AWARD NUMBER: W81XWH-22-1-0714

TITLE: Advanced Patient-Derived Microglia Assay for Preclinical and Clinical Trial Drug Validation

PRINCIPAL INVESTIGATOR: Associate Professor Anthony White

CONTRACTING ORGANIZATION: QUEENSLAND INSTITUTE OF MEDICAL RESEARCH
Herston, QLD, Australia

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PREPARED FOR: U.S. Army Medical Research and Development Command
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14. ABSTRACT

In this proposal, we are investigating the potential of a new model system to culture microglia from people with ALS, and demonstrate that this model is ideal for patient pre-selection for clinical trials, and for ongoing monitoring of drug action during trials. The model involves preparation of microglia directly from cells in the blood called monocytes. The presence of pathological changes in ALS monocyte-derived microglia provides us with the unique opportunity to determine if drugs are able to induce normal outcomes in these cells, and therefore, more likely improve function in people with ALS. The initial aim is to benchmark monocyte-derived microglia against other microglia commonly used for drug screening (iPSC-derived microglia, and mouse SOD1 ALS model microglia). The annual report predominantly covers this aim, and preparations for the subsequent aims of demonstrating that patient monocyte-derived microglia provide a clinically relevant platform for assessment of ALS drug efficacy, and demonstrating that patient microglia TDP-43 aggregation and phagocytosis are qualifiable biomarkers for microglia-targeted drug development.

15. SUBJECT TERMS
None listed.

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1. INTRODUCTION:

In this proposal, we are investigating the potential of a new model system to culture microglia from people with ALS, and demonstrate that this model is ideal for patient pre-selection for clinical trials, and for ongoing monitoring of drug action during trials. The model involves preparation of microglia directly from cells in the blood called monocytes. The presence of pathological changes in ALS monocyte-derived microglia provides us with the unique opportunity to determine if drugs are able to induce normal outcomes in these cells, and therefore, more likely improve function in people with ALS. The project involves proof of concept screening of drugs using these microglia.

2. KEYWORDS:

Monocyte microglia, TDP-43, phagocytosis, drug screening, biomarker,

3. ACCOMPLISHMENTS:

p

What were the major goals of the project?

Specific Aim 1: Benchmarking of human monocyte-derived microglia against current microglia for ALS drug testing.

Major Task 1 Establishment of ALS patient monocyte-derived microglia, ALS patient iPSC-derived microglia, and SOD1G93A mouse microglia (to benchmark monocyte-derived microglia against the other two currently used ALS microglia models).

Subtask 1 - QIMRB-MRI institutional review board (IRB) and USAMRDC Human Research Protection Office (HRPO) approval for generating human monocyte-derived microglia.
Projected date: (1-4 months) 30th Nov 2022.
Completion date and percentage completed: 23rd June 2022 (100% completed)

Subtask 2 - QIMRB-MRI institutional review board (IRB) and USAMRDC Human Research Protection Office (HRPO) approval for generating human iPSC-derived microglia.
Projected date: (1-4 months) 30th Nov 2022.
Completion date and percentage completed: 23rd June 2022 (100% completed)

Subtask 3 – The University of Melbourne institutional animal care and use committee (IACUC) and USAMRDC Animal Care and Use Review Office (ACURO) approval for generating mouse primary microglia.
Projected date: (1-4 months) 30th Nov 2022.
Completion date and percentage completed: 23rd June 2022 (100% completed)

Subtask 4 - Generate cultures of ALS patient monocyte-derived microglia from PBMCs.
Projected date: (4-9 months) 30th April 2023
Completion date and percentage completed: Expected completion 31st Oct 2023 (50% completed)

Subtask 5 - Generate cultures of ALS patient iPSC-derived microglia.
Projected date: (4-9 months) 30th April 2023
Completion date and percentage completed: Expected completion 31st Oct 2023 (50% completed)

Subtask 6 - Generate cultures of SOD1G93A mouse primary microglia.
Projected date: (4-9 months) 30th April 2023
Completion date and percentage completed: Expected completion 31st Oct (50% completed)

Major Task 2 Compare gene and protein expression, and functional behavior of microglia markers in monocyte-derived, iPSC-derived, and mouse-derived microglia.

Subtask 1 - Treat cultures with test drugs as listed in Table 1 of Project Narrative.
Projected date: (10-13 months) 31st August 2023
Completion date and percentage completed: Expected completion 30th Nov 2023 (50% completed)

Subtask 2 - Measure microglia gene marker expression by qRT-PCR
Projected date: (10-13 months) 31st August 2023
Completion date and percentage completed: Expected completion 30th Nov 2023 (50% completed)

Subtask 3 - Measurement of microglia migration, ramification (branching) state and process movement using a Spinning Disk confocal microscope**.**
Projected date: (10-13 months) 31st August 2023
Completion date and percentage completed: Expected completion 31st Dec 2023 (25% completed)

Subtask 4 - Measurement of phagocytosis of fluorescently-labelled particles using Cytell Cell Imaging System) and/or IncuCyte Zoom.
Projected date: (10-13 months) 31st August 2023
Completion date and percentage completed: Expected completion 31st Dec 2023 (25% completed)

Subtask 5 - Measurement of cytokine responses by cytometric bead array and qPCR.
Projected date: (10-13 months) 31st August 2023
Completion date and percentage completed: Expected completion 31st Dec 2023 (25% completed)

Major Task 3 Measure gene expression in each type of microglia with and without drug treatment.

Subtask 1 - Collect RNA from cultures after treated with drug or vehicle.
Projected date: (10-12 months) 31st July 2023
Completion date and percentage completed: Expected completion 30th Nov 2023 (50% completed)

Subtask 2 - Perform RNA-seq analysis.
Projected date: (10-12 months) 31st July 2023
Completion date and percentage completed: Expected completion 31st Dec 2023 (25% completed)

Subtask 3 - Compare gene expression profiles for monocyte-derived, and iPSC-derived, microglia under basal and drug treated conditions.
Projected date: (10-12 months) 31st July 2023
Completion date and percentage completed: Expected completion 31st Jan 2024 (10% completed)

Specific Aim 2: Demonstrate that patient monocyte-derived microglia provide a clinically relevant platform for assessment of ALS drug efficacy.

Major Task 4 Demonstrate that monocyte-derived ALS patient microglia can provide a platform for rapid assessment of clinically efficacious drugs.

Subtask 1 - Generate cultures of ALS patient monocyte-derived microglia from PBMCs.
Projected date: (13-16 months) 30th Nov 2023
Completion date and percentage completed: Expected completion 31st Jan 2024 (10% completed)

Subtask 2 - Treat all cultures with test drugs as listed in Project Narrative.
Projected date: (13-16 months) 30th Nov 2023
Completion date and percentage completed: Expected completion 31st Jan 2024 (10% completed)

Subtask 3 - Measurement of phagocytosis of fluorescently-labelled particles as above.
Projected date: (13-16 months) 30th Nov 2023
Completion date and percentage completed: Expected completion 31st Jan 2024 (0% completed)

Subtask 4 - Measurement of cytokine responses by cytometric bead array and qPCR.
Projected date: (13-16 months) 30th Nov 2023
Completion date and percentage completed: Expected completion 28th Feb 2024 (0% completed)

Subtask 5 - Measurement of TDP-43 aggregation.
Projected date: (13-16 months) 30th Nov 2023
Completion date and percentage completed: Expected completion 28th Feb 2024 (0% completed)

Specific Aim 3: Demonstrate that patient microglia TDP-43 aggregation and phagocytosis are qualifiable biomarkers for microglia-targeted drug development.

Major Task 5 Demonstrate the impact of selected example drugs to modify ALS hallmark pathology (TDP-43 and impaired phagocytosis) in ALS patient monocyte-derived microglia.

Subtask 1 - Generate cultures of ALS patient monocyte-derived microglia from PBMCs.
Projected date: (17-20 months) 31st Mar 2024
Completion date and percentage completed: Expected completion 31st May 2024 (0% completed)

Subtask 2 - Treat all cultures with test drugs as listed in Project Narrative and any additional drugs shown to have significant effect on TDP-43 or phagocytosis in Aims 1 or 2).
Projected date: (17-20 months) 31st Mar 2024
Completion date and percentage completed: Expected completion 31st May 2024 (0% completed)

Subtask 3 - Measurement of phagocytosis of fluorescently-labelled particles as above.
Projected date: (17-20 months) 31st Mar 2024
Completion date and percentage completed: Expected completion 31st May 2024 (0% completed)

Subtask 4 - Measurement of TDP-43 aggregation by immunofluorescence (and Imaris software).
Projected date: (17-20 months) 31st Mar 2024
Completion date and percentage completed: Expected completion 31st May 2024 (0% completed)

Major Task 6 Measure global gene expression of drug-treated cultures to determine if biomarker genes of TDP-43 aggregation or phagocytosis impairment can be identified.

Subtask 1 - Collect RNA from cultures after treated with drug or vehicle. 17-20 X
Projected date: (17-20 months) 31st Mar 2024
Completion date and percentage completed: Expected completion 30th June 2024 (0% completed)

Subtask 2 - Perform RNA-seq analysis. 17-20 X
Projected date: (17-20 months) 31st Mar 2024
Completion date and percentage completed: Expected completion 30th June 2024 (0% completed)

Subtask 3 - Compare gene expression profiles for basal and drug-treated conditions.
Projected date: (17-20 months) 31st Mar 2024
Completion date and percentage completed: Expected completion 30th June 2024 (0% completed)

Major Task 7 Measure global protein expression (proteomics) of drug-treated cultures to determine if biomarker proteins of TDP-43 aggregation or phagocytosis impairment can be identified.

Subtask 1 - Collect protein from cultures after treated with drug or vehicle.
Projected date: (20-24 months) 31st July 2024
Completion date and percentage completed: Expected completion 30th Sept 2024 (0% completed)

Subtask 2 - Perform proteomics analysis.
Projected date: (20-24 months) 31st July 2024
Completion date and percentage completed: Expected completion 30th Sept 2024 (0% completed)

Subtask 3 - Compare protein expression profiles for basal and drug-treated conditions.
Projected date: (20-24 months) 31st July 2024
Completion date and percentage completed: Expected completion 30th Sept 2024 (0% completed)

What was accomplished under these goals?

Specific Aim 1: Benchmarking of human monocyte-derived microglia against current microglia for ALS drug testing.

Major Task 1 Establishment of ALS patient monocyte-derived microglia, ALS patient iPSC-derived microglia, and SOD1G93A mouse microglia (to benchmark monocyte-derived microglia against the other two currently used ALS microglia models).

Major Task 2 Compare gene and protein expression, and functional behavior of microglia markers in monocyte-derived, iPSC-derived, and mouse-derived microglia.

Major Task 3 Measure gene expression in each type of microglia with and without drug treatment.

The first major activity was Specific Aim 1, Major Task 1 as indicated above. This aim seeks to establish how monocyte-derived microglia (generated from ALS and control blood peripheral monocytes (PBMCs)) compare to other common ALS microglia models, including from induced pluripotent stem cells (iPSCs), and ALS murine model microglia. The initial 3 subtasks under Major Task 1 were to establish the human and animal institutional ethics (and HRPO) approval for undertaking the research on human and animal microglia respectively. The ethics approvals were all achieved. Subtasks 4-6 under Major Task 1 then seeks to establish the monocyte-derived, iPSC-derived, and murine-derived microglia cultures for which the subsequent drug testing and analysis requires. As described above under 'Major goals and percentage completion', we are 50% completed for these Subtasks. The Subtasks requires the cell culture of microglia generated from each of the three sources (monocytes, iPSC cells, and ALS model mice). This also necessarily requires the complex coordination of all three approaches to help achieve a valid platform for comparison of drug testing outcomes in the subsequent Major Task 2 and Major Task 3. We have collected all the required cell lines for ALS patients (monocytes and iPSC lines), and bred the SOD1 ALS model mice for generation of the murine microglia. We experienced some difficulties associated with establishing all three approached including some delay in obtaining additional ALS patient monocytes from the National ALS Biorepository,

some issues with generation of consistent iPSC-microglia, and additional time to establish the SOD1 ALS mouse numbers required (all detailed further in Section 5 below). However, we have overcome these issues and have ALS patient and control monocyte-derived microglia (Site 1), iPSC-derived microglia (Site 1), and SOD1 ALS mouse microglia (Site 2) all currently growing in culture, and being treated with the drugs listed in the Project Narrative for this Aim. Examples of these cultures are shown in Figures 1-3 below, including data from monocyte-derived microglia cultures (Figure 1). This shows that in the initial analysis of ALS patient and healthy control monocyte-derived microglia, we can induce inflammatory stimulation with LPS. Upon co-treatment with Ibudilast or HU-308, we see inhibition of a key pro-inflammatory cytokine, interleukin 8 (IL-8) secretion. We are now proceeding with further testing of the drugs outlined below and undertaking broader analysis of the anti-inflammatory effects.

We also show the pre-screening analysis of iPSC-derived microglia (IMG) compared to monocyte-derived microglia (MDMI) and the original hematopoietic progenitor cell line (IHPC) for comparison (Figure 2). Interestingly, there are a number of differences in the expression levels of different microglia marker genes between IMG and MDMI, suggesting that this could potentially lead to differences and broader gene expression changes in responses to drug treatment when the analysis for Major Tasks 2 and 3 are complete. Figure 3 shows microglia growing from murine SOD1 ALS model and ready for drug treatment. All required drugs have been pre-screened for optimal testing concentration to ensure no toxic effects are induced. These include:
1) Ibudilast (10μM, 1μM, 0.1μM)
2) HU-308 (1μM, 0.1μM, 0.001μM)
3) TG101209 (0.1μM, 0.05μM, 0.01μM)
4) Masitinib (10uM, 5uM, 1uM, 0.1)
5) BLZ945 (10μM, 1μM, 0.1μM, 0.01μM)
6) Cu(atsm) (0.1 μM, 1μM, 2 μM)

BLZ945 (CSR1 receptor targeting anti-inflammatory) has replaced RNS60 due to difficulty in obtaining the latter. We have also established in preliminary pre-screening that these concentrations are optimal to inhibit pro-inflammatory action, based on initial determination of cytokine IL-8 expression levels in monocyte-derived microglia (MDMI) cultures. The drugs are now being tested at Site 1 (ALS MDMI and iMG) and Site 2 (SOD1 ALS murine microglia). Due to the issues as outlined briefly above and in more detail in Section 5 below, we have adjusted the anticipated completion date for Major Task 1 to 31st Oct 2023. This will allow us to complete the establishment and treatment of all cultures for the subsequent comparisons in Major Tasks 2 and 3.

Although Major Tasks 2 has an expected completion date of 31st August 2023 (just beyond the current reporting period), we have included this Major Task in the report as it is tied closely with Major Tasks 1 and 3. Major Task 2 covers the following subtasks:
Subtask 1 - Treat cultures with test drugs as listed in Table 1 of Project Narrative.
Subtask 2 - Measure microglia gene marker expression by qRT-PCR.
Subtask 3 - Measurement of microglia migration, ramification (branching) state and process movement using a Spinning Disk confocal microscope**.**
Subtask 4 - Measurement of phagocytosis of fluorescently-labelled particles using Cytell Cell Imaging System) and/or IncuCyte Zoom.
Subtask 5 - Measurement of cytokine responses by cytometric bead array and qPCR.
As indicated above, these are 25-50% complete with analyses being undertaken across these assays.
Due to the delays, we have shifted the expected completion date of Major Task 2 to 30th Nov and 31st Dec for the Subtasks. While this is some months later than anticipated, we are able to make up time on the rest of the aims, with only a small projected over-run of the completed project in 2024. The ability to make up the time is because of the repetitive nature of the culturing, testing, and analysis across the different aims, and being able to run a number of assays in parallel to streamline outcomes. Since submission of the application, we have established a more streamlined data processing and analysis approach which helps up to generate the desired outcomes sooner. This has been reported for analysis of drug testing on Alzheimer's disease microglia by us (see Cuni-Lopez, bioRxiv 2023.08.17.552618).

The progress of Major Task 3 has also been affected by the delays and we now anticipate completion of the 3 subtasks between Nov 2023 and Jan 2024. This includes:
Subtask 1 - Collect RNA from cultures after treated with drug or vehicle.
Subtask 2 - Perform RNA-seq analysis.
Subtask 3 - Compare gene expression profiles for monocyte-derived, and iPSC-derived, microglia under basal and drug treated conditions.
As for Major Task 2, we do not expect this to have a great deal of impact on the overall project due to our current streamlining of testing protocols and analysis which will allow us to finish the remaining aims more quickly than anticipated. As reported by us (Rantanen, Cells. 2022 PMID: 36291125; Lampinen, Cells. 2022 PMID: 35203328; Wasielewka, Theranostics. 2022 PMID: 36276649) we now have considerable expertise in performing RNA-seq analysis and are able to run samples, and analyse data on gene expression more rapidly than anticipated when the application was being submitted.

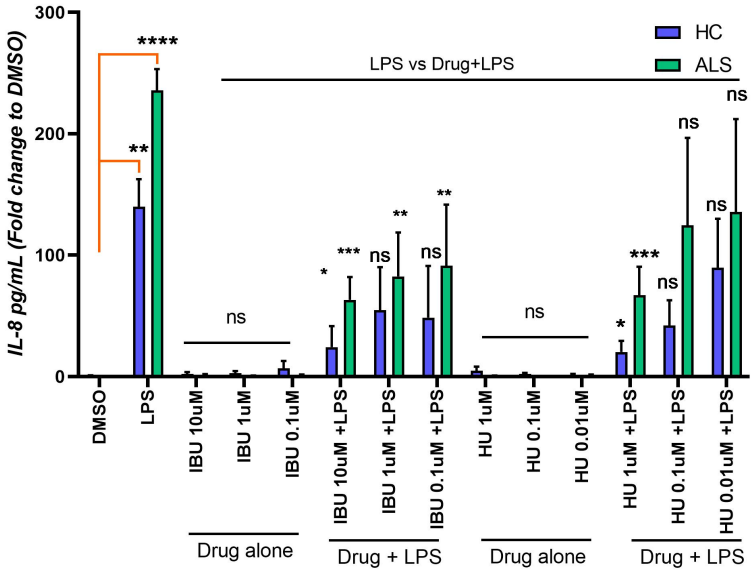

Figure 1: IL-8 secretion from ALS and healthy control (HC) monocyte-derived microglia with LPS alone or co-treatment with Ibudilast (IBU) or HU-308 (HU). *p<0.05 -***p<0.0001.

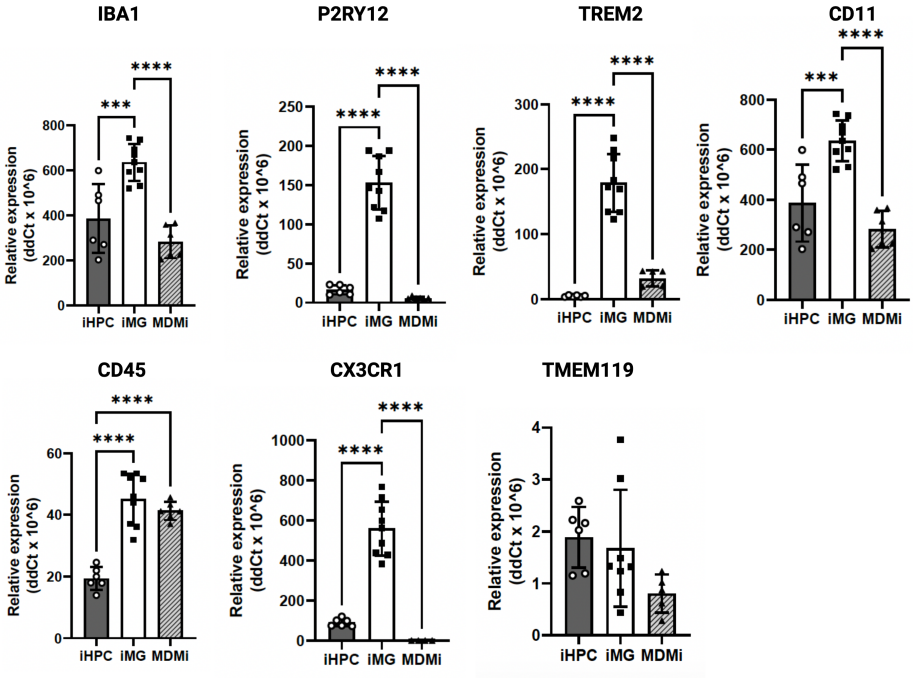

Figure 2: induced microglia (IMG) grown from human hematopoietic progenitor cells (IHPC), and compared to human monocyte-derived microglia (MDMI). Expression of key microglia marker genes determined by qRT-PCR. ***p<0.05, ****p<0.01.

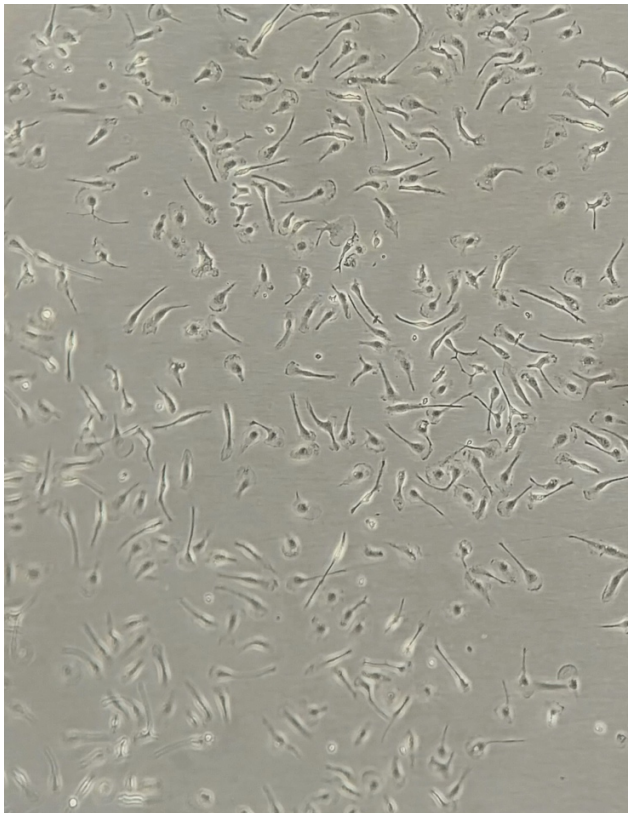

Figure 3: Microglia from SOD1 ALS model mice immediately after removal of astrocytes.

What opportunities for training and professional development has the project provided?

This project has provided training to PhD student (now Dr) Carla Cuni-Lopez, and PhD graduate Fazeleh Etebar. Dr Hazel Quek (post-doc) has provided key training to Carla and Fazeleh, including culture of monocyte-derived microglia, assays to analyse pathological changes, and measurement of responses to drugs. This has also involved development and training in how to analyse data from multiple assays to determine drug effect at a personalized level and has led to a manuscript submitted to Brain Behaviour and Immunity for analysis of drug effects on Alzheimer's disease monocyte-derived microglia.

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?

We have all the cells needed to complete the project, and all techniques have been developed and validated. We have determined all the optimal concentrations for testing of the drugs for all aims. To complete the project goals we are routinely generating microglia cultures, treating with drugs, and performing assays and data analysis. We will make up time by running more cultures and analysis in parallel. This will be enhanced by the addition of Dr Fazeleh Etebar to our team. Dr Etabar has skills in microglia culture, and data analysis, and has been further trained by our post-doc Dr Hazel Quek. Dr Etebar will help Dr Quek with all aspects of the project, which will expedite the research towards our goals. We have also developed a rapid data analysis process as reported in our manuscript submitted to Brain Behaviour and Immunity. This will allow us to generate final data analysis on large data sets from drug treatments more rapidly to provide key outcomes for the remaining goals.

4. IMPACT:

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

Our research using this ALS monocyte-derived microglia platform is rapidly establishing this as an optimal new model for drug screening and disease understanding in ALS and other neurodegenerative diseases. The number of publications using monocyte-derived microglia instead of iPSC-derived microglia is increasing. Within Australia, we are now in discussions with the major ALS research groups around the country to establish a national biorepository of ALS PBMCs as a source for drug screening for the ALS research community. In addition, the bioanalysis approach we have developed and is currently under review at Brain Behaviour and Immunity, has the potential to provide a new means of interpreting drug effects at the personalized level, rather than assessing drug effects on cohorts where there is a wide variety of drugs responses.

What was the impact on other disciplines?

Nothing to Report.

What was the impact on technology transfer?

Although this research has not yet led to a start-up, it has gained interest from a small biotech firm in Australia (*GenieUs*) who are working with us to develop their leading drug compound for treatment of ALS. While they are funding this aspect of the work, the assessment of the drug in parallel to our CDMRP-funded studies has the potential to develop new commercial outcomes.

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS:

While there have been some minor problems and delays (outlined below), there have been no major problems. There has been one change which was discussed with our grant contact, and agreed upon. We have been approached by a small biotech company in Australia to test a drug in our ALS monocyte-microglia platform. As this is an opportunity for a 'real world' drug test, we wished to compare this drug alongside the drugs listed in the Project Narrative to determine how this proprietary drug faired against the ones we selected. It was agreed that provided the costs for this new drug were covered by the external company then there were no issues with this. We are finalizing the contract with the external company and will be testing their drug alongside the ones we have selected (their drug funded by the drug company). This has led to a slight delay as we await the delivery of the drug and determine final concentrations to be tested. However, we believe this is worth it as the whole reason for this project is to determine if our platform can be used for drug testing purposes so it is an excellent opportunity to demonstrate this using a newly developed drug from a drug company. This will increase the impact of the outcomes from our work.

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

The protocol used to generate iPSC-derived microglia was found to be inconsistent making it difficult to obtain reproducible cultures of iPSC-derived microglia for Aim 1. To rectify this, we have switched to using StemDiff™ Microglia Maturation Kit for iPSC microglia. We have found this to generate far more consistent and reproducible cultures of iPSC-derived microglia (Figure 2) and this has allowed us to advance with the iPSC-derived microglia aspect of Aim 1.

We had originally planned to obtain additional ALS PBMCs from CReATe Consortium Biorepository. However, when we requested access to samples, we were told to contact them again in six months due to their backlog of work. As this gave no indication that things would improve in six months, we instead contacted the National Amyotrophic Lateral Sclerosis Registry (as was also included in the Project Narrative) to access ALS PBMCs. They were able to provide the samples, however, it was a lengthy process as we had to obtain Australian Federal Government approval for the access, and prepare an MTA. There were a number of delays with this and final approval and shipment was only obtained in June 2023. We have obtained a change to the Institutional Human Ethics approval to use the cells from the National Amyotrophic Lateral Sclerosis Registry rather than CReATe. We have the cells in storage ready to be used. We are currently waiting on the National Amyotrophic Lateral Sclerosis Registry to provide key patient details to allow planning of cultures to ensure the optimal comparisons with our other PBMC-derived microglia (age, sex, disease classification as slow, fast etc.).

There were also some delays with generation of the ALS SOD mice microglia at Site 2 (Subaward). The colony needed to be re-established by our collaborators so this slowed down the generation of mice for Aim 1. The colony has bred well with adequate numbers of animals and cultures are now being generated for this aim, and cultures are currently growing and drugs being tested as outlined in Section 3 above.

Changes that had a significant impact on expenditures

There have been no changes that have led to significant impact on expenditure.

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

Significant changes in use or care of human subjects

There are no significant changes in use of human-derived materials. We have had to obtain additional PBMCs from the National Amyotrophic Lateral Sclerosis Registry instead of CReATe Consortium Biorepository. As both are nationally registered biorepositories, this is not a major change and a minor amendment has been approved by our Institutional Human Research Ethics committee.

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.

Nothing to Report

- **Website(s) or other Internet site(s)**

Nothing to Report

- **Technologies or techniques**

We have published a related paper on Alzheimer's patient monocyte-derived microglia. While not derived from the current research activities, it is relevant to the future outcomes of the current grant. The manuscript, currently in pre-print at bioRxiv by Cuni-Lopez et al, describes a preclinical drug testing strategy by assessing the efficacy of anti-inflammatory drugs in 2D and 3D *in vitro* models of monocyte-derived microglia-like cells (MDMi) derived from AD and mild cognitive impairment (MCI) patients, and matched healthy individuals. We observed that the cytokine inflammatory profiles of MDMi in response to drugs clustered separately between cohorts. By ranking donor and cytokine responses to drugs, we identified that drug efficacy was limited in AD patients and involved cohort-specific responsive cytokines. Our findings suggest that MDMi models have the potential to predict disease progression, stratify responders and identify biomarkers for estimating the efficacy of microglia-targeted drugs.

The approach we have applied in the manuscript confirms that we can rapidly generate cohort and patient-based stratification of drug responses and potential biomarkers in microglia cultures treated with potential therapeutic drugs, a key aim of the current project. The manuscript is: A novel patient-derived cellular platform for validating microglia-targeted therapeutics for Alzheimer's disease. Carla Cuní-López, Romal Stewart, Satomi Okano, Garry L. Redlich, Mark W. Appleby, Anthony R. White, Hazel Quek.
bioRxiv 2023.08.17.552618; doi: https://doi.org/10.1101/2023.08.17.552618

- **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?

Name:	Anthony White
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	(https://orcid.org/0000-0003-1802-9891)
Nearest person month worked:	3
Contribution to Project:	Anthony White has supervised the establishment of the project, and overseen the planning of the experiments and collection of data, as well as submitting the required ethics approval paperwork.
Funding Support:	This award.
Name:	Vincenzo La Bella
Project Role:	CI
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0003-2045-1864
Nearest person month worked:	2
Contribution to Project:	Vincenzo La Bella has provided support in provision of ALS patient PBMCs for this project.
Funding Support:	ALS Clinical Research Center, Neurology Unit University Hospital Policlinico, Palermo, Italy.

Name:	Hazel Quek
Project Role:	Post-doctoral researcher
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0001-5597-5372
Nearest person month worked:	12
Contribution to Project:	Dr Hazel Quek has established the ALS and control patient PBMC-derived microglia cultures, determined optimal drug concentrations for testing, and undertaken experimental work described in Section 3 above.
Funding Support:	This award.
Name:	Fazelah Etebar
Project Role:	Post-doctoral researcher
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0002-1380-832X
Nearest person month worked:	2
Contribution to Project:	Dr Fazelah Etebar is a new post-doc in my group and has helped Dr Quek to establish the ALS and control patient PBMC-derived microglia cultures.
Funding Support:	QIMR Berghofer Institute near-miss grant.
Name:	Lotta Oikari
Project Role:	Post-doctoral researcher
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0001-7821-8622
Nearest person month worked:	12
Contribution to Project:	Dr Lotta Oikari has established ALS and control patient iPSC-derived microglia cultures, determined optimal drug concentrations for testing, and undertaken experimental work described in Section 3 above.
Funding Support:	This award.
Name:	Jeffrey Liddell (Site 2)
Project Role:	Post-doctoral researcher
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0002-0798-5338
Nearest person month worked:	1.25
Contribution to Project:	Dr Jeffrey Liddell has been breeding the ALS mouse colony and started to establish and treat ALS SOD1 mouse microglia cultures for Aim1.
Funding Support:	This award.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

PI White has been awarded a grant from FightMND Australia (USD$160,000) for 2022-2024. This grant was for a project titled: ***Development of a human MND Neurovascular Unit model to improve therapeutic translation in drug testing.*** The aims are:
1. Develop a model of the human MND NeuroVascular Unit (MND-NVU).
2. Demonstrate applicability of the MND-NVU for pre-clinical drug development.
3. Harness the HTP capacity of MND-NVU to screen for re-positioned drugs.

There is no overlap with the current CDMRP ALS Program project.

PI White has also received a grant from the QIMR Berghofer MRI called a 'near-miss' grant. This is to help secure funding from our National Health and Medical Research Council (Australia) for research into the role of PILRB in Alzheimer's disease microglia. The funds are USD$60,000 for 2023. There is no overlap with the current project.

What other organizations were involved as partners?

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES: