start-up and logistical planning, conducting all study visits, and data entry.

Name: Hanien Amer
Project Role: Technician
Researcher Identifier (e.g. ORCID ID): n/a
Nearest person month worked: 1
Contribution to Project: Ms. Amer has performed work in the areas of preparation for sample collection and analysis by single-cell RNA/TCR-seq, Luminex and Quanterix

Name: Samuel Levy
Project Role: Student
Researcher Identifier (e.g. ORCID ID): n/a
Nearest person month worked: 4
Contribution to Project: Dr. Levy has performed work in the areas of preparation for sample collection and analysis by single-cell RNA/TCR-seq, Luminex and Quanterix

Name: Marisha Liliah
Project Role: Technician
Researcher Identifier (e.g. ORCID ID): n/a
Nearest person month worked: 1
Contribution to Project: Dr. Liliah has performed work in the areas of preparation for sample collection and analysis by single-cell RNA/TCR-seq, Luminex and Quanterix

- 4. Changes/Problems:** The 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:

a. Actual Problems or delays and actions to resolve them

Provide a description of current problems or issues that may impede performance or progress of this project along with proposed corrective action. Also 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.

For an award that includes the recruitment of human subjects for clinical research or a clinical trial, discuss any problems or barriers encountered, if applicable, and what has been done to mitigate those issues. Discussion may highlight enrollment problems, retention problems, and actions taken to increase enrollment and/or improve retention.

Nothing to report

b. Anticipated Problems/Issues

Provide a description of anticipated problems or issues that have a potential to impede performance or progress. Also provide course of actions planned to mitigate problems or to take should the problem materialize.

5. Special Reporting Requirements:

Quad Charts: If applicable, the Quad Chart (available on <https://www.usamraa.army.mil>) should be updated and submitted with attachments.

Treg DoD Report data

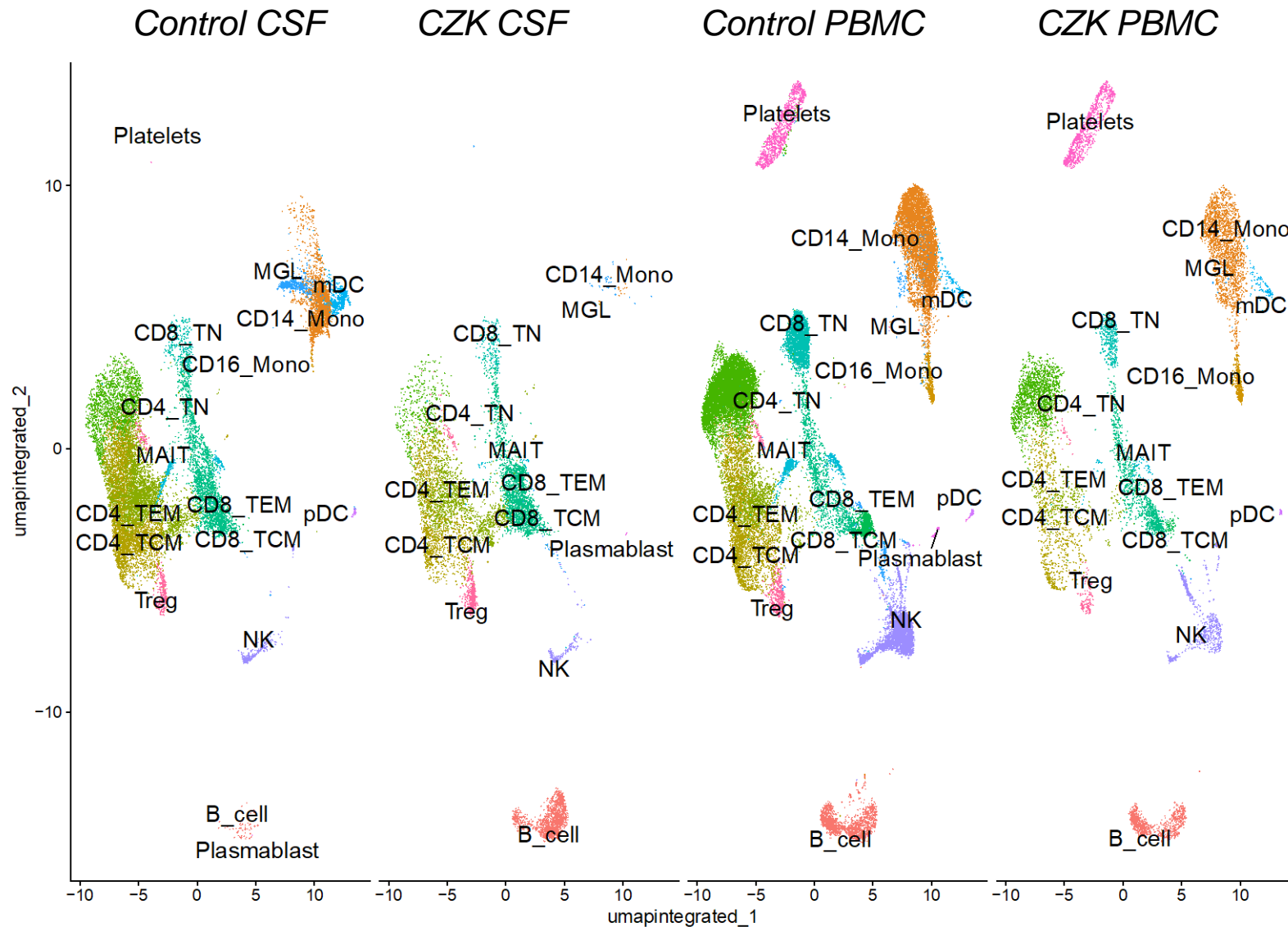
22 January 2024

Olivia Rifai

Patient CZK characterization

- DoD-funded patient CZK had blood and CSF samples taken on 08/15/2024 as part of a screening visit before infusion (i.e., baseline sample). Paired PBMC and CSF samples were submitted for scRNA-seq and scTCR-seq.
- Data were analyzed to characterize patient baseline cell populations and T cell clonality as part of the analysis pipeline that will be used on further patient samples. Data were compared with paired PBMC and CSF data from four healthy controls.
- Analyses included:
 - Seurat UMAP clustering and marker analysis to identify immune cell subtypes
 - TCR (T cell receptor) analysis to quantify clonal expansion

Clustering of patient and control samples

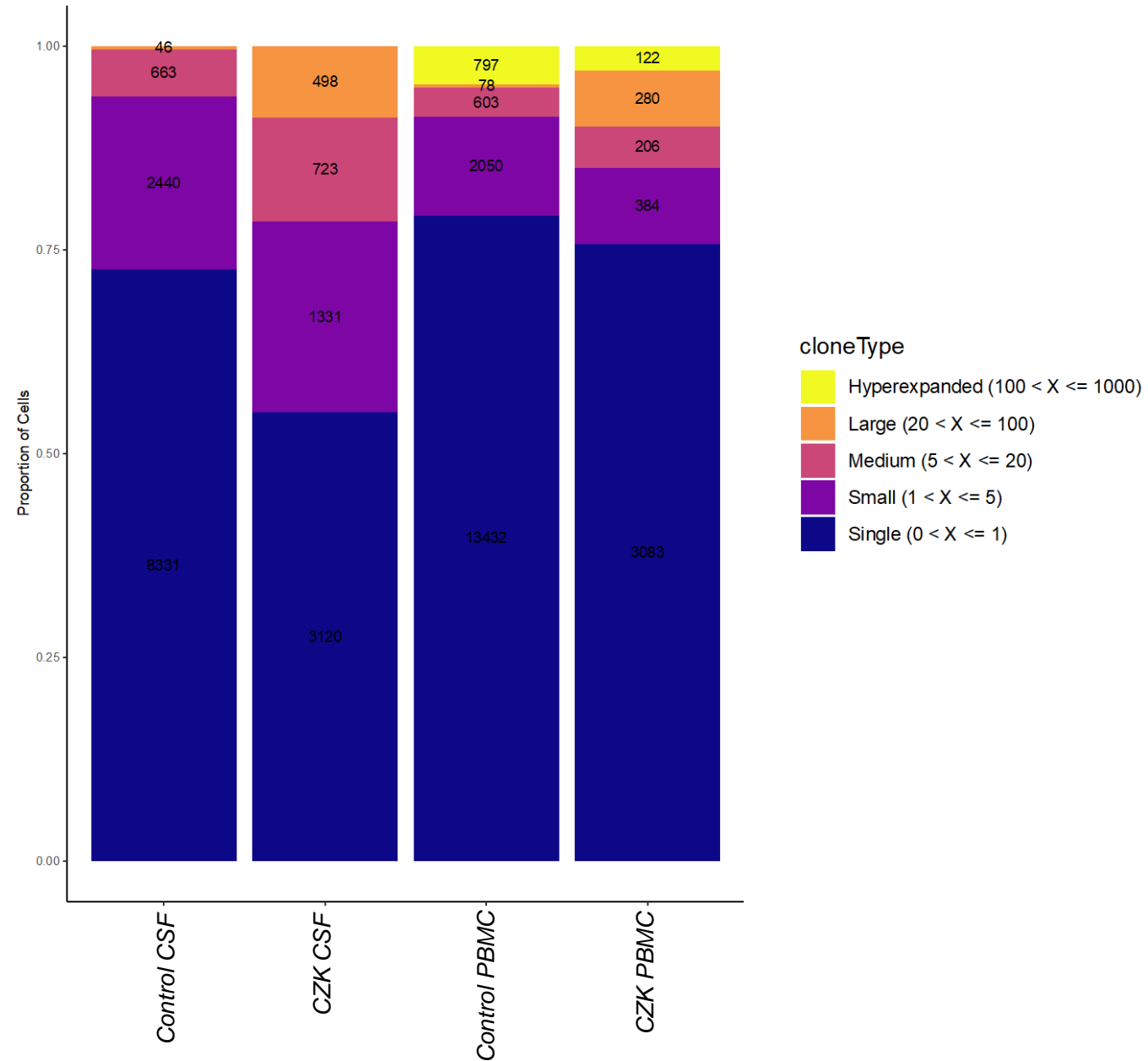


Data were integrated and clustered using Seurat in R and cell identities were assigned based on expression of a predefined list of cell type-specific marker genes.

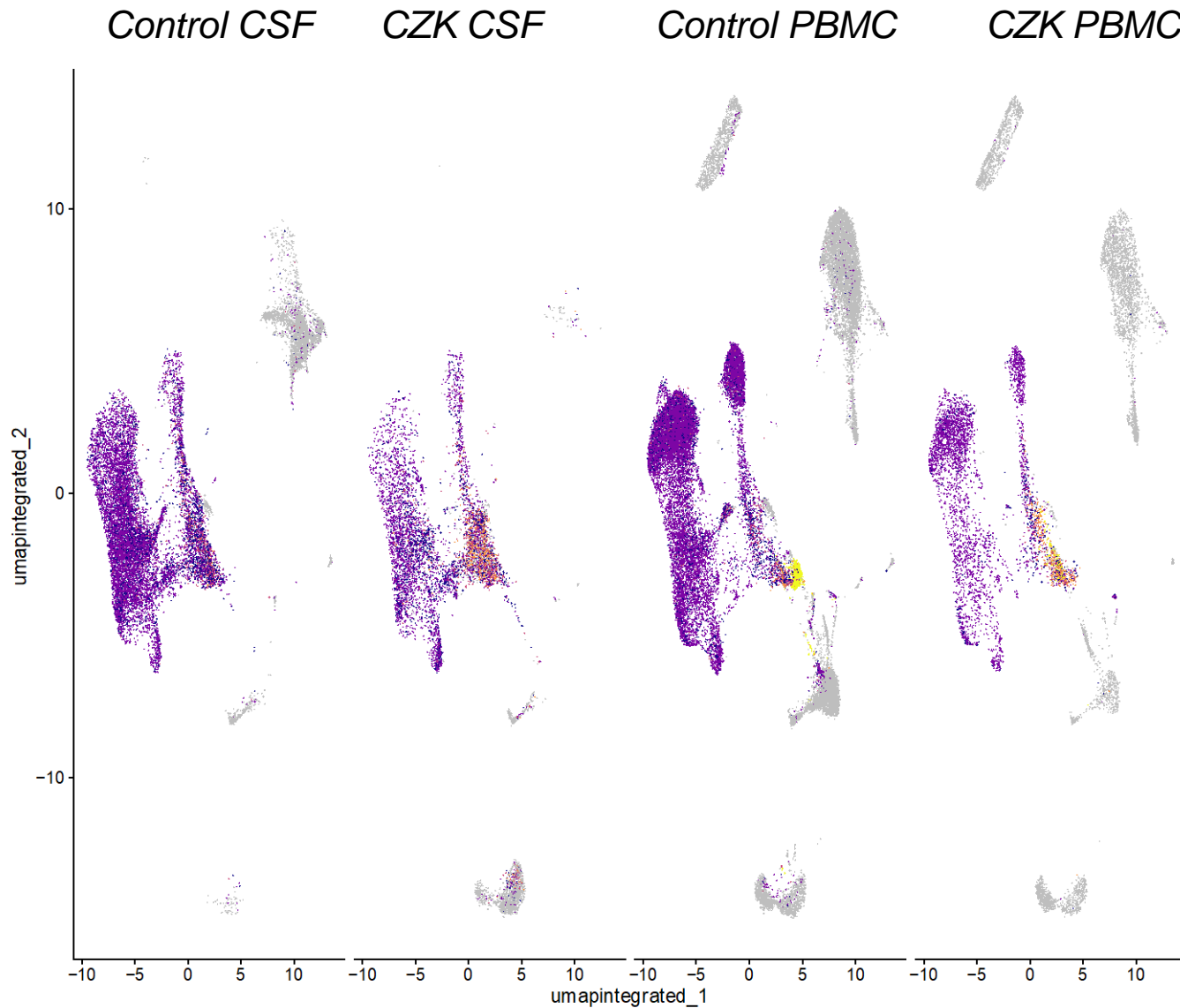
Clusters are shown on the right, stratified by group (Control, or CZK Baseline) and tissue (CSF, PBMC)

TCR clonality analysis

T cell receptor (TCR) sequences were evaluated for small (1-5), medium (5-20), large (20-100) and hyperexpanded (100-1000) clones in the CZK baseline samples compared to the control samples.



TCR clonality analysis



Clonal expansion levels were projected onto scRNA-seq UMAPs to determine which cell populations contained clonal expansion in each condition, showing expansion predominantly in CD8+ effector memory and central memory T cell populations (see ref in slide 3).

cloneType

- Hyperexpanded ($100 < X \leq 1000$)
- Large ($20 < X \leq 100$)
- Medium ($5 < X \leq 20$)
- Small ($1 < X \leq 5$)
- Single ($0 < X \leq 1$)

Conclusions and Next Steps

- Characterization of DoD-funded patient CZK baseline PBMC and CSF scRNA-seq and scTCR-seq data revealed apparent differences in clonal expansion in CD8+ T cell populations compared to a group of four healthy controls.
- The included images are part of our analysis pipeline that will be used on all future DoD-funded participants receiving CK0803 to monitor treatment-relevant changes.
- When the sample size is increased, statistical comparisons between groups measuring differences in immune cell proportions and differential expression of cell subtypes across the treatment course will also be possible.