Final Report

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Title of Project: ***Blocking neuroinflammation to treat ALS***

Summary:

Accumulation of a protein known as TDP-43 is a hallmark of disease in almost all patients with amytrophic lateral sclerosis, and many individuals with frontotemporal dementia, Alzheimer's disease, Parkinson's disease, or diffuse Lewy body disease. TDP-43-associated neurodegeneration in general has been linked to inflammation, and interestingly, these inflammatory signals precede symptoms of disease. This suggests that inflammation contributes to disease pathogenesis, rather than simply acting as a marker of disease. We have now identified the primary innate immune pathway in cells that triggers neuroinflammation due to TDP-43. Unexpectedly this is driven by an innate immune pathway which is an immune sensor that recognises stray DNA in the cytoplasm of cells, cGAS/Sting.

TDP-43 releases mitochondrial DNA to activate cGAS/Sting
Normally, DNA remains within the nucleus or mitochondria of cells, but if stray DNA gets into the cytoplasm it can be recognised by cGAS/Sting, leading to inflammation. Indeed, it was already known that TDP-43 can get into mitochondria, and we found that this is what causes mitochondrial DNA to leak into the cytoplasm and trigger inflammation.

Pharmacologic inhibition of cGAS/Sting protects prevents TDP-43 driven inflammation
Critically, we have obtained a small molecule inhibitor of Sting (H-151), and found that it can block neuroinflammation triggered by TDP-43 for cell lines in vitro. H-151 is being commercialised by the company IFM therapeutics (Boston), who have agreed to support our project, looking towards first in man clinical trials.

Therefore, in summary, we suggest that patients with accumulation of TDP-43 suffer from neuroinflammation caused by the cGAS/Sting pathway. This occurs because TDP-43 destabilises mitochondria and the DNA leaks into the cytoplasm, activating cGAS/Sting. We propose to inhibit Sting and thus treat a mouse model of ALS caused by TDP-43, and confirm our results in primary human cells, as important steps towards getting this therapy into the clinic.

Hypothesis vs Findings

Aim 1: Validate small molecules inhibitors of Sting in models of ALS:

1.1 ALS patient iPSC derived neurons

We have obtained iPSC from healthy controls and patients with ALS either sporadic or carrying a range of TDP-43 mutations. These were differentiated into motor neurons and tested for the release of mtDNA, and we quantified cGAMP as a biomarker of cGAS/Sting pathway activation, both of which were elevated in ALS as expected. We also quantified Type I IFN by ELISA as a biomarker of target engagement in response to the small molecule Sting inhibitor H-151, which worked as expected.

1.2 Mouse models of ALS

We treated established disease in a mouse model of ALS using H-151 and it was found to prevent motor neuron loss, together with the reduction of inflammation as observed in vitro.

Aim2: cGAS/Sting biomarkers in ALS:

2.1 cGAMP as a biomarker to stratify ALS

We performed an ELISA to detect cGAMP, the metabolite synthesised by cGAS to trigger STING, using serum and cerebrospinal fluid. So far we have been unable to detect elevated levels that were observed in spinal cord samples of patients with ALS.

2.2 Type I IFN as a biomarker of Sting target engagement

We employed serum IFNb detection initially in the mouse models of ALS, treated with H-151 which confirmed target engagement.

Unanswered Questions

We still have not validated a biomarker for target engagement in serum of CSF of patients with ALS. Our next steps will be to try IFNb detection, which was successful in the mouse model of disease.

What these research outcomes mean

Our project specifically addresses the role of the innate immune system in ALS, which has hitherto been underappreciated. Our work dramatically redefines this landscape and identifies pathways and molecules that can be broadly investigated in the field of ALS to answer long standing questions regarding disease pathogenesis. We do not argue that neuroinflammation underlies all disease pathology in this complex neurodegenerative disease, but the insights that we have generated are nonetheless critical players that are pharmacologically tractable.

This resulted in **extremely significant contributions** to scientific knowledge and informs future practice of ALS treatment.

Our research led to **significant research outputs**. These include, but are not restricted to:

- **Intellectual property** – We have filed a method of use patent regarding the application of cGAS/STING inhibitors in TDP-43-opathies (ALS and a subset of FTLD patients). Our patent contends that TDP-43 aggregates and cGAMP as a biomarker define a specific patient population for which no prior art exists regarding cGAS/STING inhibition.
- **Publications** – Our manuscript based on this work was recently published in Cell: https://www.cell.com/cell/fulltext/S0092-8674(20)31161-2 . This work was widely reported in the print media (front page herald-sun), on radio, and syndicated nationally on channels 7, 9 and 10.
- **Conferences** – We organised the 2019 Immunology Group of Victoria (IgV) symposium on neuroinflammation. This emerging field has been the topic of too few conferences in Australia and the community will benefit from more interaction between immunologists and neurologists to bring the agenda forward, and we will help to achieve this.
- **Consulting, contract research, spin-offs, licensing** – Our team consults for several companies interested to apply cGAS/STING inhibitors to the field of neurodegeneration.