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TITLE: Neuropathology and Immune Biomarker Discovery in a Rat Model of Alzheimer's Disease, TgF344-AD, with Single or Repetitive Traumatic Brain Injury

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14. ABSTRACT

Purpose: The major goals of this project were to develop better models to investigate mechanisms by which traumatic brain injury (TBI) is a risk factor for developing Alzheimer's disease (AD) and related neurodegenerative diseases, including chronic traumatic encephalopathy (CTE). To accomplish these goals, we used wild-type (WT) F344 control and the transgenic (Tg) AD rat model, Tg-F344-AD, to conduct and contrast two paradigms of closed-head controlled cortical impact TBI (i.e. 1X-CCI and 2X-CCI), and one paradigm of repetitive moderate blast TBI (i.e. rmTBI), after which cohorts of varying ages post-injury were studied for neuropathology and immune system changes in the periphery and CNS to determine their role in AD pathogenesis.
Scope: The study was limited to developing and characterizing these new TBI-AD models, and did not incorporate treatments or translational efforts for human clinical settings.
Major Findings: The 1X- and 2X-CCI experiments in 6-month-old animals did not induce detectable AD pathologies in the brain or in pathology-associated plasma biomarker profiles. However, experiments in 12-month-old TgF344-AD rats revealed that 2X-CCI induced maturation of diffuse plaques into β-sheet positive dense-cored plaques, coincident with induction of astrogliosis and tauopathy in the vicinity of the impact site, of which occurred about four months earlier than expected from previous characterizations of the TgF344-AD model (Cohen et al., 2013). These data indicate that TBI may not induce detectable AD pathologies, but that it may exacerbate or accelerate existing AD pathology, thus providing evidence of mechanisms that may underlie TBI's risk for the onset of clinical presentation of AD and related dementias (ADRD). These data also indicate that the 2X-CCI paradigm of TBI in aged TgF344-AD rats may be a useful model for further research studies examining the interrelationship between TBI and subsequent AD/ADRD for the military, Veteran, and civilian communities.

15. SUBJECT TERMS
Alzheimer's disease, AD, Aβ, amyloid, blood-brain barrier, chronic traumatic encephalopathy, CTE, closed-head injury, controlled cortical impact, CCI, gadolinium, mild cognitive impairment, MCI, neurofibrillary tangles, p-Tau, repetitive moderate TBI, traumatic brain injury

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1. **INTRODUCTION:**

The purpose of this project was to develop better models for investigating potential mechanisms by which traumatic brain injury (TBI) is a risk factor for developing Alzheimer's disease (AD) and related neurodegenerative diseases, such as chronic traumatic encephalopathy (CTE). To accomplish the project's objectives, we proposed to use wild-type (WT) control rats and a transgenic (Tg) rat model of AD to conduct, compare, and contrast two paradigms of closed-head controlled-cortical impact (CCI) TBI (CCI/TBI), and one paradigm of repetitive moderate blast TBI (rmTBI), after which cohorts of rats of varying ages post-injury were to be studied for neuropathological changes and for immune system changes within both the periphery and the central nervous system (CNS) to determine their potential role(s) in AD pathogenesis. Specifically, immune system changes were to be determined by examining changes in leukocyte populations, plasma protein biomarkers, and extracellular vesicle profiles. Neuroimaging was proposed to examine *in vivo* one-month post-injury chronic neuroinflammatory effects, together with confirmation by postmortem histochemical analyses. Notably, the study's scope was to be limited only to developing and characterizing these new TBI-AD rat models, and the project did not incorporate any treatment regimens or any other translational efforts towards use in a human clinical setting.

2. **KEYWORDS:**

Alzheimer's disease, AD, Aβ, amyloid, blood-brain barrier, chronic traumatic encephalopathy, CTE, closed-head injury, controlled cortical impact, CCI, gadolinium, mild cognitive impairment, MCI, neurofibrillary tangles, p-Tau, repetitive moderate TBI, traumatic brain injury

3. **ACCOMPLISHMENTS:**

- **What were the major goals of the project?**
  - To develop new models of AD, CCI/TBI, and rmTBI.
    - Milestone target date of 36 months, which included four subtasks: 1) induce 1X- and 2X-CCI (76% completed), 2) induce rmTBI (0% completed), 3) collecting and analyzing neuroimaging data (35% completed), and 4) collecting and analyzing post-mortem neuropathology data (46% completed). Also, milestones for obtaining local IACUC approval at three months and ACURO approval at six months were achieved.
  - To identify new peripheral immune system biomarkers associated with AD in CCI/TBI and rmTBI-AD.
    - Milestone target date of 36 months (46% completed).
  - To identify novel neuroimmune signaling biomarkers associated with AD in CCI/TBI and rmTBI-AD.
    - Milestone target date of 36 months (0% completed).
- **What was accomplished under these goals?**
  - **Major activities:** The major activities of the project included the breeding and aging of AD and WT control rats until either 6 or 12 months, at which age the animals underwent either two paradigms of closed-head controlled cortical impact TBI (i.e. 1X-CCI and 2X-CCI), or one paradigm of repetitive moderate blast TBI (i.e. rmTBI). There were a total of 600 experimental animals included in this project, of which 360 were assigned to the CCI procedures and 240 to the rmTBI procedures. The updated Statement of Work (SOW) is included below in Appendix 1 and shows that 274 animals completed the 1X- and 2X-CCI procedures. However, because there were no rmTBI experiments performed, and because their total of 240 animals were used in the updated SOW to calculate percentages completed, we included an alternative SOW in Appendix 2 to show percentages completed for just the CCI cohorts. Thus, the 274 animals that completed the study comprise 76% of those Subtasks. We have also included below in Appendix 3, the tables that show exactly which cohorts remain. As we have reported previously, issues with generating enough animals to conduct these TBI experiments impacted the early part of the project, and as described in detail below in Section 5, the pandemic greatly inhibited our ability to complete project goals. Although we were unable to complete any animals in the rmTBI cohorts for various reasons described in previous reports and below in this report, the rmTBI part of this project was in fact the catalyst for perhaps one of this project's greatest achievements, in that a dedicated TBI blast room has now been constructed within the University of Colorado Anschutz vivarium, which will have a lasting impact in the scientific field by

enabling these blast types of research projects to be performed on our campus for years to come. More discussion on this achievement is provided below in the "Other Achievements" part of this Section 1.

Additional major activities of this project included MRI neuroimaging and proteomic analyses of blood sample components, in an attempt to measure and potentially identify specific biomarkers that may correlate with TBI damage or the progression of AD pathologies. Below in Appendix 4, we have included some MRI results of the 42 animals that underwent imaging. As described below, we are not confident that MRI neuroimaging generates reliable data that can provide live *in vivo* data as a proxy for determining leakage of the blood brain barrier (BBB). For instance, preliminary 1X-CCI data suggest that AD animals had increased BBB leakage, whereas there were no significant differences between any of the 2X-CCI groups, and possibly even a diminutive trend in the 2X-CCI WT animals, as compared to WT sham animals. These MRI data do not correlate with the histochemical pathology data of the project. However, as described below, ongoing immunohistochemical analyses of these animals' brain tissues will clarify whether or not CCI does in fact cause damage to the BBB. Also, with many of the 30-day post-CCI cohorts not being performed yet, including all 6-month-old 2X-CCI cohorts, these preliminary results and our conclusions of MRI not being very useful may change.

Similar to the MRI results, our analyses of blood plasma biomarkers using the Meso Scale Discovery (MSD) proinflammatory cytokine panel also did not reveal any reliable data towards being useful in monitoring TBI damage or AD disease progression (see Appendix 5). Again, we conclude that we are not confident that these proteomic analyses will be very useful in new research experiments using our TBI-AD model. In addition to these MSD results, we also do not have confidence that we can reliably isolate and measure brain-derived exosomes in these rat models. The field of exosomes in still in its infancy, and we have not yet been able to identify specific rat brain exosome markers or to validate procedures for their isolation. As such, we are continuing to work with human blood samples to validate neuronal, astrocytic, and microglial exosome isolation procedures, and we chose not to perform non-validated exosome isolation procedures on the biobanked rat plasma samples, until we are confident that these procedures are reliable.

- **Specific objectives:** The overall objectives for all three major goals were dependent upon performing the sham, CCI/TBI, and blast rmTBI procedures, and then aging the animals to scheduled time points, perfusing them, and collecting tissues, including blood processing procedures for obtaining plasma and leukocyte populations. Thereafter, these tissues (i.e., brain and blood components) were examined for neuropathology and both peripheral and CNS immune effects due to the three TBI paradigms in an AD versus control setting, to identify potential mechanism(s) by which TBI is a risk factor for AD and related dementias, and to determine which was the optimal TBI paradigm, including animal TBI model, age, and time point post-injury wherein the findings were best elucidated.

- **Significant results or key outcomes:** The major findings of this project are that 2X-CCI in 12-month-old TgF344-AD rats led to exacerbation or acceleration of AD amyloid pathology and the early induction of tau pathology, which was paralleled by the global activation of astrocytes. As we reported previously in our quarterly report on 12/16/2020, immunohistochemical analyses by our collaborator, Dr. Natalia Vergara, demonstrated within eye tissues through co-staining with the immunohistochemical markers, 6E10 or 4G8, which label amyloid-beta (Aβ) species (including Aβ fibers, amorphous plaques, and dense-core plaques containing β-pleated sheet motifs), plus staining with specific dyes (Thio-S or NIAD-4), which only label β-pleated sheets, that there was increased Thio-S and NIAD-4 staining in the 2X-CCI animals. These results indicated that repeat moderate TBI may induce the maturation of amorphous Aβ plaques into dense-cored plaques with β-pleated sheets. These data also indicated that TBI, by itself, may not be sufficient to induce AD amyloid pathology, but that it might exacerbate or accelerate progression of existing AD amyloid pathology, since we did not observe any amyloid within the 2X-CCI 6-month-old cohorts. Our initial immunohistochemical analyses of brain tissues from 2X-CCI in 12-month-old TgF344-AD rats, using the 6E10 marker, did not reveal increased amounts of amyloid deposition. However, we did discover that there was global induction of astrogliosis and impact site-specific induction of tauopathy, which occurred earlier than had been characterized in the Tg-F344-AD rat model (Cohen et al., 2013). These results were presented at the 2020 Alzheimer's Association International Conference (AAIC) and are included below in Appendix 6. In subsequent analyses with our other collaborator, Dr. Mingxia Huang, we found that there was increased Thio-S staining in the 2X-CCI animals at specific time points after injuries (at 30 days and 60 days post-TBI), as compared to 12-month-old TgF344-AD sham procedure animals. Specifically, at the 30-day time point, we observed that the Thio-S plaque load was increased in the ipsilateral impacted hippocampus, as compared to the non-impacted contralateral hemisphere, thus indicating that repeat moderate TBI may also be inducing the maturation of amorphous Aβ plaques into dense-cored plaques with β-pleated sheets in the brain, similar to the results that we found in the eyes. In correlation with these amyloid results, we also found that 2X-CCI induced the expression of C-terminal binding protein (CtBP)-2, which is an early inflammation mediator after injury, in the brains at three days

post-TBI, as well as global induction of astrogliosis. These results were shown in our 3/15/2021 quarterly report. All of these results taken together indicate that repetitive moderate TBI induces the maturation of existing amyloid plaques and the local induction of tauopathy, along with global astroglial neuroinflammation that may be playing a significant role in these pathogenic processes. Ongoing additional analyses of these CNS tissues are expected to reveal further biological mechanisms that may underlie TBI as a risk factor for AD and ADRD, and we conclude that 2X-CCI in 12-month-old TgF344-AD rats is an appropriate new TBI model in which to conduct further TBI studies. Thus, these findings directly address one of the overarching FY16 PRARP CSRA challenges and provides evidence for a new research resource to counteract the paucity of research resources for examining the interrelationship between TBI and subsequent AD/ADRD for the military, Veteran, and civilian communities.

Additional key outcomes from this project include the use of these neuropathological findings as preliminary evidence for new grant applications and for peer-reviewed manuscripts. Both Dr. Vergara and Dr. Huang are in the revision process for resubmitting their manuscripts, and both have submitted NIH grant applications to continue these studies, which unfortunately did not get funded on their first submission, but are being resubmitted in Q1 2022.

- **Other achievements:** Perhaps the most important achievement of this project is that it initiated the construction of a blast room within the CU Anschutz vivarium, which is a critical infrastructure resource for all current and future CU Anschutz researchers who want to investigate blast-induced pathophysiological diseases, including other affected organ systems throughout the body, as well as in the CNS. Moreover, these new facilities allow for translational therapeutic studies of blast injuries, which require behavioral studies and/or animal imaging procedures to be conducted with the experimental animal cohorts. It should be noted that blast experiments were not previously possible in the CU Anschutz vivarium, because the very loud noise that comes from performing the blast procedures would affect numerous other animals in the vivarium, and therefore, any blast experiments were required to be performed only in designated research laboratories outside the vivarium. Accordingly, animal behavior experiments in blast studies were also not feasible, as the animal behavior rooms and testing apparatus reside within the vivarium, and because of strict quarantine procedures for reintroducing animals back into the vivarium space after they have been exposed to the outside environment. Furthermore, due to the 30-day required quarantine for reintroducing animals back into the vivarium, any small animal imaging (i.e., MRI, PET, etc.) studies could not be performed on blast-exposed animals until at least 30 days post-injury, thereby severely limiting research capabilities during the critical timeframe post-injury, in which interventions and therapeutics might be best employed. Currently, there are very few vivarium facilities within the United States, wherein these types of blast studies can be conducted. Therefore, with this project's grant being awarded in 2017 and coinciding with a planned vivarium expansion at CU Anschutz, a unique opportunity became available for us to develop collaborations with other interested research colleagues and to lobby for the special construction of facilities within the new vivarium space wherein blast experiments could be performed. We are happy to report here that construction of the blast room has already been finished and that planning for the additional construction of the noise attenuation chamber within the room is nearing completion, along with additional collaborating researchers joining these efforts. Thus, above all else, this project has generated a significant lasting accomplishment through its initiation and development of critical infrastructure, which is already showing success by attracting new researchers who are interested in conducting blast experiments within the new vivarium blast room, as well as strengthening their new grant applications and development of projects. These ongoing new research projects, and many others anticipated in the future, are expected to help generate valuable preclinical data needed for the translation of therapeutics into human trials, which will not only lead to advancements in medical research for battlefield blast injuries affecting soldiers, but also be applicable to Veteran and civilian communities as well, such as in studies investigating CTE and other AD related dementias.

- **What opportunities for training and professional development has the project provided?**
  - Nothing to Report
- **How were the results disseminated to communities of interest?**
  - Nothing to Report.
- **What do you plan to do during the next reporting period to accomplish the goals?**
  - Nothing to Report.

4. **IMPACT:**

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

  - As mentioned above in Section 3, the construction of the blast room within the new vivarium space is already beginning to have a positive effect on research focused on blast-induced injuries. New researchers have recently expressed interest in contributing to the final construction of the blast attenuation chamber to support their new research projects for which they are actively developing and writing grants. We are also working to continue this project's research into mechanisms underlying traumatic brain injury (TBI) as a risk factor for Alzheimer's disease (AD) and AD-related dementias (ADRD), including translational research for interventions that may ameliorate controlled cortical impact (CCI)- and blast TBI-induced pathogenesis. We expect that these new vivarium research facilities will continue to positively impact and enable expansion of the scientific field's base of knowledge, theory, and research in blast-induced damage to the central nervous system (CNS), as well as to other organ systems.

- **What was the impact on other disciplines?**

  - This project led to a research collaboration with Dr. Natalia Vergara to study TBI-induced changes in AD pathologies within the eyes of the 12-month-old Tg-F344-AD rats that had undergone 2X-CCI procedures. Immunohistochemical studies of the animal eyes revealed that 2X-CCI induces the maturation of amyloid plaques in the eyes, as demonstrated by evidence of dense core staining within diffuse amyloid accumulations, as compared to sham injured Tg-F344-AD rats, which was not observed by 2X-CCI in the WT rats. These data indicated that TBI, by itself, was not sufficient to induce AD amyloid pathology, but that it could exacerbate or accelerate the progression of existing AD amyloid pathology. These data from Dr. Vergara also correlated with our data showing that 2X-CCI induced tauopathy and astrogliosis at the CCI impact site in the 12-month-old Tg-F344-AD rats, which occurred earlier than had been characterized in the Tg-F344-AD rat model. With these findings, a manuscript is currently in preparation for peer review, and a grant was submitted to the NIH for expanded research into TBI-induced exacerbation of AD pathologies in the eye, as well as for testing proposed therapeutics. Although the grant was not awarded, favorable scoring has led to its revision and plans to resubmit in Q1 2022. As such, this project is impacting new research in the ophthalmic field, which is not typically studied in correlation with neurodegenerative diseases or with TBI and other injuries to the CNS. Thus, the findings from this project are helping address the overarching FY16 PRARP CSRA challenge of a paucity of research resources for examining the interrelationship between TBI and subsequent AD/ADRD for the military, Veteran, and civilian communities, and if the resubmitted grant is awarded, we are hopeful that one of the proposed therapeutic will provide positive and translatable data towards addressing the paucity of clinical studies for examining the interrelationship between TBI and subsequent AD/ADRD for the military, Veteran, and civilian communities.

- **What was the impact on technology transfer?**

  - Nothing to Report.

- **What was the impact on society beyond science and technology?**

  - Nothing to Report.

5. **CHANGES/PROBLEMS:**

- **Changes in approach and reasons for change**

  - Nothing to Report.

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

  - As noted in previous reporting, the SARS-CoV-2 pandemic greatly interfered with the performance of this project. The University of Colorado Anschutz Medical Campus was shut down at the beginning of the pandemic, which prevented any further CCI experiments from being performed. However, we were allowed to complete our work on the majority of the experimental animals that had already undergone TBI procedures. When personnel were allowed back on campus and laboratory activities could resume, personnel numbers were still reduced and social distancing rules mandated. Because at least two personnel are required to conduct the TBI experiments for the perfusion and post-mortem collection of tissues, including a dedicated person for timely blood processing procedures and a second person for perfusing the animals and collecting their tissues, the uncertainties of the duration of social distancing and reduced

personnel number mandates also impacted our decisions for when to resume conducting TBI procedures on new animal cohorts. The pandemic also hindered our ability to analyze tissues for neuropathology from animals that had already completed the study, because our laboratory shares the immunofluorescence microscope with other laboratories, who similarly had backlogged experiments waiting. Additional pandemic-related issues included impacts on animal husbandry, breeding, and generating sufficient colony numbers to resume TBI experiments, especially because a large colony size is required to have enough breeders producing offspring to generate progeny of correct genotypes for specified experimental groups and to then age them according to our protocol's procedures. As this is the final report of this project and there are no actions or plans that can currently resolve these ongoing pandemic issues, we do want to note that we have stored all of the tissues from animals that completed the study in the University of Colorado Alzheimer's and Cognition Center (CUACC) Biorepository, and which we are continuing to analyze and study. The data that we are generating from these experiments are being used to support new grant applications that will allow us to continue this research and pursue the remaining goals of this project. When laboratory conditions allow, we intend to resume TBI animal experiments and to complete many of the cohorts that remain (as shown in experimental animals tables in Appendix 3), and conduct blast TBI experiments when the new vivarium blast room and noise attenuation chambers are completely finished and available for use.

- **Changes that had a significant impact on expenditures**
  - As just mentioned above, this project requires the breeding and maintenance of large animal colonies to generate enough progeny of the correct genotype, which then need to be aged to 6 and 12 months before TBI procedures can be performed on them. As such, a large number of animals that are not be used in the study are generated, as well as per diem costs for the ones that are selected for these aging time periods, which together generate a significant amount of expenditures to resume and complete this project's experiments. As we have previously reported and can be seen in the Quad Chart below (Appendix 7), we have supplemented this project's expenditures with other funds that were available to our laboratory, and that were raised through philanthropy and other sources. We are also continuing to analyze the tissues from the animals that have already completed the study, and we will be using these data as well as data from our collaborators' projects as preliminary evidence for new grant applications. While the pandemic effects described above caused changes to our project and resulted in significant impacts on our expenditures, we are confident that new grant awards and obtaining other funding will allow the resumption of TBI experiments and continuance of this project.
- **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**
  - Nothing to Report.
- **Significant changes in use or care of vertebrate animals.**
  - Nothing to Report.
- **Significant changes in use of biohazards and/or select agents**
  - Nothing to Report.

6. **PRODUCTS:**

- **Publications, conference papers, and presentations**.
  - **Journal publications.** Nothing to Report.
  - **Books or other non-periodical, one-time publications.** Nothing to Report.
  - **Other publications, conference papers, and presentations.** We presented an abstract and poster at the 2020 Alzheimer's Association International Conference (Poster # 46103) showing neuropathology findings of 2X-CCI-induced tauopathy and astrogliosis in 12-month-old TgF344-AD rats. This poster is appended below in Appendix 6.
- **Website(s) or other Internet site(s)**
  - Nothing to Report.
- **Technologies or techniques**
  - Nothing to Report.
- **Inventions, patent applications, and/or licenses**
  - Nothing to Report.
- **Other Products**
  - As mentioned above in Section 3, this project initiated the construction of a blast room within the CU Anschutz vivarium, which is a critical infrastructure resource for all current and future CU Anschutz researchers who want to investigate blast-induced pathophysiological effects, including other affected organ systems throughout the body, as well as the CNS. Moreover, these new facilities will also allow for therapeutic studies of blast injuries, which require behavioral studies and/or animal imaging procedures to be conducted with the experimental animal cohorts.
  - We collected peripheral and CNS tissues and whole blood components from all animals that underwent sham or CCI/TBI procedures. These biospecimens will continue to be banked within the CUACC Biorepository, which includes digital specimen management and continuous temperature-monitored refrigerators and freezers, until these specimens are used in biomarker assays and histochemical analyses. The results from these tissues will continue to be used by our laboratory and our collaborators to provide preliminary data for future grant applications and publications.

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7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

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

Name: *Huntington Potter, PhD*
Project Role: *Principal Investigator*
Researcher Identifier (e.g. ORCID ID): *N/A*
Nearest person month worked: *no change*
Contribution to the project: *no change*

Name: *Timothy Boyd, PhD*
Project Role: *Co-Principal Investigator*
Researcher Identifier (e.g. ORCID ID): *N/A*
Nearest person month worked: *0.0*
Contribution to the project: *Dr. Boyd resigned from his position at the University of Colorado for an industry position. Although he has provided some unpaid consulting time, he did not contribute to any person month time since the previous reporting period.*

Name: *Neil Markham, MBA*
Project Role: *Senior Professional Research Assistant*
Researcher Identifier (e.g. ORCID ID): *N/A*
Nearest person month worked: *no change*
Contribution to the project: *no change*

Name: *Athena Ching Jung Wang, PhD*
Project Role: *Post-Doctoral Fellow*
Researcher Identifier (e.g. ORCID ID): *N/A*
Nearest person month worked: *no change*
Contribution to the project: *no change*

Name: *Vanesa Adame*
Project Role: *Professional Research Assistant*
Researcher Identifier (e.g. ORCID ID): *N/A*
Nearest person month worked: *no change*
Contribution to the project: *no change*

Name: *Polly Serrano*
Project Role: *Administration*
Researcher Identifier (e.g. ORCID ID): *N/A*
Nearest person month worked: *no change*
Contribution to the project: *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?**
  - Nothing to Report.
- **What other organizations were involved as partners?**
  - Nothing to Report.
    - **Organization Name.** Nothing to Report.
    - **Location of Organization.** Nothing to Report.

- **Partner's contribution to the projec**t. Nothing to Report.
    - **Financial support.** Nothing to Report.
    - **In-kind support.** Nothing to Report.
    - **Facilities.** Nothing to Report.
    - **Collaboration.** Nothing to Report.
    - **Personnel exchanges.** Nothing to Report.
    - **Other.** Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

- **COLLABORATIVE AWARDS:** Nothing to Report.
- **QUAD CHARTS:** An updated Quad Chart is provided below in Appendix 7.

9. APPENDICES:

APPENDIX 1.

Statement of Work (updated 31 December 2021).

STATEMENT OF WORK - Start Date Approval 01-04-2018

Specific Aim 1(specified in proposal)	Timeline	Start Date: 1-4-2018	Progress to Date		Site 1
Major Task 1: To develop new models of AD, TBI, and rmTBI.	Months	Time Range	# Animals Completed	Percent Completed	Total # Animals to Complete Task
Subtask 1: Induce TBI (1X and 2X Unilateral CCI)	1 - 24	1/4/18 - 1/4/20	274	76%	360
Subtask 2: Induce rmTBI (TBI Blast Model)	1 - 30	1/4/18 - 7/4/20	0	0%	240
Subtask 3: Collect and analyze MRI neuroimaging data from use of two contrast agents: Multihance (for BBB integrity) and Feraheme (for microglial density).	6 - 24	7/4/18 - 1/4/20	42	35%	120
Subtask 4: Collect and analyze post-mortem neuropathology data: amyloid, tau, markers of neurodegeneration, markers of vascular integrity, synaptic markers, markers of microglial/astroglial morphology and activation, and neuronal cell counts.	6 - 30	7/4/18 - 7/4/20	274	46%	600
Milestone(s) Achieved	36	1/4/2021		39%	
Local IACUC Approval (8/7/17)					
Milestone Achieved: ACURO Approval (12/28/17)					
Major Task 2: To identify new peripheral immune system biomarkers associated with TBI/rmTBI-AD.	Months	Time Range	# Animals Completed	Percent Completed	
Subtask 1: Analyze whole blood samples using to assess alterations in immune cell populations.	3 - 30	4/4/18 - 7/4/20	274	46%	600
Subtask 2: Analyze plasma samples to examine levels of: translational biomarkers, Aβ, tau, P-tau, and inflammatory biomarkers, using the rat Meso Scale Discovery (MSD) inflammation panel.	3 - 30	4/4/18 - 7/4/20	274	46%	600
Milestone(s) Achieved:	36	1/4/2021		46%	
Major Task 3: To identify novel neuroimmune signaling biomarkers associated with TBI/rmTBI-AD.	Months	Time Range	# Animals Completed	Percent Completed	
Subtask 1: Immunopurify extracellular vesicles from the total plasma vesicle pool to obtain exosomes and microvesicles derived from neurons, astrocytes, microglia.	3 - 30	4/4/18 - 7/4/20	0	0%	600
Subtask 2: Analyze Aβ, tau, P-tau, and inflammatory translational biomarkers using the MSD inflammation panel.	3 - 30	4/4/18 - 7/4/20	0	0%	600
Milestone(s) Achieved:	36	1/4/2021		0%	

The Statement of Work, as of 12/31/2021, which shows that 274 animals have completed 1X-CCI and 2X-CCI procedures, with histochemical analyses performed and MRI procedures performed on 42 of these animals. The CBC/diff data and MSD analyses have also been conducted on these 274 animals.

APPENDIX 2.

SOW (Excluding rmTBI Cohorts):

STATEMENT OF WORK - excluding blast TBI experiments

Specific Aim 1(specified in proposal)	Timeline	Start Date: 1-4-2018	Progress to Date		Site 1
Major Task 1: To develop new models of AD, TBI, and rmTBI.	Months	Time Range	# Animals Completed	Percent Completed	Total # Animals to Complete Task
Subtask 1: Induce TBI (1X and 2X Unilateral CCI)	1 - 24	1/4/18 - 1/4/20	274	76%	360
Subtask 2: Induce rmTBI (TBI Blast Model)	1 - 30	1/4/18 - 7/4/20	0		240
Subtask 3: Collect and analyze MRI neuroimaging data from use of two contrast agents: Multihance (for BBB integrity) and Feraheme (for microglial density).	6 - 24	7/4/18 - 1/4/20	42	58%	72
Subtask 4: Collect and analyze post-mortem neuropathology data: amyloid, tau, markers of neurodegeneration, markers of vascular integrity, synaptic markers, markers of microglial/astroglial morphology and activation, and neuronal cell counts.	6 - 30	7/4/18 - 7/4/20	274	76%	360
Milestone(s) Achieved	36	1/4/2021		69%	
Local IACUC Approval (8/7/17)					
Milestone Achieved: ACURO Approval (12/28/17)					
Major Task 2: To identify new peripheral immune system biomarkers associated with TBI/rmTBI-AD.	Months	Time Range	# Animals Completed	Percent Completed	
Subtask 1: Analyze whole blood samples using to assess alterations in immune cell populations.	3 - 30	4/4/18 - 7/4/20	274	76%	360
Subtask 2: Analyze plasma samples to examine levels of: translational biomarkers, Aβ, tau, P-tau, and inflammatory biomarkers, using the rat Meso Scale Discovery (MSD) inflammation panel.	3 - 30	4/4/18 - 7/4/20	274	76%	360
Milestone(s) Achieved:	36	1/4/2021		76%	
Major Task 3: To identify novel neuroimmune signaling biomarkers associated with TBI/rmTBI-AD.	Months	Time Range	# Animals Completed	Percent Completed	
Subtask 1: Immunopurify extracellular vesicles from the total plasma vesicle pool to obtain exosomes and microvesicles derived from neurons, astrocytes, microglia.	3 - 30	4/4/18 - 7/4/20	0	0%	600
Subtask 2: Analyze Aβ, tau, P-tau, and inflammatory translational biomarkers using the MSD inflammation panel.	3 - 30	4/4/18 - 7/4/20	0	0%	600
Milestone(s) Achieved:	36	1/4/2021		0%	

Alternative SOW concentrating on CCI cohorts. There were 240 animals (40%) of this project that were allocated to undergo repetitive moderate blast TBI (rmTBI) or sham control experiments. Because of several technical issues and pandemic restrictions during the course of this project, none of the animals allocated to these cohorts underwent rmTBI or sham procedures. However, their numbers are included within the calculation for the Statement of Work above in Appendix 1, and also for the results reported above in the Final Report. If we were to instead determine our progress just for the 1X- and 2X-CCI cohorts (60%) of the project, calculations in this theoretical SOW show that we have completed 69% of Major Task 1 and 76% of Major Task 2.

APPENDIX 3.

Tables for 1X-CCI and 2X-CCI groups. Cells that are filled in show the sacrifice dates of animals that have completed the study, and cells with blank spaces are reserved for animals that have not yet undergone CCI procedures.

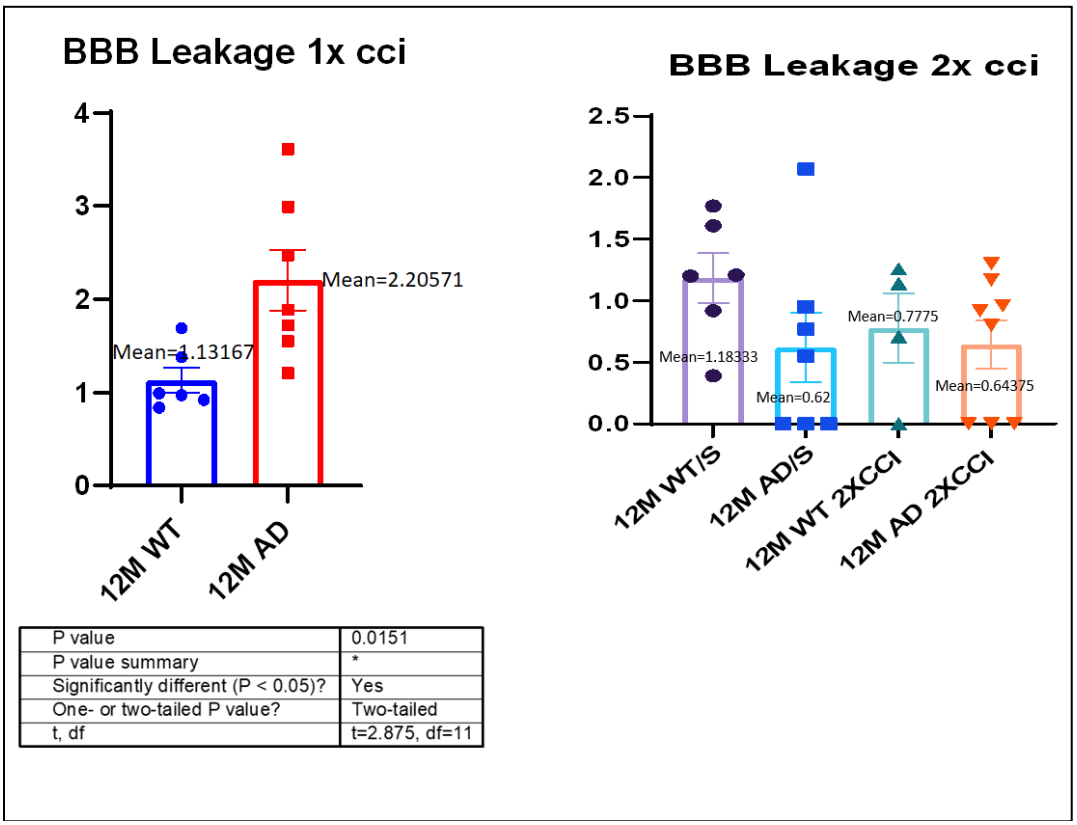
Injury paradigm	Age:	Groups: (n=6 each)	Days Post Final TBI injury				
			3	15	30	60	120
			Sac Date	Sac Date	Sac Date	Sac Date	Sac Date
1X Unilateral CCI/TBI	6 Months	TgF344-AD + injury*	2/8/19		12/19/19	2/7/20	10/11/19
			2/8/19		12/19/19	2/7/20	10/11/19
			2/8/19				7/3/20
			3/8/19				7/3/20
			3/8/19				7/3/20
			3/8/19				7/3/20
		F344 control + injury	10/12/18	10/25/18	12/19/19	12/18/18	2/26/19
			10/12/18	10/25/18	12/19/19	12/18/18	2/26/19
			10/12/18	10/25/18	12/19/19	12/18/18	2/26/19
			10/12/18	10/25/18	12/19/19	12/18/18	2/26/19
			10/12/18	10/25/18		12/18/18	2/26/19
			10/12/18	10/25/18		12/18/18	2/26/19
	12 Months	TgF344-AD + injury	5/24/19	8/1/18	8/8/19	9/9/19	11/20/19
			5/24/19	10/7/19	8/8/19	9/9/19	10/11/19
			9/20/19	10/7/19	8/8/19	9/9/19	3/10/20
			9/20/19	10/7/19	8/8/19	11/22/19	3/10/20
			9/20/19	10/7/19	8/8/19	2/7/20	3/10/20
			9/20/19	10/7/19	8/8/19	4/27/20	3/10/20
		F344 control + injury	4/26/19	5/23/19	1/22/19	9/10/19	10/11/19
			4/26/19	5/23/19	1/22/19	9/10/19	10/11/19
			4/26/19	5/23/19	1/22/19	9/10/19	10/11/19
			4/26/19	5/23/19	1/22/19	9/10/19	11/20/19
			4/26/19	5/23/19	1/22/19	9/10/19	11/20/19
			4/26/19	5/23/19	1/22/19	11/22/19	11/20/19

Injury paradigm	Age:	Groups: (n=6 each)	Days Post Final TBI injury				
			3	15	30	60	120
			Sac Date	Sac Date	Sac Date	Sac Date	Sac Date
2X Unilateral CCI/TBI	6 Months	TgF344-AD + injury		12/4/19		5/11/20	3/18/20
						5/11/20	5/21/20
							7/13/20
							6/17/20
							6/17/20
		TgF344-AD + sham injury					
		F344 control + injury		6/13/19		4/17/20	3/18/20
				6/13/19		4/17/20	3/18/20
				6/13/19		4/17/20	5/21/20
				12/4/19		5/11/20	5/21/20
				12/4/19		5/11/20	5/21/20
				12/4/19		5/11/20	6/17/20
		F344 control + sham injury	10/22/18	10/18/18		12/14/18	1/31/19
			10/22/18	10/18/18		12/14/18	1/31/19
			10/22/18	10/18/18		12/14/18	1/31/19
			10/22/18	10/18/18		12/14/18	1/31/19
			10/22/18	10/18/18		12/14/18	1/31/19
			10/22/18	10/18/18		12/14/18	1/31/19

Injury paradigm	Age:	Groups: (n=6 each)	Days Post Final TBI injury				
			3	15	30	60	120
			Sac Date	Sac Date	Sac Date	Sac Date	Sac Date
2X Unilateral CCI/TBI	12 Months	TgF344-AD + injury	9/30/19	9/18/19	10/3/19	5/28/19	1/16/20
			9/30/19	11/7/19	10/3/19	5/28/19	1/16/20
			9/30/19	11/7/19	10/3/19	1/21/20	1/16/20
			9/30/19	11/7/19	10/3/19	1/21/20	2/21/20
			9/30/19	11/7/19	10/18/19	2/14/20	2/21/20
			9/30/19	12/4/19	10/18/19	2/14/20	2/21/20
		TgF344-AD + sham injury	10/24/19	8/1/18	11/21/19	5/28/19	2/21/20
			10/24/19	11/5/19	11/21/19	5/28/19	2/21/20
			10/24/19	11/5/19	11/21/19	1/7/20	2/21/20
			10/25/19	11/6/19	11/21/19	1/7/20	2/21/20
			10/25/19	11/6/19	11/21/19	1/21/20	2/21/20
			3/16/20	11/6/19	12/16/19	1/21/20	2/21/20
		F344 control + injury	9/30/19	9/18/19	10/3/19	5/28/19	2/18/20
			9/30/19	9/18/19	10/3/19	1/16/20	4/15/20
			9/30/19	9/18/19	10/3/19	1/21/20	4/15/20
			3/16/20	9/18/19	10/18/19	5/12/20	4/15/20
			3/16/20	11/7/19	10/18/19	5/12/20	4/15/20
			3/16/20	11/7/19	**4/13/20**	5/12/20	4/15/20
		F344 control + sham injury	7/16/18	5/30/19	12/16/19	5/28/19	1/31/19
			7/16/18	5/30/19	12/16/19	1/7/20	1/31/19
			10/24/19	5/30/19	12/16/19	1/7/20	2/18/20
			10/24/19	5/30/19	12/16/19	1/21/20	2/18/20
			10/24/19	11/6/19	12/16/19	1/21/20	2/18/20
			10/25/19	11/6/19	**4/13/20**	5/12/20	4/15/20

APPENDIX 4.

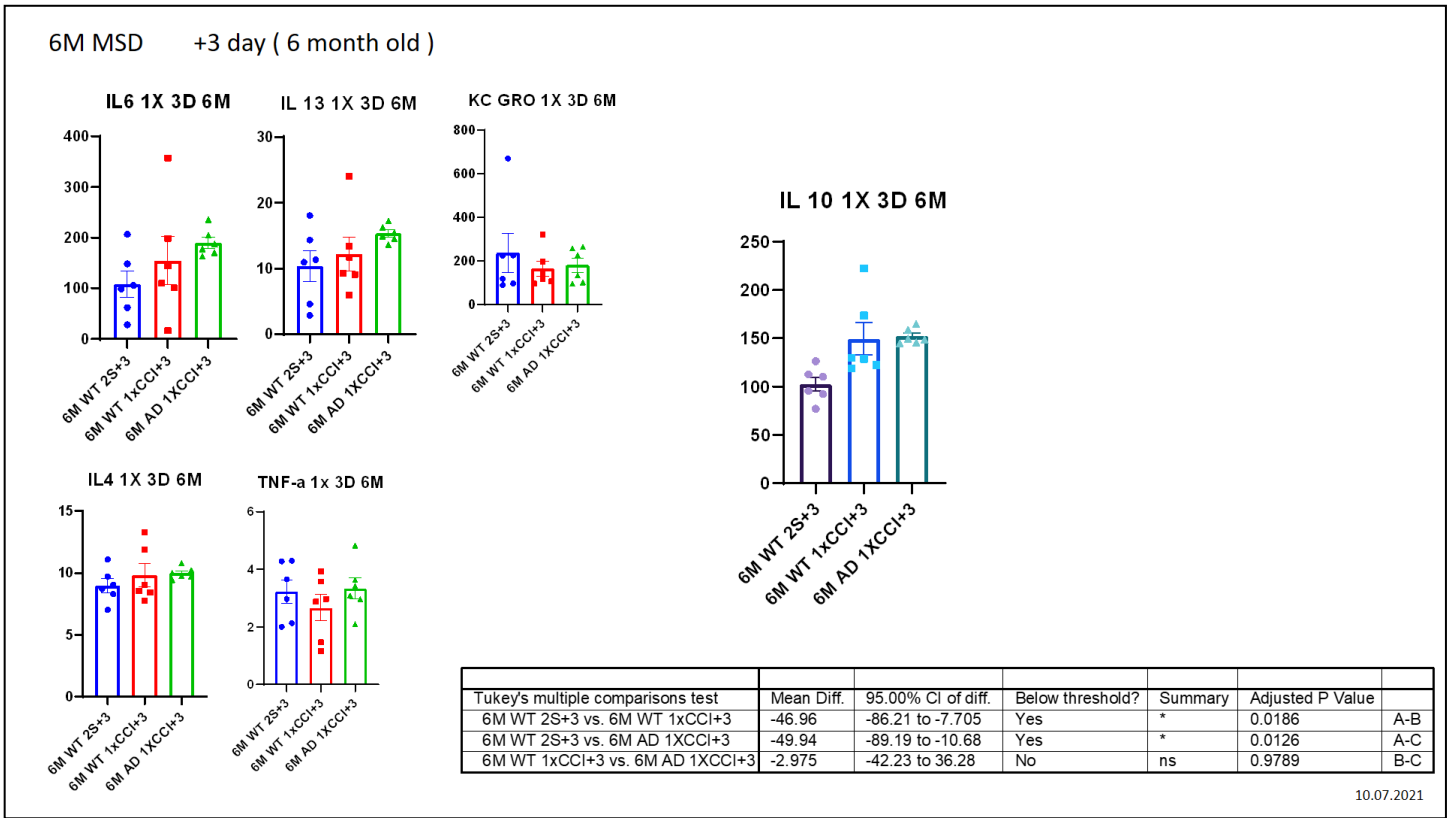
Preliminary summary of MRI T1-weighted imaging with Gadolinium contrast. The left panel indicates that 1X-CCI might increase permeability of the blood brain barrier (BBB) in the 12-month-old TgF344-AD rats, as compared to 1X-CCI wild-type (WT) F344 control rats. However, in the right panel, there were no significant differences between any groups and in comparing sham (S) vs. CCI or WT vs. AD groups. There are apparent wide variances between individual animals of each group, and much larger cohorts would be needed to determine by MRI whether 2X-CCI can indeed affect BBB integrity. Additional analyses are ongoing, including MRI quantification of lesion volumes and immunohistochemistry of brain tissues, in order to verify whether the increased BBB leakage by 1X-CC is indeed correct, and whether there was any BBB damage attributable to 2X-CCI that was not able to be detected by MRI scans.



P value	0.0151
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=2.875, df=11

APPENDIX 5.

Meso Scale Discovery (MSD) data for 6-month-old cohorts at various time points post-injury. MSD analyses for 12-month-old cohorts are not included below as there were no significant differences or consistent trends between any of the groups due to high variability for each group across all of the analytes measured below. However, in the results of 6-month-old cohorts below, the cytokines, interleukin (IL)-4, IL-10, and IL-13, which have been generally characterized in the literature as anti-inflammatory mediators, showed significant differences between some of the 1X-CCI groups at different post-injury time points. However, no pro-inflammatory markers, such as IL-6 and TNFα, of which numerous literature indicate should be affected by TBI, showed any significant differences between groups. Large variances between individual animals in many groups suggest that much larger cohorts of these CCI/AD models would be needed to determine whether any of these circulating biomarkers are indeed reliable proxies for monitoring disease progression, either from TBI, AD, or TBI and AD combined. Thus, based on these results below, we are not confident that measuring the specific plasma cytokine biomarkers below in our TBI-AD/ADRD models will provide reliable mechanistic and/or translational data for enhancing neuropathology and behavioral results in future TBI-AD studies.



Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value	
6M WT 2S+3 vs. 6M WT 1xCCI+3	-46.96	-86.21 to -7.705	Yes	*	0.0186	A-B
6M WT 2S+3 vs. 6M AD 1XCCI+3	-49.94	-89.19 to -10.68	Yes	*	0.0126	A-C
6M WT 1xCCI+3 vs. 6M AD 1XCCI+3	-2.975	-42.23 to 36.28	No	ns	0.9789	B-C

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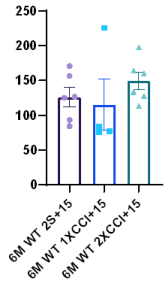
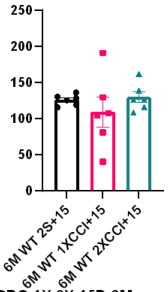
6M MSD +15 day (6 month old)

IL6 1X 2X 15D 6M

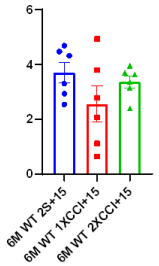


IL10 1X 2X 15D 6M

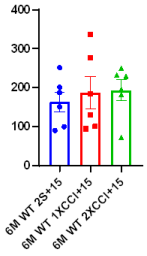


ANOVA summary	
F	3.985
P value	0.0409
P value summary	*
Significant diff. among means (P < 0.05)?	Yes
R squared	0.3470

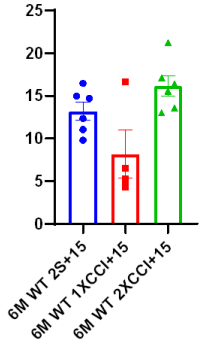
TNF-a 1x 2x 15D 6M



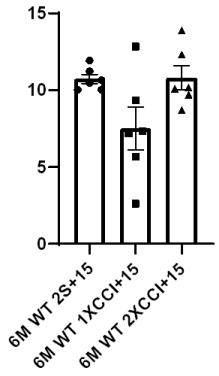
KC GRO 1X 2X 15D 6M



IL 13 1X 2X 15D 6M



IL4 1X 2X 15D 6M



Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
6M WT 2S+15 vs. 6M WT 1XCCI+15	5.043	-1.199 to 11.29	No	ns	0.1215
6M WT 2S+15 vs. 6M WT 2XCCI+15	-2.926	-8.509 to 2.658	No	ns	0.3774
6M WT 1XCCI+15 vs. 6M WT 2XCCI+15	-7.969	-14.21 to -1.726	Yes	*	0.0130

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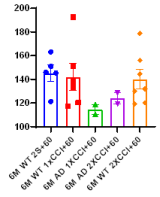
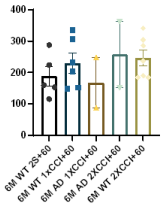
6M MSD +60 day (8 month old)

IL10 1X.2X 60D 6M

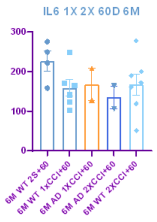


KC GRO 1X 2X 60D 6M

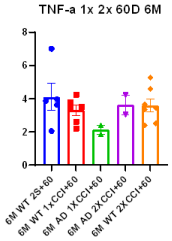


Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
6M WT 2S+60 vs. 6M WT 1xCCI+60	3.241	0.2843 to 6.197	Yes	*	0.0280
6M WT 2S+60 vs. 6M AD 1XCCI+60	4.715	0.6303 to 8.799	Yes	*	0.0196
6M WT 2S+60 vs. 6M AD 2XCCI+60	1.946	-2.139 to 6.030	No	ns	0.6063
6M WT 2S+60 vs. 6M WT 2XCCI+60	0.4822	-2.376 to 3.341	No	ns	0.9849
6M WT 1xCCI+60 vs. 6M AD 1XCCI+60	1.474	-2.512 to 5.461	No	ns	0.7912
6M WT 1xCCI+60 vs. 6M AD 2XCCI+60	-1.295	-5.281 to 2.691	No	ns	0.8570
6M WT 1xCCI+60 vs. 6M WT 2XCCI+60	-2.758	-5.474 to -0.04226	Yes	*	0.0456
6M AD 1XCCI+60 vs. 6M AD 2XCCI+60	-2.769	-7.651 to 2.113	No	ns	0.4454
6M AD 1XCCI+60 vs. 6M WT 2XCCI+60	-4.233	-8.147 to -0.3184	Yes	*	0.0307
6M AD 2XCCI+60 vs. 6M WT 2XCCI+60	-1.464	-5.378 to 2.451	No	ns	0.7848

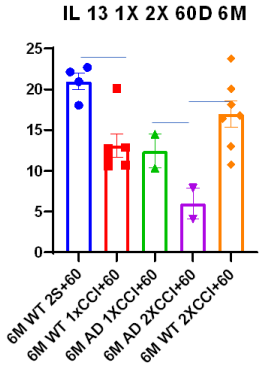
IL6 1X 2X 60D 6M



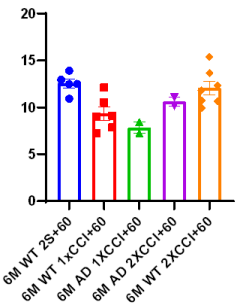
TNF-a 1x 2x 60D 6M



IL 13 1X 2X 60D 6M



IL4 1X 2X 60D 6M



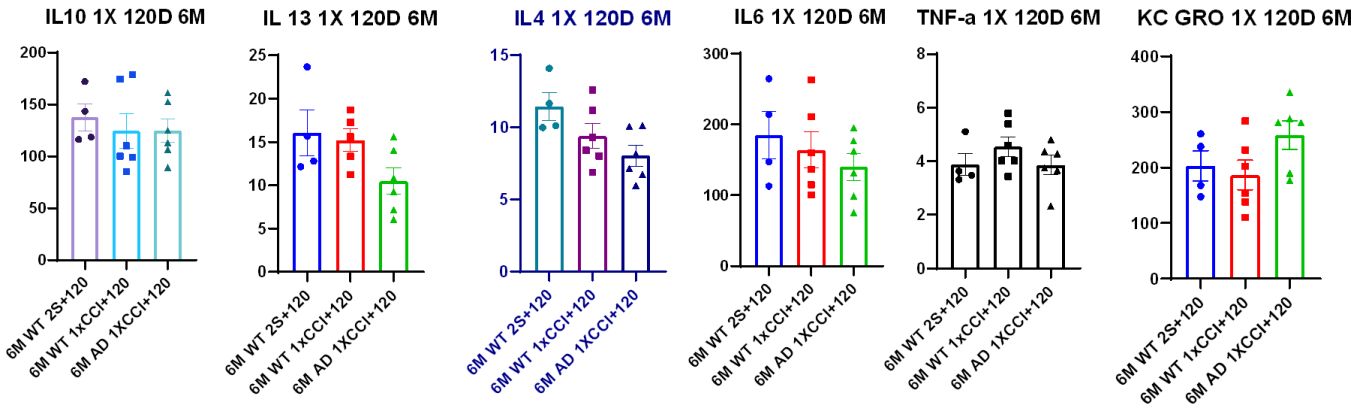
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value	
6M WT 2S+60 vs. 6M WT 1xCCI+60	7.854	0.8023 to 14.90	Yes	*	0.0254	A-B
6M WT 2S+60 vs. 6M AD 1XCCI+60	8.534	-0.9266 to 17.99	No	ns	0.0876	A-C
6M WT 2S+60 vs. 6M AD 2XCCI+60	14.97	5.509 to 24.43	Yes	**	0.0014	A-D
6M WT 2S+60 vs. 6M WT 2XCCI+60	3.967	-2.880 to 10.81	No	ns	0.4202	A-E
6M WT 1xCCI+60 vs. 6M AD 1XCCI+60	0.6801	-8.239 to 9.599	No	ns	0.9993	B-C
6M WT 1xCCI+60 vs. 6M AD 2XCCI+60	7.116	-1.803 to 16.04	No	ns	0.1537	B-D
6M WT 1xCCI+60 vs. 6M WT 2XCCI+60	-3.887	-9.964 to 2.191	No	ns	0.3280	B-E
6M AD 1XCCI+60 vs. 6M AD 2XCCI+60	6.436	-4.488 to 17.36	No	ns	0.4043	C-D
6M AD 1XCCI+60 vs. 6M WT 2XCCI+60	-4.567	-13.33 to 4.192	No	ns	0.5195	C-E
6M AD 2XCCI+60 vs. 6M WT 2XCCI+60	-11.00	-19.76 to -2.244	Yes	*	0.0107	D-E

10.07.2021

Page contains data that should be protected

6M MSD +120 day (10 month old)

There is no significant cytokine changes 120 days after TBI between the groups



10.07.2021

APPENDIX 6.

2020 AAIC Poster Presentation:

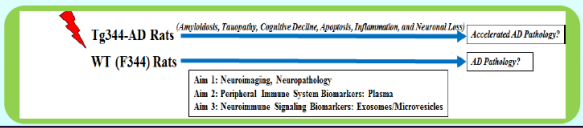
Neuropathology and Immune Biomarker Discovery in a Rat Model of Alzheimer's disease, TgF344-AD, with Controlled Cortical Injury model of Traumatic Brain Injury

Athena Ching-Jung Wang[1], Vanesa Adame[1], Neil Markham[1], Heidi J. Chial[1], Timothy D. Boyd[1], Huntington Potter[1]

[1]University of Colorado Alzheimer's and Cognition Center (CUACC), Department of Neurology, and the Linda Crnic Institute for Down Syndrome, University of Colorado, Anschutz Medical Campus

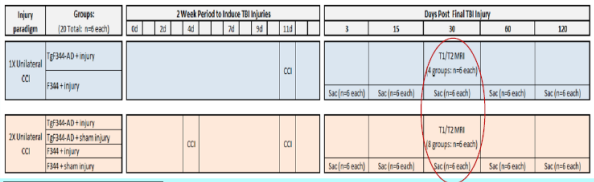
Background

People with a history of traumatic brain injury (TBI) have a higher risk of developing Alzheimer's disease (AD) and AD-related dementias (ADRD), such as chronic traumatic encephalopathy (CTE). However, the relationship(s) between TBI, AD, and CTE remain poorly understood and markedly understudied. Most mouse models of AD exhibit either amyloidosis or tauopathy independently, which confounds research into TBI-AD-ADRD pathogenic mechanisms. In this study, we used the transgenic TgF344-AD rat model of AD (Cohen et al., 2013) in which amyloid deposition drives endogenous tauopathy and neurodegeneration, serving as a more representative model of human AD. This study is focused on dynamic changes in AD-related neuropathology and neuroinflammation in TgF344-AD and wild-type (WT) rats at different time points post-TBI injury versus sham controls.



Methods

In this study, we used the closed-head controlled cortical impact (CCI) model of TBI, which is similar to mild TBI, the most common form of TBI. The CCI was performed once (1X-CCI) or twice (2X-CCI) over two weeks in both six-month-old and 12-month-old TgF344-AD and wild-type (F344) rats in order to investigate the effects of TBI on acute and chronic neuroinflammation, on blood brain barrier (BBB) integrity, and on ADRD neuropathology. Brains and terminal blood draws were collected at different time points after the 1X- and 2X-CCI TBI procedures, with cohorts ending on days 3, 15, 30, 60, and 120 days after the final CCI procedure. Thus, the ending ages of the animals across the project allow for analyses of TBI-induced ADRD-related pathological and immune system changes at 6, 7, 8, 10, 12, 13, 14, and 16 months of age. MRI scans for cohorts at 30 days post-CCI were conducted to examine in vivo BBB leakage and chronic neuroinflammation, with subsequent post-mortem immunohistochemical confirmation. Terminal blood samples were used for complete blood cell counts with differential and multiple biomarker analyses.



Experimental Design:

Tail bleed (CBC manual count) ➡ CCI (1X or 2X) or Sham

Different time points: day of harvest ➡ Tail bleed (CBC manual count) ➡ 0.9% Saline Perfusion (tissue collection) ➡ IHC

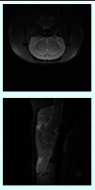
Method Cont.

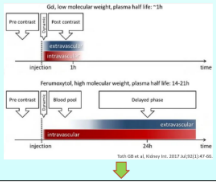
MRI imaging at 30 days post-CCI/Sham (AD vs WT) to examine BBB leakage and iron oxide phagocytosis

Day 1: Gadolinium (Gd) injection followed by MRI imaging

Day 2: Injection of ferumoxytol

Day 3: MRI imaging





Tissue embedding in paraffin and sectioning at 4 µm

Immunofluorescence microscopy (amyloid [6E10], tau [AT8], GFAP, and S-100β)

Imaging on Olympus IX83 scanning microscope ➡ Analysis

Results

- No differences in upright time (UT) in WT versus AD rats (data not shown)
- After second anesthesia, WT 2X-Sham animals develop tolerance to isoflurane and wake up faster than WT 2X-CCI animals

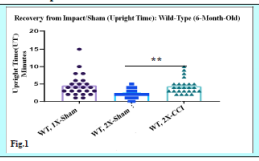


Fig.1

Neutrophil response to injury decreases with age

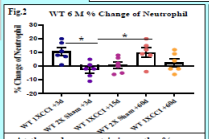


Fig.2

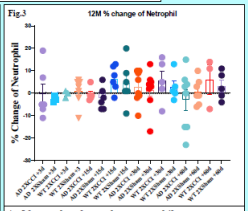


Fig.3

- At three days post-injury, the % change in neutrophils is significantly higher in six-month-old WT 1X-CCI than in WT 1X-Sham rats
- Older animals (12-month-old) show greater variability in the % neutrophil change
- At 60 days post-injury, the % neutrophil change is lower in the 1X-CCI rats and higher in the 1X-Sham rats

At 12 months of age, the neutrophil response to injury does not show a significant difference between 2X-CCI and 2X-Sham groups in AD or WT rats

% Difference = (% Neutrophils after CCI/Sham) - (% Neutrophils before CCI/Sham)

Results Cont.

Amyloidosis: Preliminary analyses suggest no significant differences in Aβ plaque density in the hippocampi of 12-month-old AD 2X-CCI rats at 3 days post-injury. The 12-month-old WT rats had no Aβ plaques regardless of CCI injury or sham. These studies are ongoing. We are analyzing additional brain regions (e.g., cortex, CA1, CA3, dentate gyrus) and ipsilateral CCI vs. contralateral hemispheres.

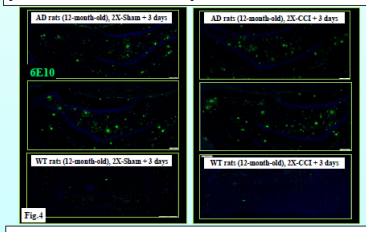


Fig.4

Tauopathy: Preliminary analyses suggest that increased phospho-tau (AT8) staining, especially in the CA1 and dentate gyrus regions, in 12-month-old AD 2x-CCI rats at 3 days post-injury compared to AD 2X-Sham rats.

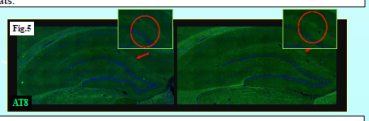


Fig.5

Neuroinflammation: Preliminary analyses suggest that increased astrogliosis (GFAP, S100β) in 12-month-old AD 2x-CCI rats at 3 days post-injury compared to AD 2X-Sham rats, which appears to include global activation of astrocytes.

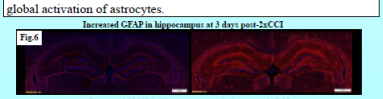


Fig.6

Fig.7

Conclusions & Future directions

- The TgF344-AD rat model of AD may be useful for studying the pathogenic relationships between genetic predisposition to AD and tauopathy, as well as environmental risk factors, such as TBI.
- CCI induces tauopathy in 12-month-old AD rats, which is earlier than reported without TBI (occurring at >16 months [Cohen et al., 2013]), suggesting that TBI in TgF344-AD rats accelerates their AD pathogenesis.
- Peripheral immune responses to TBI (i.e., the % increase in neutrophils) decrease with age, suggesting that deficits may be due to an aging immune system or due to chronic TBI-induced neuroinflammation. Ongoing analyses of cryopreserved leukocytes and plasma biomarkers will be used to compare with the manual CBC with differential results.
- We have generated a large tissue biobank (e.g., brain, spleen, eyes, buffy coat, plasma) of TgF344-AD and WT rats from the 1X-CCI, 2X-CCI, and Sham groups at six and 12 months of age, collected at different time points post-injury that will allow future analyses of TBI risk in AD and ADRD pathogenesis, including TBI- and Aβ-induced tauopathy, chronic systemic inflammation, chronic neuroinflammation, BBB integrity, and neurodegeneration.

Acknowledgements: CUACC Donors and DoD Grant Award # W81XWH-17-1-0583; (1) the U.S. Army Medical Research Acquisition Activity, 820 Chandler Street, Fort Detrick MD 21702-5014 is the awarding and administering acquisition office. (2) This work was supported by the Office of the Assistant Secretary of Defense for Health Affairs, through the Peer Reviewed Alzheimer's Research Program under Award No. W81XWH-17-1-0583. Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the Department of Defense. (3) In conducting research using animals, the investigator(s) adhere to the laws of the United States and regulations of the Department of Agriculture.

APPENDIX 7.

Quad Chart (updated 31 December 2021).

Neuropathology and Immune Biomarker Discovery in a Rat Model of Alzheimer's Disease, TgF344-AD, with Single or Repetitive Traumatic Brain Injury

LOG AZ160059, FY16 Peer Reviewed Alzheimer's Research Program, Convergence Science Research Award W81XWH-17-1-0583

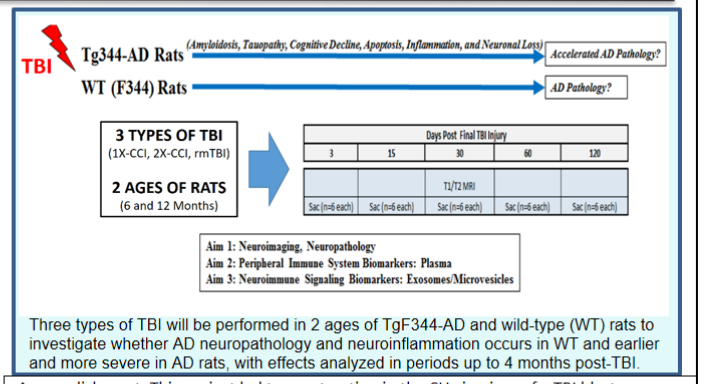
PI: Huntington Potter **Org:** Regents of the University of Colorado **Award Amount:** $555,870.73

Study/Product Aim(s)

- Aim 1. To develop new models of Alzheimer's disease (AD), controlled cortical impact traumatic brain injury (CCC/TBI), and blast repetitive moderate TBI (rmTBI).
- Aim 2. To identify new peripheral immune system biomarkers associated with CCI/TBI-AD or rmTBI-AD.
- Aim 3: To identify novel neuroimmune signaling biomarkers associated with CCI/TBI-AD or rmTBI-AD.

Approach

Three methods of TBI (either single (1X)- or twice (2X)-CCI or rmTBI) or Sham will be performed on two ages of TgF344-AD or WT rats and then cohorts aged to post-TBI time points of 3, 15, 30, 60, and 120 days (n=6 each). MRI neuroimaging for BBB damage and brain inflammation will be performed on cohorts at 30-day post-TBI. Blood will be collected at sacrifice to analyze leukocyte populations and for plasma and exosome biomarkers between cohorts. Histochemical analyses will be performed on brain tissue for various markers of AD neuropathology between groups and to confirm MRI data, as well as correlate with blood biomarkers.



Three types of TBI will be performed in 2 ages of TgF344-AD and wild-type (WT) rats to investigate whether AD neuropathology and neuroinflammation occurs in WT and earlier and more severe in AD rats, with effects analyzed in periods up to 4 months post-TBI.

Accomplishment: This project led to construction in the CU vivarium of a TBI blast room for future blast TBI studies . Neuropathology results indicate TBI exacerbates AD pathology and these data are being used in grant applications and in preparation for publication.

Timeline and Cost

Activities CY	17	18	19	20
Obtain IACUC/ACURO approvals				
Major Task 1				
Major Task 2				
Major Task 3				
Estimated Budget ($K)	**$000**	**$213**	**$293**	**$49**

Updated: 31 December 2021

Goals/Milestones

Major Task 1: To develop new models of AD, TBI, and rmTBI.

☐ Subtask 1: Induce TBI (1X and 2X Unilateral CCI)
☐ Subtask 2: Induce rmTBI (TBI Blast Model)
☐ Subtask 3: Collect and analyze MRI neuroimaging data
☐ Subtask 4: Collect and analyze post-mortem neuropathology

Major Task 2: To identify new peripheral immune system biomarkers associated with TBI/rmTBI-AD.

☐ Subtask 1: Analyze leukocyte populations from whole blood.
☐ Subtask 2: Analyze plasma samples for AD-related biomarkers

Major Task 3: To identify novel neuroimmune signaling biomarkers associated with TBI/rmTBI-AD.

☐ Subtask 1: Immunopurify (IP) extracellular vesicles from plasma
☐ Subtask 2: Analyze AD-related biomarkers in IP'd exosomes

Comments/Challenges/Issues/Concerns

- Early breeding issues, technical issues, and pandemic restrictions have delayed project. We received a year of No Cost Extension for project.
- Entire allotted budget plus voluntary committed cost share of $172,848.
- **Budget Expenditure to Date**

Projected Expenditure: $555,870.73
Actual Expenditure: $555,870.73