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TITLE: Novel Therapeutic Target Identification through analysis of Convergent AD and TBI Pathogenic Mechanisms

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14. ABSTRACT: We plan to identify and provide preliminary validation for novel targets for the TBI-AD interrelationship discovered through our molecular level profiling. The goal here is to target validation and provision of these therapeutic targets for future studies in drug discovery and preclinical efficacy; i.e. IND enabling studies of new therapeutic approaches to the AD sequelae of TBI.

15. SUBJECT TERMS: AD, TBI, Pathology, Animal Models, Novel Therapeutic Targets

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

   In this new project we plan to identify and provide preliminary validation for novel targets for the TBI-AD interrelationship discovered through our molecular level profiling. The goal here is target validation and provision of these therapeutic targets for future studies in drug discovery and preclinical efficacy; i.e. IND enabling studies of new therapeutic approaches to the AD sequelae of TBI.

2. **KEYWORDS**: AD, TBI, Pathology, Animal Models, Novel Therapeutic Targets
3. **ACCOMPLISHMENTS:**

   The most important activity for the first Aim of this project is to validate up to 10 different TBI-AD molecular targets and/or pathways ex vivo and *in vivo*, and to select the top performing therapeutic targets in vivo with recovery of normal molecular phenotype.

   We began the year by evaluation of bioactive lipids and downstream signaling pathways in stored frozen TBI and AD brains from mouse models to further interrogate the Leukotriene and Sphingomyelin signaling pathways and expression of lipid nuclear receptors RXR and PPAR. We have generated time dependent profiles of changes in these molecular targets to support and implicate their role in TBI pathogenesis.

   From our other Roskamp-funded efforts we have now generated a sizeable number of human AD brain tissue (frozen and tissue slices) that we plan to use in our interrogation and demonstration of translational relevance from the mouse to human tissue. We now have approx. 32 AD brains (at two different Braak stages; 0-III to IV-VI), and 16 controls samples. With respect to our TBI request we have only been able to acquire 4 TBI brain tissue samples and are reaching out to other collaborators and brain banks with autopsy cases of patients with a history of repetitive TBI with chronic post-injury time points. Once we obtain these samples our plan is to probe them for the same markers significantly regulated in the brains of TBI-AD mouse models.

   We have obtained regulatory approval for both our IACUC and full version ACURO protocols for this project. We began our first series of *in vivo* treatment and target engagement studies a few months ago in our TBI mouse models. This study involves testing four main targets with drugs that modulate the cysteinyl leukotrienes, 5-lipoxygenase, insulin signaling and shingosine-1-phosphate signaling from which we have preliminary data from our models implicating a role in TBI pathogenesis. These studies used the compounds Monteleukast (leukotriene receptor antagonist), Zileuton (5-lipo-oxygenase inhibitor), intranasal Insulin and Fingolimod (sphingosine-1-phosphate receptor modulator). Mice were exposed to our injury paradigms and were left to age until 3 months post-TBI time points, whereby these compounds were administered over the last 14days prior to euthanasia for analyses of target engagement and modulation of molecular targets and acute TBI neuropathology. We have completed two out of the four drug treatments mentioned above for this part of the study. We are currently analyzing

these datasets using flow cytometry, immunoblotting or ELISA of brain homogenates and isolated single cell analyses, and histopathology. We will also explore bioavailability and toxicity of the compounds. The remaining two drug treatments we initiated will complete within the next month and will be ready for analyses. We plan to start a new cohort for our injury paradigm for analyses of efficacy of another four drug compounds – PTEN, recombinant IGF1 (Iplex), Baxoretene, and pioglitazone. We anticipate these datasets will be ready within the next four months.

We have also developed in a separate Roskamp-funded project, TWO drug assay screening platform for studying efficacy of our drugs prior to in vivo analyses. This platform involves isolation and culturing of glial cells from our mouse models under a 24hr window, and exposure to our drug compounds. The second platform involves collecting brain slices in media from our TBI models and exposing them to our drug compounds. We are using this platform as a supplement to direct our in vivo studies.

We are also developing a novel mass spectrometry method for analyses of bio-active lipids for this current study. This assay will potentially be used to screening >25 bioactive lipids such as – lipoxins, arachidonic acid, leukotrienes, D and E –series resolvins, decosahexaenoic acid, eicosapentaenoic acid, 18-HEPE, prostaglandins, and primary eicosanoids e.g. thromboxanes etc.

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

Major Task 1: Validation of 6 potential targets for therapeutic intervention in the pathogenic TBI-AD interrelationship - DHA imbalance; Oleic Acid levels; peroxisomal function (RXR/PPAR signaling); PI3K/Akt/mTOR pathway; sphingomyelin signaling; eicosanoid pathway

Major Task 2: Chronic evaluation of the efficacy of three potential therapeutics against the pathogenic TBI-AD interrelationship

- **What was accomplished under these goals?**

We have accomplished several of the subtasks related to Major task 1 i.e. validation of 6 potential targets for therapeutic intervention in the pathogenic TBI-AD interrelationship.

Efforts from SUBTASK 1 and 2 have been completed.

Efforts from SUBTASK 3, 4 4e, 4f and 5 and 6 are currently either completed or underway.

See below for subtask descriptions.

Subtask 1: Obtained ACURO approval in March 20th, 2018 (Roskamp Institute IACUC approval for TBI procedures is already in place, and have been amended to include the treatment paradigms).

Subtask 2: Evaluation of bioactive lipids and downstream signaling in stored TBI and AD mouse model samples to identify proposed targets from Leukotriene and Sphingomyelin signaling.

Subtask 3: Administration of r-mTBI or r-sham injury to 180-220 male hTau mice (C57BL/6 humanized tau transgenics) at 2-3 months of age

Subtask 4: Administration of therapeutic compounds to mice for 2 weeks immediately prior to 3 month timepoint post TBI/sham.

Subtask 4a: Administration of therapeutic compounds to target DHA imbalance

Subtask 4b: Administration of therapeutic compounds to target Oleic Acid levels

Subtask 4c: Administration of therapeutic compounds to target peroxisomal function (RXR/PPAR signaling)

Subtask 4d: Administration of therapeutic compounds to target PI3K/Akt/mTOR pathway

Subtask 4e: Target exploration of downstream eicosanoid pathway and administration of therapeutic compounds to target leukotriene signaling

Subtask 4f: Target exploration of downstream sphingomyelin pathway and administration of therapeutic compounds to target sphingomyelin signaling

Subtask 5: Euthanasia of hTau mice at 3 months post-TBI/sham, followed by brain omic and antibody based analyses

Subtask 6: Validation of identified targets in human TBI brains with history of repetitive concussions

Below demonstrate the biochemical analyses of TBI/AD brain (cortex) tissue samples.

Below demonstrate biochemical analyses of PSAPP AD brain (cortical) tissue samples at different ages, to compliment previous data proposed in our preliminary findings for the identified pathways/markers, and to show correlation with the TBI (cortical) tissue. (We focused on sphingomyelin signaling markers, 5-lipoxygenase and cysteinyl leukotrienes, and PPAR/RXR signaling markers).

Methodology – Animals were previous subjected to our injury TBI paradigm (see Mouzon et al., 2012; 2014) and euthanized at 24hrs to 12 months post-injury, and PSAPP mice were euthanized at 3, 9 and 15 months of age. Western blotting was conducted using established protocols as previously demonstrated (see Ojo et al., 2016; Mouzon et al., 2018). ELISA/ EIA kits followed manufacturers guidelines.

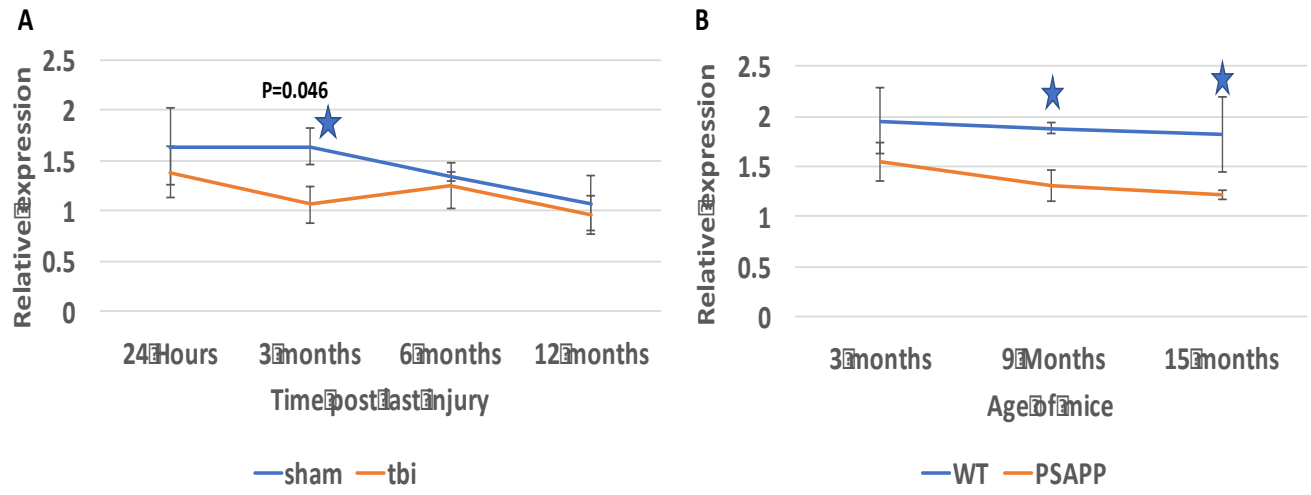

Fig 1.1. The effect of repetitive mild traumatic brain injury and amyloid pathology on the expression of SPHK1. Showing the average change in the relative expression of SPHK1 between sham injury or wild-type littermate mice (blue line) and r-mTBI or PSAPP mice (orange line) across various ages or time points post injury assessed by western blot. N= 4 mice per group. Statistical significance was assessed using a students T-test. Asterisks (*) denote P<0.05.

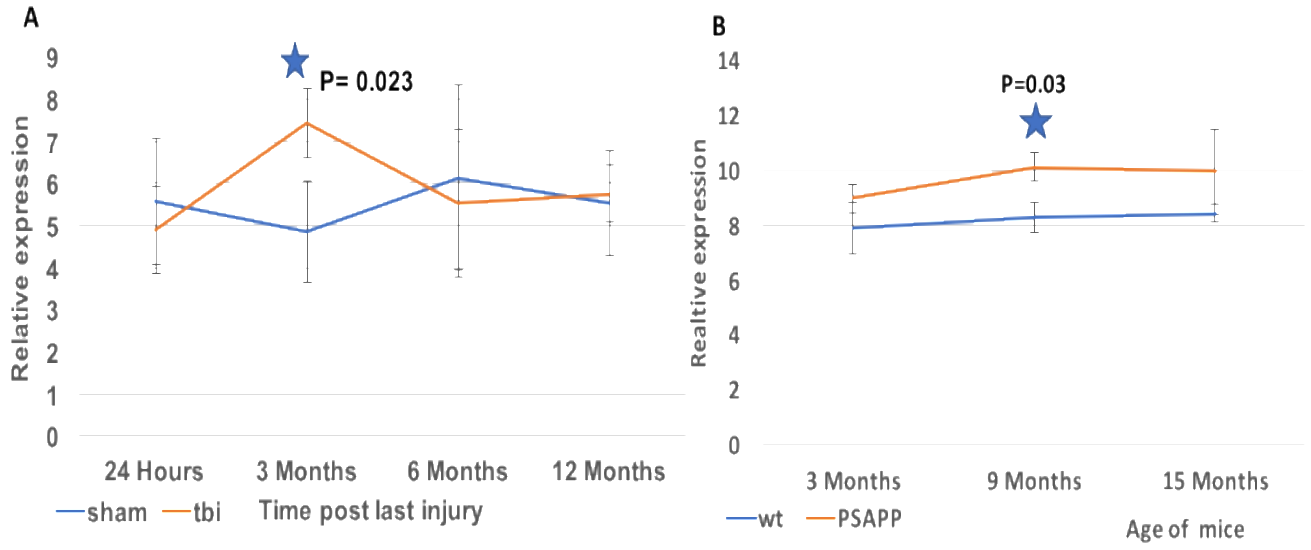

Fig 1.2. The effect of repetitive mild traumatic brain injury and amyloid pathology on the expression of S1P1. Showing the average change in the relative expression of S1P1 between sham injury or wild-type littermate mice (blue line) and r-mTBI or PSAPP mice (orange line) across various ages or time points post injury assessed by western blot. N= 4 mice per group. Statistical significance was assessed using a students T-test. Asterisks (*) denote P<0.05.

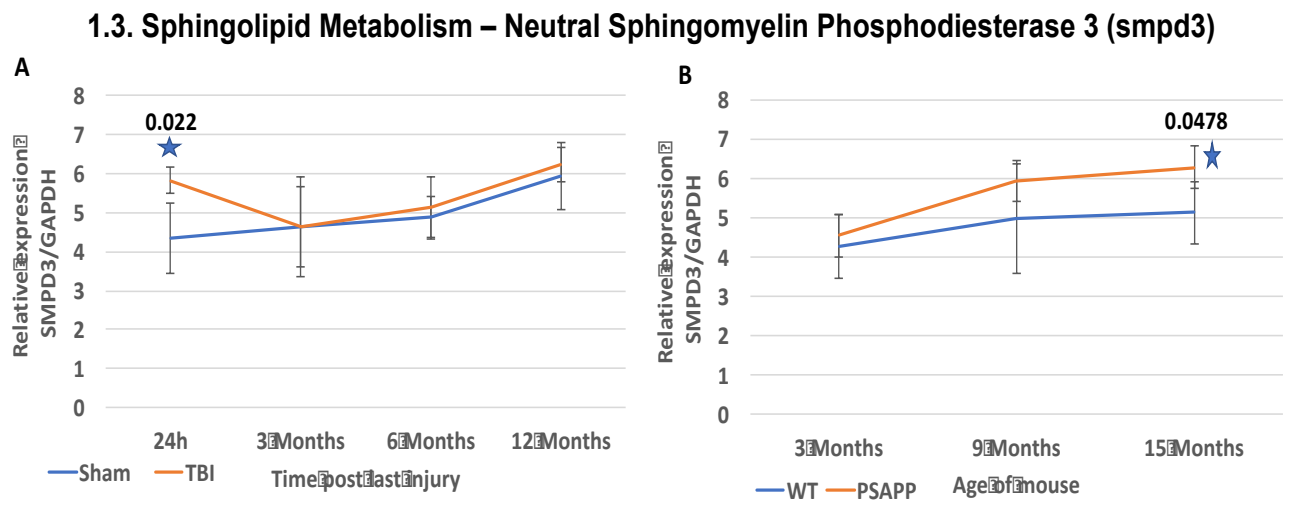

Fig 1.3. The effect of repetitive mild traumatic brain injury and amyloid pathology on the expression of Neutral Sphingomyelin Phosphodiesterase 3 (SMPD3). Showing the average change in the relative expression of SMPD3 between sham injury or wild-type littermate mice (blue line) and r-mTBI or PSAPP mice (orange line) across various ages or time points post injury assessed by western blot. N= 4 mice per group. Statistical significance was assessed using a students T-test. Asterisks (*) denote P<0.05. Together, these data suggest that the targeting of sphingolipid metabolism may have promising outcomes. In TBI/AD research, Given the roles of sphingolipids such as Ceramide and sphingosine 1 phosphate in the promotion of neuronal cell apoptotic signaling and cell survival pathways respectively.

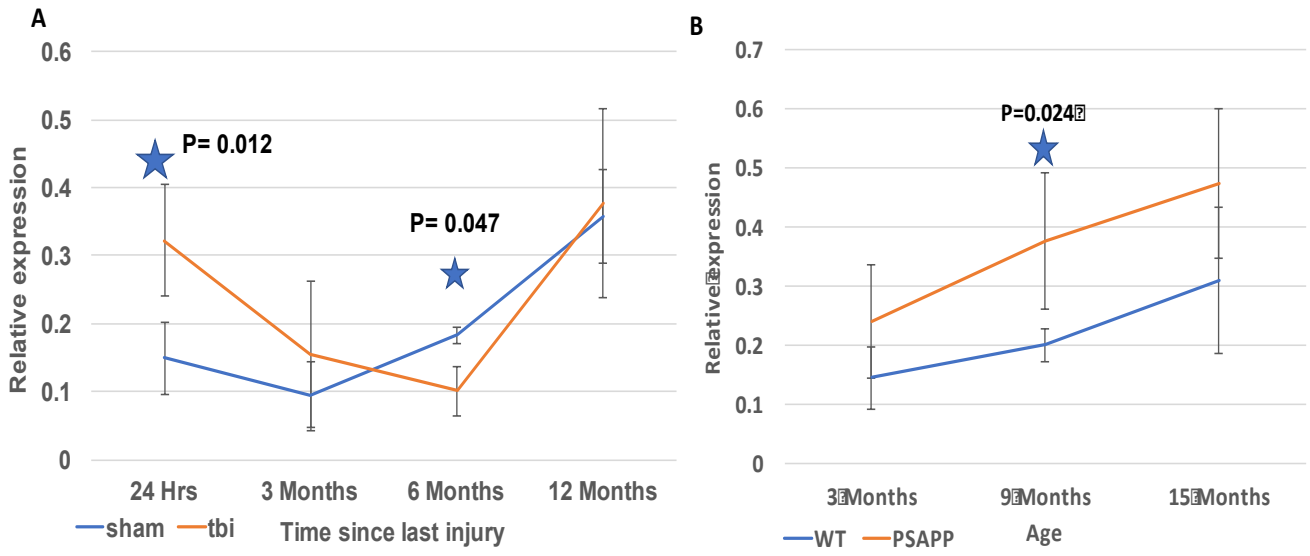

Fig 1.4. The effect of repetitive mild traumatic brain injury and amyloid pathology on the expression of RXRα. Showing the average change in the relative expression of **RXRα** between sham injury or wild-type littermate mice (blue line) and r-mTBI or PSAPP mice (orange line) across various ages or time points post injury assessed by western blot. N= 4 mice per group. Statistical significance was assessed using a students T-test. Asterisks (*) denote P<0.05.

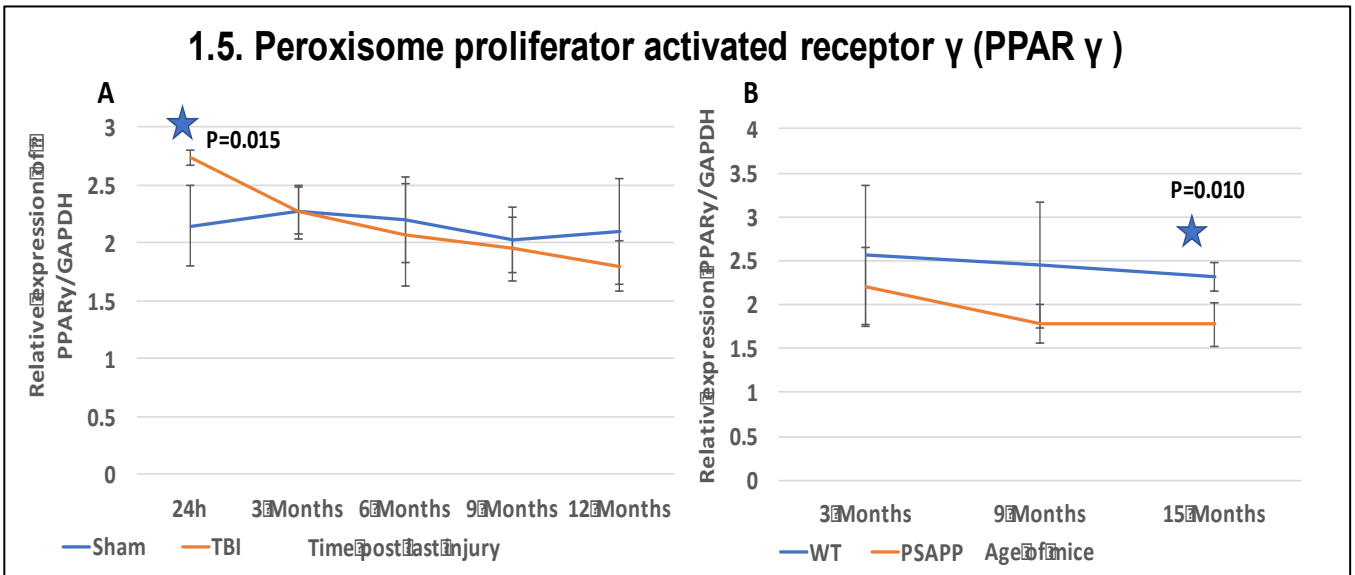

Fig 1.5. The effect of repetitive mild traumatic brain injury and amyloid pathology on the expression of PPARγ. Showing the average change in the relative expression of **PPAR γ** between sham injury or wild-type littermate mice (blue line) and r-mTBI or PSAPP mice (orange line) across various ages or time points post injury assessed by western blot. N= 4 mice per group. Statistical significance was assessed using a students T-test. Asterisks (*) denote P<0.05. These data suggest that while following an initial increase in the TBI model, potentially promoting cell survival, injured or diseased animal phenotypes show a trend towards a decrease in the expression of the PPARγ, supporting the potential intervention with thiazolinediones (TZDs). Within the literature it is well recognized that the promotion of PPARγ activation has the potential to reduce neuroinflammation and ameliorate AD associated pathologies. The next confirmatory step will be to assess the levels of phospho-PPARs and the levels Peroxisome proliferator activated receptor gamma coactivator 1 alpha (PGC1a).

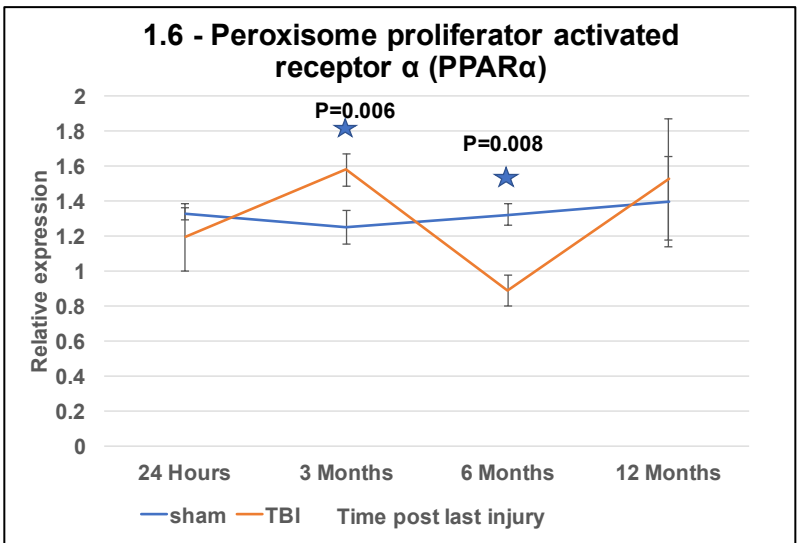

Fig 1.6. The effect of repetitive mild traumatic brain injury on the expression of PPARα. Showing the average change in the relative expression of PPARα between sham mice (blue line) and mice exposed to r- mTBI (orange line) across various time points post injury assessed by western blot. N= 4 mice per group. Statistical significance was assessed using a students T-test.

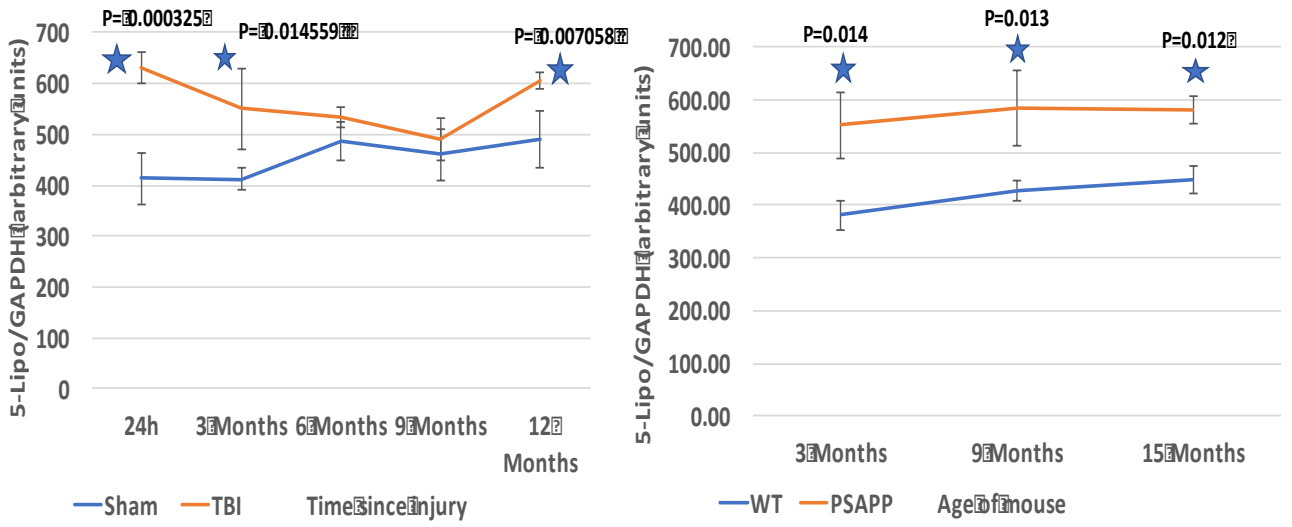

Fig 1.7. The effect of repetitive mild traumatic brain injury and amyloid pathology on the expression of 5-lipoxygenase. Showing the average change in the relative expression of **5-lipoxygenase** between sham injury or wild-type littermate mice (blue line) and r-mTBI or PSAPP mice (orange line) across various ages or time points post injury assessed by western blot. N= 4 mice per group. Statistical significance was assessed using a students T-test. Asterisks (*) denote P<0.05. These data suggest that 5-lipoxgenase is chronically upregulated in both TBI and AD. 5-Lipo plays a crucial role in the generation of inflammatory bio-lipids from Arachidonic acid, this enzyme has been suggested to be of great therapeutic potential in ameliorating neuroinflammation.

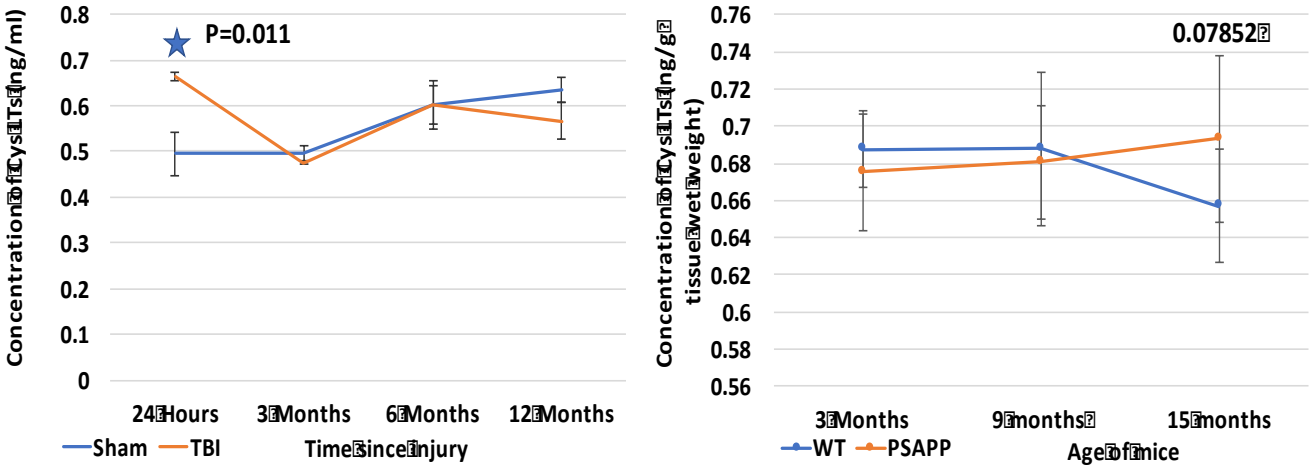

Fig 1.8. The effect of repetitive mild traumatic brain injury and amyloid pathology on the expression of Cysteinyl leukotrienes (LTC4,LTD4,LTE4). Showing the average change in the relative expression of Cysteinyl leukotrienes (LTC4,LTD4,LTE4) between sham injury or wild-type littermate mice (blue line) and r-mTBI or PSAPP mice (orange line) across various ages or time points post injury assessed by western blot. N= 4 mice per group. Statistical significance was assessed using a students T-test. Asterisks (*) denote P<0.05.

Below in Figure 1.9 and 1.10, we show cell suspensions from our TBI brain injury model that we are planning to use as a drug screening tool to demonstrate significant changes with our identified compounds. We plan to investigate changes in inflammatory cells in our treated mice for evaluation of impact of our compounds on glial reactivity and inflammatory phenotypes which are prominent features of our TBI model This study is currently ongoing, and data will be presented in the next quarterly reporting period.

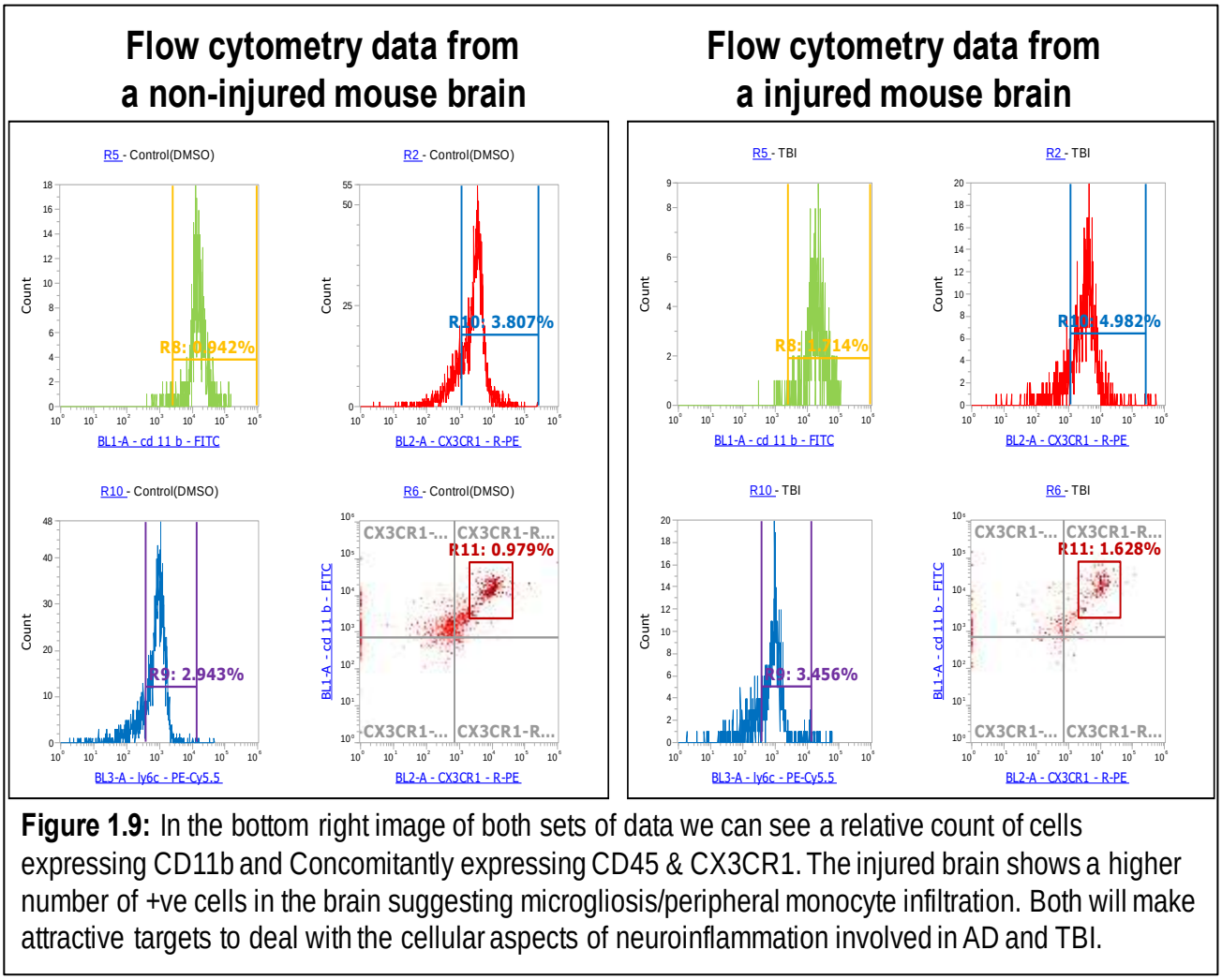

Figure 1.9: In the bottom right image of both sets of data we can see a relative count of cells expressing CD11b and Concomitantly expressing CD45 & CX3CR1. The injured brain shows a higher number of +ve cells in the brain suggesting microgliosis/peripheral monocyte infiltration. Both will make attractive targets to deal with the cellular aspects of neuroinflammation involved in AD and TBI.

Development of a glial screening assay to assess identified compound effect on reactive gliosis and inflammatory phenotype following injury and treatment

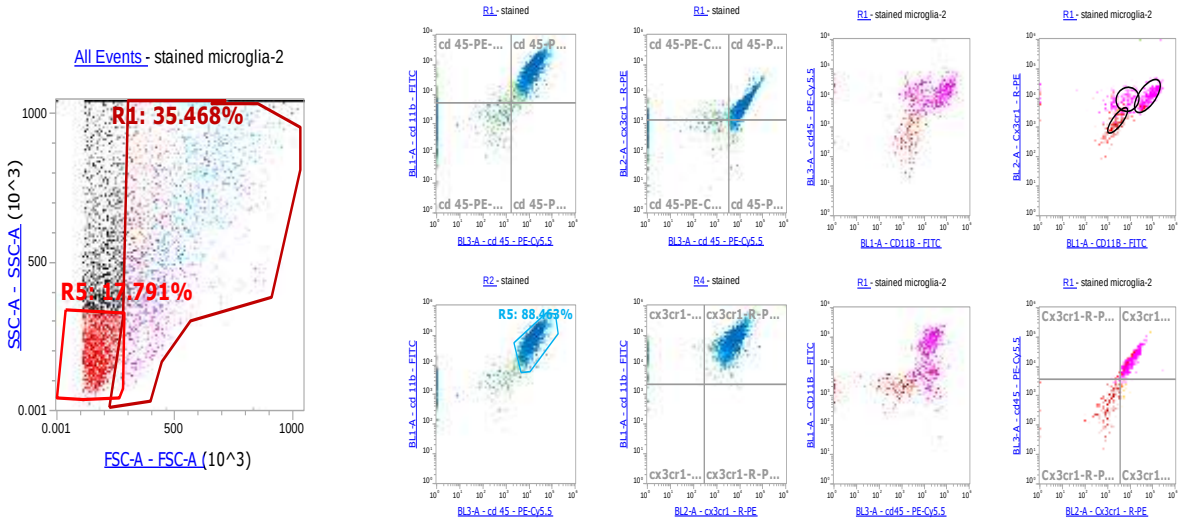

Figure 1.10: Flow cytometry figures show the potential to generate a single cell suspension from brain tissue, and with the aid of immunopanning we are able to isolate cells based on the CD11b epitope and identify 3 distinct cell populations within the brain, representing Microglia, perivascular macrophages and blood derived monocytes. We aim to use these cell suspension in a short-term ex vivo drug screen assay with our compounds to monitor impact on inflammatory phenotype and glial reactivity.

In the next series of figures/Tables, we present our experience with developing a mass spectrometry protocol for analyses of bioactive lipids. We have identified up to >20 bioactive lipids which we plan to screen in mice treated with compounds targeting lipid species. These bioactive lipids are part of the lipoxins, mediators of polyunsaturated fatty acids, leukotrienes, D and E series resolvins, prostaglandins, primary eicosanoids, and cysteinyl leukotrienes.

Table 1: list of bioactivie lipids for mass spectrometry analyses

	Name	Group	Exact mass	Found?
1	Lipoxin A4	lipoxin	351.2171	Y
2	Lipoxin B4	lipoxin	351.2171	Y
3	15(R)-Lipoxin A4	lipoxin	351.2171	Y
4	Arachidonic acid	FFA	303.2324	Y
5	Leukotriene b4	leukotriene	335.2222	Y
6	Resolvin E1	E-series resolvins	349.2015	Y
7	18-HEPE	E-series resolvins	317.2117	?
8	Eicosapentaenoic acid	FFA	301.2168	Y
9	Resolvin D1	D-series resolvins	375.2171	?
10	Resolvin D2	D-series resolvins	375.2171	?
11	Resolvin D3	D-series resolvins	375.2171	?
12	Resolvin D5	D-series resolvins	359.2222	N
13	17R-Resolvin D1	D-series resolvins	375.2171	?
14	Docosahexaenoic acid	FFA	327.2324	Y
15	Leukotriene C4	Cysteinyl-Leukotriene	624.2955	Y
16	Leukotriene D4	Cysteinyl-Leukotriene	495.2529	Y
17	Leukotriene E4	Cysteinyl-Leukotriene	438.2314	Y
18	Leukotriene F4	Cysteinyl-Leukotriene	567.274	Y
19	N-acetyl-Leukotriene E4	Cysteinyl-Leukotriene	480.242	N
20	Prostaglandin E1	Prostaglandin	353.2328	Y
21	Prostaglandin E2	Prostaglandin	351.2171	Y
22	Prostaglandin F1α	Prostaglandin	355.2484	Y
23	6-keto-Prostaglandin F1α	Prostaglandin	369.2277	Y
24	Prostaglandin F2α	Prostaglandin	353.2328	Y
25	Prostaglandin D2	Primary-Eicosanoids	351.2171	Y
26	Thromboxane B2	Primary-Eicosanoids	369.2277	Y

Table 2: LC-MS conditions used for mass spectrometry analyses for bioactive lipids

Liquid Chromatography conditions		Gradient profile		MS conditions	
A	Water + 0.1% FA	T=0	40% B	Resolution	35k
B	ACN:100mM AmFm:water 90/5/5 + 0.05%FA	4	75% B	AGC target	2e5
column	Kinetex 1.7u EVO C18 100A, LC Column 100 x 1.0 mm	4.5	99% B	Maximum IT	100ms
Temperature	40°C	4.98	99% B	Isolation window	2Da
Injection volume	1uL	4.99	40% B	Fixed first mass	50Da
Flow	50ul/min	9	40% B		

Example 1: Lipoxins Species: LXB4 mass spectra identified

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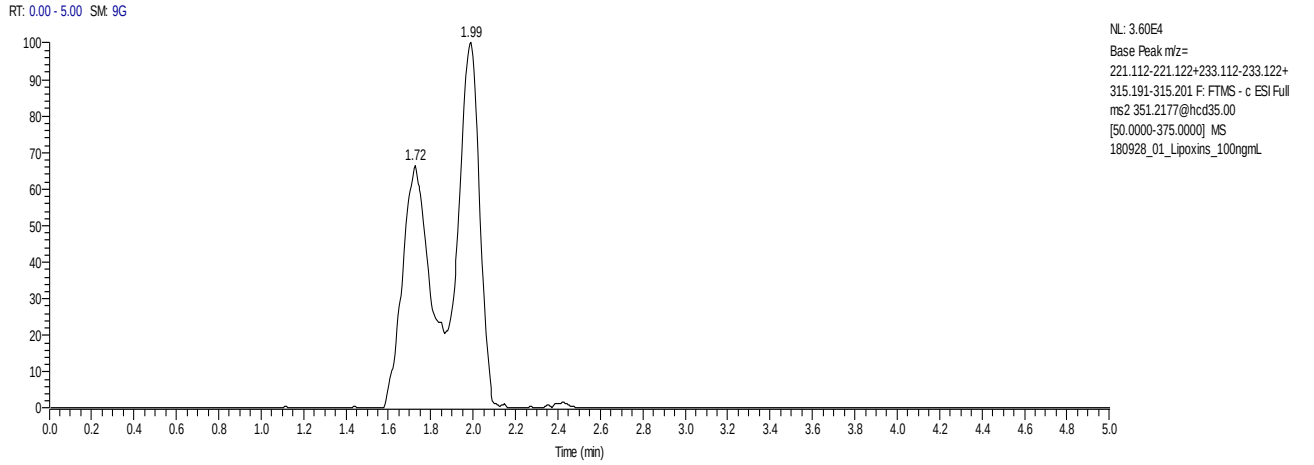

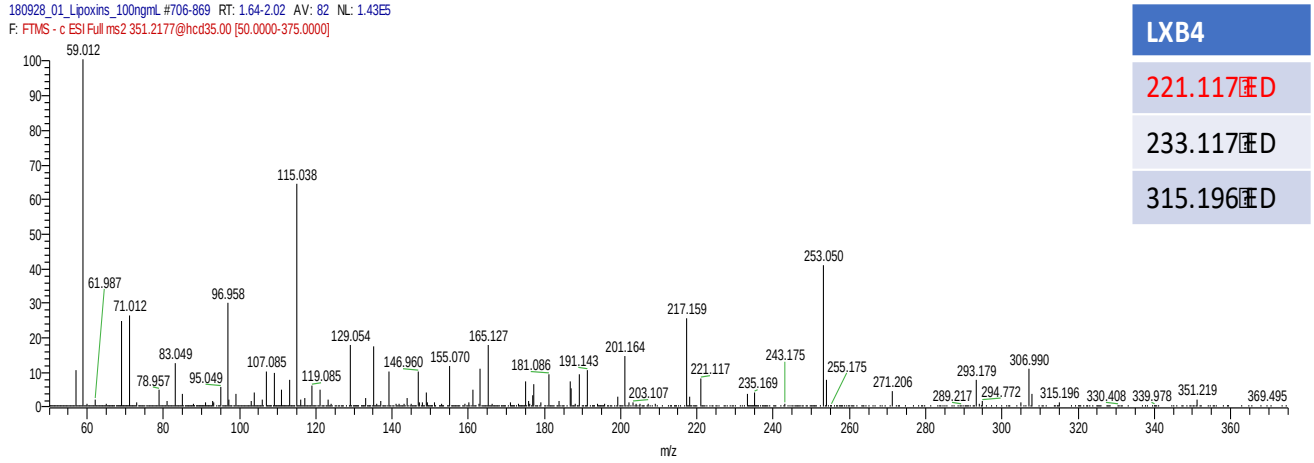

LXB4
221.117 ED
233.117 ED
315.196 ED

Example 2: E-series resolvins Species: Resolvin E1 mass spectra identified

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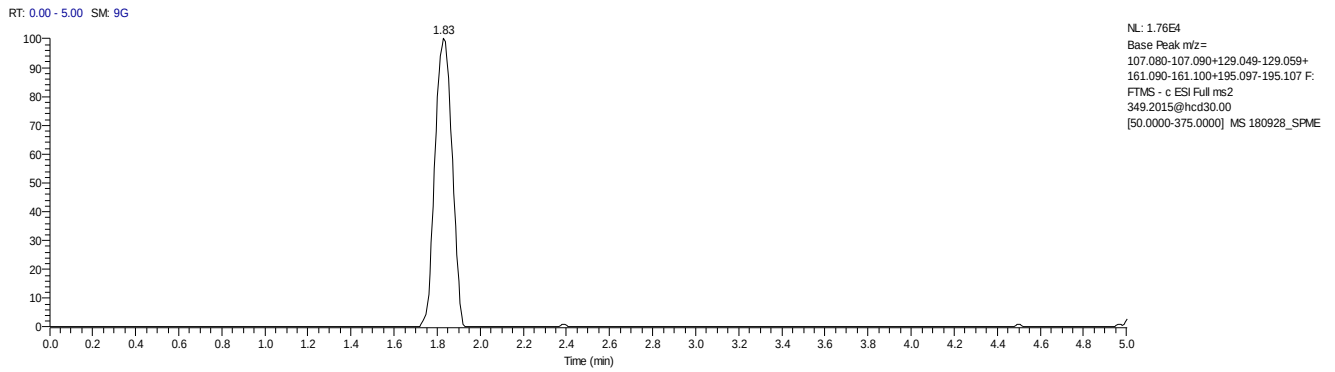

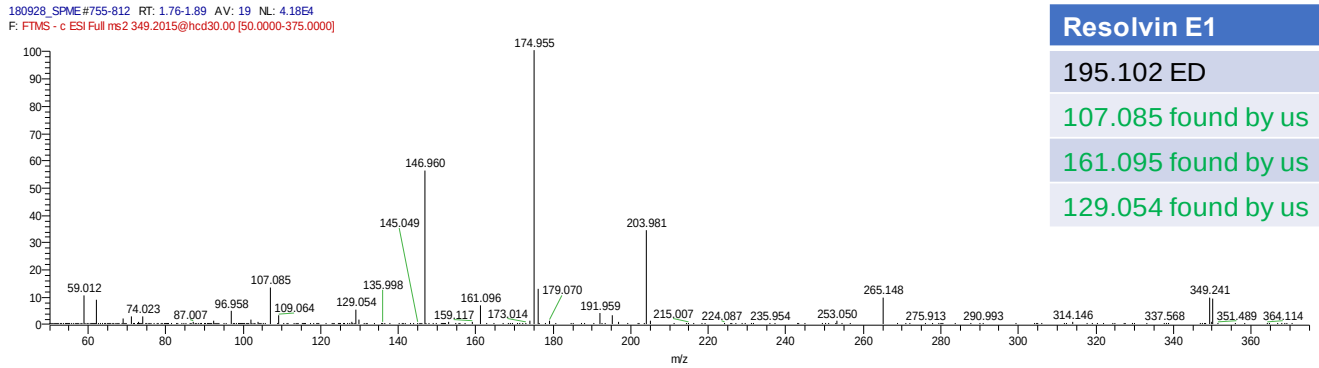

Resolvin E1

195.102 ED

107.085 found by us

161.095 found by us

129.054 found by us

Example 3: Cysteinyl leukotrienes Species: N-acetyl leukotriene E4 mass spectra identified

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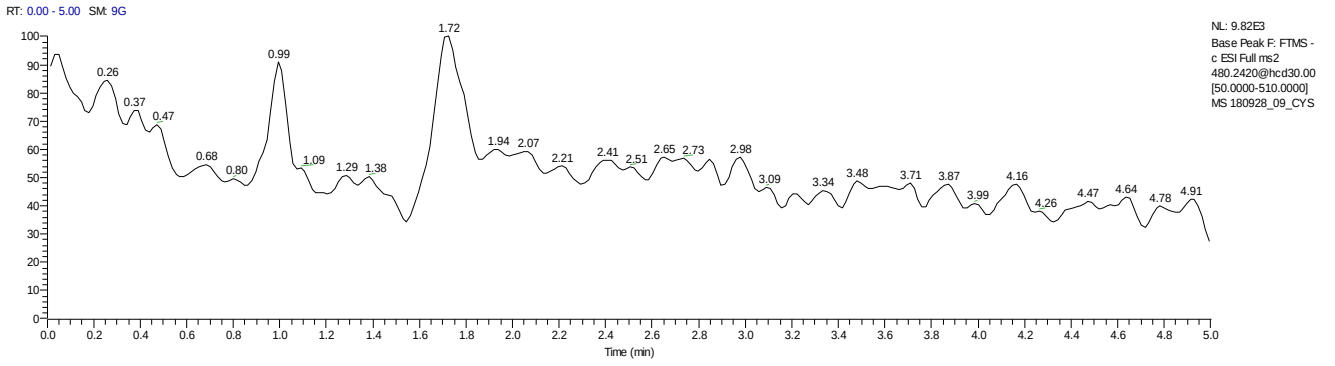
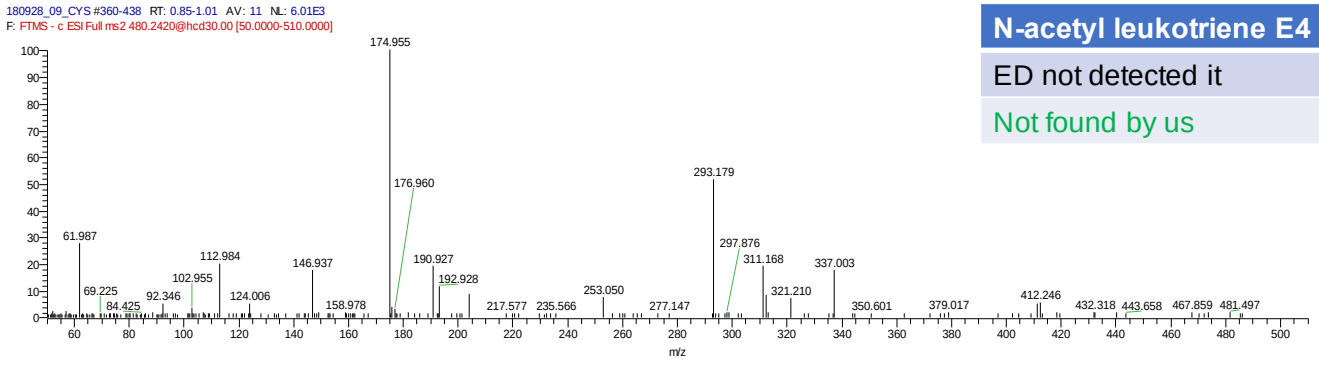


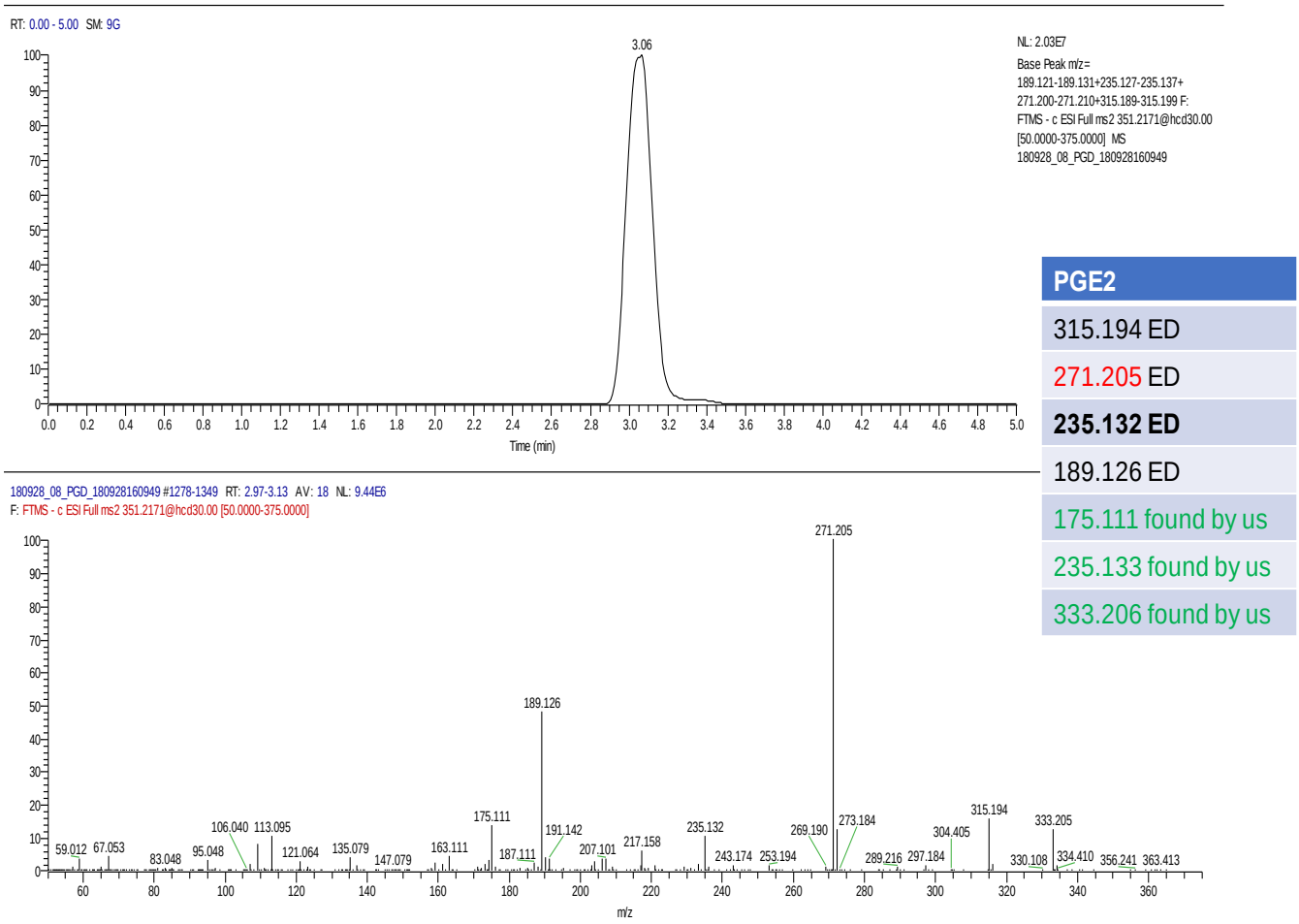


N-acetyl leukotriene E4

ED not detected it

Not found by us

Example 4: Prostaglandins Species: Prostaglandin E2 mass spectra identified



PGE2
315.194 ED
271.205 ED
235.132 ED
189.126 ED
175.111 found by us
235.133 found by us
333.206 found by us

We are currently conducting Subtask 3, 4, 5 and 7 which require staggering our mouse studies and administering TBI paradigms and compounds to modulate potential pharmacological molecular targets in our model in an acute study. No molecular data has been generated yet (currently ongoing), however we anticipate probing samples from this study and generating target engagement datasets within the next three months.

Subtask 3: Administration of r-mTBI or r-sham injury to 180-220 male hTau mice (C57BL/6 humanized tau transgenics) at 2-3 months of age. [96 mice of this request have been fulfilled].

Subtask 4: Administration of therapeutic compounds to mice for 2 weeks immediately prior to the 3-month time point post TBI/sham.

i. Administration of therapeutic compounds to target PI3K/Akt/insulin pathway – **Initiated currently underway**

ii. Target exploration of downstream eicosanoid pathway and administration of therapeutic compounds to target leukotriene signaling- **Completed, target engagement underway**

iii. Target exploration of downstream sphingomyelin pathway and administration of therapeutic compounds to target sphingomyelin signaling – **Completed, target engagement underway**

Subtask 5: Euthanasia of hTau mice at 3 months post-TBI/sham, followed by brain omic and antibody based analyses.

Subtask 7: Evaluation and selection of therapeutics for each target for use in Major Task 2

We began our first series of *in vivo* treatment and target engagement studies in our TBI mouse models. This study involves testing four main targets with drugs that modulate the cysteinyl leukotrienes, 5-lipoxygenase, PI3K/Akt insulin signaling and shingosine-1-phosphate signaling from which we have preliminary data from our models implicating a role in TBI pathogenesis. These studies used the compounds Monteleukast (leukotriene receptor antagonist), Zileuton (5-lipo-oxygenase inhibitor), intranasal Insulin and Fingolimod (sphingosine-1-phosphate receptor modulator). Mice were exposed to our injury paradigms and were left to age until 3 months post-TBI time points, and these compounds will be administered over the last 14days prior to euthanasia for analyses of target engagement and modulation of molecular targets and acute TBI neuropathology. There were 4 groups– [r-mTBI treated; r-mTBI vehicle; r-sham treated; r-sham control] each with 6 mice per group, totaling 96 mice. We anticipate completion of this first part of the study in the next three months. During this period, we plan to begin (and stagger) a separate batch of analyses involving an additional four compounds using the same TBI paradigms and treatment regimen described above.

We are also validating banked human tissue (in subtask 6) in our lab for markers related to the pathways above.

Subtask 6: Validation of identified targets in human TBI brains with history of repetitive concussions

- **What opportunities for training and professional development has the project provided?**
  Nothing to Report
  - *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.

- **How were the results disseminated to communities of interest?**

Nothing to report

- *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.*

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

Over the next reporting period, we plan to accomplish the subtasks below:

Subtask 4: **Molecular analyses for target engagement studies** of mice treated with Monteleukast, Zilueton, intranasal insulin and fingolimod.

Finish development of a mass spectrometry protocol for measuring bioactive lipids in the brain.

Utilize our single cell suspension method (i.e. flow cytometry, magnetic cell sorting, immunoblotting) to closely assess changes in inflammatory phenotype following treatment.

Analyses of acute-TBI histopathological outcome (assessing glial activation and neuroinflammatory markers, extent of axonal injury, and white matter thinning, and impact on tauopathy).

Subtask 7: **Complete molecular (ELISA, immunoblotting, and LC-MS) studies for identified pathways of interest** in human autopsy tissue from TBI and AD patients.

Over the next reporting period, we also plan to initiate the subtasks below:

Subtask 4: Administration of therapeutic compounds to target:

Peroxisomal function (RXR/PPAR signaling)

(iv) Pioglitazone a PPARγ agonist administered at 20mg/kg orally in chow; ii) Oleoylethanolamide 10mg/kg i.p. daily; iii) Baxerotene administered by oral gavage at 5mg/kg daily. For each therapeutics there will be 4 groups– [r-mTBI treated; r-mTBI vehicle; r-sham treated; r-sham control] each with 6 mice.

PI3K/Akt/mTOR pathway

(v) IPLEX will be administered at 60 mg/kg/day via i.p injection; (ii) intranasal insulin delivery, 1.75U/17.5ul insulin; (iii) PTEN inhibitor administered at 0.2 mg/kg of BPV(pic) by I.P injection. For each therapeutics there will be 4 groups– [r-mTBI treated; r-mTBI vehicle; r-sham treated; r-sham control] each with 6 mice.

DHA imbalance

(vi) fish oil 15 mL/kg by daily oral gavage; (ii) DHA-LPC 8mg/kg/daily in chow. For each therapeutics there will be 4 groups– [r-mTBI treated; r-mTBI vehicle; r-sham treated; r-sham control] each with 6 mice.

ALL plans are consistent with the original approved SOW.

These studies will involve administration of r-mTBI or r-sham injury to a fraction of the 180-220 male hTau mice required in this subtask at 2-3 months of age

Animals will receive closed head injuries with an electromagnetic impounder. For each compound, there will be 4 groups– [r-mTBI treated; r-mTBI vehicle; r-sham treated; r-sham control] each with 5-6 mice per group. All mice will be on the C57BL/6 background.

4. **IMPACT:** *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

- **What was the impact on the development of the principal discipline(s) of the project?** Nothing to Report
- **What was the impact on other disciplines?** Nothing to Report
- **What was the impact on technology transfer?** Nothing to Report
- **What was the impact on society beyond science and technology?** Nothing to Report

5. **CHANGES/PROBLEMS:**

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

We propose to conduct our acute treatment studies in a similar chronic repetitive mTBI model paradigm (see Ojo et al., 2016, Lynch et al., 2016) as we consider this paradigm to recapitulate more of the human neuropathology of repetitive mTBI compared to our 5hit paradigm. Most of our current work has shifted in this direction towards this chronic repetitive mTBI mouse model paradigm. We have observed more prominent involvement of lipid dysregulation in this model which we believe will be a better platform for this current project. This newer model will be administered over a period of one month with a total

of 20 exposures (5 days per week). An amendment has been accepted for our ACURO modification. Time point of analyses will still be the same, i.e at 3 months post injury, with treatment regimen remaining the same over the last 14days prior to euthanasia.

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

  - We have been having issues with generating sufficient number of hTau mice in our acute treatment studies which require a total of 220 mice. We require male hTau mice which are generated from a cross between TauKO and hTau heterozygotes; 25% off the offspring's will be male hTau mice. Giving that the hTau mice were generated on the C57BL/6 background, we have proposed changing the initial acute studies to C57BL6 mice, as similar molecular effects particularly in lipid profiling are shown in our preliminary data from our previous PRARP award project. We have put in an amendment for the IACUC and ACURO protocol which we believe will facilitate and expedite the efficient screening of compounds in a timely fashion in a more readily and commercially available C57BL6 mouse model. For our chronic studies we will still use the hTau model, to demonstrate the TBI-AD link and role of tau pathology in the TBI pathobiological process which requires chronic time points for seeding pathology and is less relevant to the acute treatment studies we are currently conducting.

  - Anticipated problem – Finishing the acute treatment studies within the first 15 months as previously proposed. Our ACURO protocol was accepted in late March and also we have sent in an additional amendment to include the use of C57BL6 mice for our acute treatment studies and to replace our TBI paradigm to a more chronic r-mTBI model. We anticipate that this will set us back by a few months from our initial timeline, and we anticipate generating all the acute treatment and target engagement data 3-4 months later than our initial schedule in the statement of work.

- **Changes that had a significant impact on expenditures** Nothing to Report
- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents** Nothing to report
- **Significant changes in use or care of human subjects:** 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:** *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."* Nothing to Report

- **Publications, conference papers, and presentations ?** Nothing to Report.

  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). Nothing to Report

- **Books or other non-periodical, one-time publications.** *Nothing to Report*
- **Other publications, conference papers, and presentations.** *Nothing to Report*

- **Website(s) or other Internet site(s)** *Nothing to Report*
- **Technologies or techniques** *Nothing to Report*
- **Inventions, patent applications, and/or licenses** *Nothing to Report*
- **Other Products** *Nothing to Report*

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

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

Personnel	**Researcher Identifier**	**Role**	**Nearest Person Month Worked**	**Contribution to Project**	**Funding Support**
Dr. Fiona Crawford	N/A	Principal Investigator	2	Principal Investigator	N/A
Dr. Joseph Ojo	N/A	Co-Principal Investigator	3	Co-Principal Investigator	N/A
Dr. Benoit Mouzon	N/A	Co-Investigator	1	Co-Investigator – TBI animal models	N/A
Dr. Daniel Paris	N/A	Co-Investigator	1	Co-Investigator – Drug Discovery	N/A
Dr. Laila Abdullah	N/A	Co-Investigator	1	Co-Investigator - Lipidomics	N/A
James Evans	N/A	Consultant	1	Consultant – lipid biology	N/A

Andrew Pearson MS	N/A	Consultant	1	Consultant	N/A
Dr. Michael Mullan	N/A	Consultant	1	Consultant	N/A
Dr. William Stewart	N/A	Consultant	1	Consultant	N/A
Dr. Elliott Mufson	N/A	Consultant	1	Consultant	N/A

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?** None
- **What other organizations were involved as partners?** Nothing to Report

8. **SPECIAL REPORTING REQUIREMENTS**
   - **COLLABORATIVE AWARDS:** ***N/A***
   - **QUAD CHARTS:** *If applicable* ***N/A***
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. Reminder: Pages shall be consecutively numbered throughout the report.* ***DO NOT RENUMBER PAGES IN THE APPENDICES.*** Nothing to Report