



Amyotrophic Lateral Sclerosis: Novel strategy to clear pathologic TDP-43 aggregates

EXECUTIVE SUMMARY

- Amyotrophic Lateral Sclerosis (ALS or MND) and Frontotemporal Dementia (FTD) are devastating neurodegenerative diseases that are untreatable. Both are characterised and caused by **abnormal cytoplasmic TDP-43** aggregates, whose prevention or clearance are a major focus of therapeutic intervention. Increasing interest in TDP-43 targeting (incl. capital investments) is evident, with ~ 10 companies with preclinical and early clinical programs. Notably, the MoA of our therapy is distinctly different to all of these pipeline therapeutics.
- This novel and **disease-modifying approach** is based on a validated Mechanism of Action involving an endogenous clearance pathway. Cyclin F directly controls TDP-43 ubiquitylation. Its disease-linked mutations lead to abnormal TDP-43 ubiquitination, which causes its pathological aggregation and neurodegeneration. Importantly, this strategy **clears only pathologic TDP-43** (cytoplasmic) and not nuclear (functional) TDP-43.
- This **AAV-based gene therapy** approach delivers a cytoplasm-localising variant of wild-type Cyclin F gene, *CCNF*, to clear cytoplasmic accumulation of pathologic TDP-43 and to prevent downstream neuronal dysfunction and improve motor and behavioural deficits in ALS and FTD.
- **IP portfolio** includes a filed patent (target and therapeutic modalities) and significant know-how.
- The team at the MND Research Centre has significant expertise, capabilities, unique animal models and access to **Australia's largest ALS Biobank and Clinic**, all in one location.

BACKGROUND

Amyotrophic Lateral Sclerosis (ALS, also known as Motor Neurone Disease (MND)) and Frontotemporal Dementia (FTD) are devastating neurodegenerative diseases that are currently untreatable. The presence of abnormal, cytoplasmic inclusions of the hyper-ubiquitylated nuclear protein TDP-43 in neurons is the primary pathological hallmark and a leading cause of neurodegeneration in almost all ALS cases and >50% of FTD [1]. TDP-43 mutations cause familial ALS and FTD [2], and transgenic animals that express human mutant TDP-43 develop progressive motor dysfunction consistent with the clinical presentation of the disease [3,4]. Therefore, strategies to prevent or clear the formation of TDP43 aggregates are currently a major focus for novel therapeutic interventions.

UNMET NEED

ALS and FTD are dreadful diseases, for which there are currently no effective therapies available. The total cost of ALS in Australia was \$2.37billion in 2015 (equating to \$1.1million per patient) [5]. In 2016, the total cost of dementia in Australia was \$14.25billion (equating to \$35,550 per patient) [6], of which FTD represents the second most common form of dementia after Alzheimer's disease. Notably FTD is considered a younger onset disease (45-65 years of age) than Alzheimer's disease (>70 years). Patients and their families are desperate for a treatment. Therefore, for social and economic reasons, identifying a tractable therapeutic strategy for ALS/FTD is an important research priority.

There are currently approximately 450,000 ALS patients worldwide (30,000 in US, 2000 in Australia). Approximately 10% have an inherited form of the disease (familial) with the majority of patients considered to have a sporadic origin. There are approximately 1-1.5million FTD patients worldwide (60-100,000 in US, 3-10,000 in Australia), with the majority of patients considered to have sporadic origin.

Hyper-ubiquitylated TDP-43 aggregates

- › *Pathological hallmark and leading cause of neurodegeneration in ALS and FTD*
 - › *Major target for novel therapeutic interventions*
-

There are currently two FDA approved drugs for ALS treatment, both of which are symptomatic and have mild benefits for some patients (riluzole (1995) and edaravone (2017)). More than 50 phase 2 and 3 ALS clinical trials have been conducted, with poor patient outcomes. Notably, we believe that none of these have a strong MoA that directly targets disease mechanism.

Regarding FTD, there are currently no FDA approved drugs.

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NOVEL THERAPEUTIC APPROACH

TDP-43 as a therapeutic target for ALS and FTD: Almost all cases of ALS and more than half of FTD cases are pathologically characterised by the presence of insoluble, cytoplasmic inclusions of TDP-43 within neurons. Furthermore, it is well described in the literature that pathological TDP-43 causes functional deficits in neurons that cause neurodegeneration in cell culture and animal models of disease. Notably, in transgenic mice that have inducible overexpression of TDP-43, the neurodegeneration phenotype is reversible upon removal of TDP-43 [3,4]. Our researchers have demonstrated that turning off the overexpression of mutant TDP-43 in an ALS/FTD mouse model resulted in rapid clearance of pathological TDP-43 from brains and, more importantly, significant improvement of motor and behavioural deficits after only 1 week of TDP-43 reduction [3,4]. Therefore, treatment that would achieve clearance of pathological TDP-43 could improve already established functional deficits in disease.

An important distinction that needs to be made is that TDP-43 performs important functions within the nucleus of cells, and that it is currently believed that the abnormal mislocalisation of TDP-43 into the cytoplasm, where it forms aggregated hyper-ubiquitylated inclusions (pathological TDP-43), is the cause of neurodegeneration in ALS and FTD [7]. Therefore, a TDP-43 targeting therapy should only clear pathological (cytoplasmic) TDP-43, and not nuclear TDP-43 (required for normal neuronal viability).

Validated MoA behind TDP-43 pathology and a novel target, Cyclin F: A key feature of cytoplasmic TDP-43 inclusions is that the TDP-43 protein is hyper-ubiquitylated. We have identified that Cyclin F (a component of a multi-protein E3 ubiquitin ligase) directly regulates ubiquitylation of TDP-43, targeting it for degradation. ALS-causing mutations in Cyclin F lead to abnormal ubiquitination of TDP-43 [8], which contributes to its pathological aggregation and causes neurodegeneration leading to ALS and FTD. This is the first disease-associated MoA that can be targeted therapeutically as a potential treatment for these diseases.

Key supporting data:

- (i) The mechanism of how **Cyclin F ubiquitylates TDP-43** and directly regulates homeostasis of TDP43 in cells was validated by using several approaches: (a) co-immunoprecipitation, (b) *in vitro* ubiquitination assay, and (c) *in vivo* proximity ligation assay whereby a modified biotin ligase is attached to Cyclin F, resulting in substrates of Cyclin F being tagged with biotin and subsequently identified by mass spectrometry.
- (ii) Cyclin F-mediated ubiquitylation of TDP-43 directly **regulates the homeostasis of TDP-43** in cultured cells: (a) siRNA knockdown of endogenous Cyclin F levels causes an increase in TDP-43 levels and (b) genetic overexpression of Cyclin F significantly reduces endogenous TDP-43 levels. AAV approach was used to overexpress wildtype cyclin F in neurons, resulting in substantial reduction in endogenous (wildtype) TDP-43 levels.
- (iii) To ensure that only pathological TDP-43 is targeted, we have engineered a **Cyclin F variant** that is specifically **localised to the cytoplasm**, where it can ubiquitylate and clear pathological TDP-43 and not nuclear TDP-43.

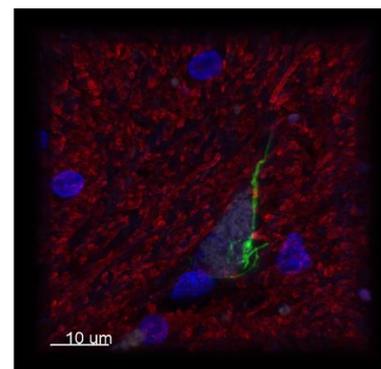


Figure 1 .TDP-43 inclusion (green) in post-mortem spinal cord section from a sporadic ALS patient

This **novel therapeutic approach** involves AAV-based gene therapy to deliver a variant of wild-type Cyclin F gene, CCNF, to clear cytoplasmic accumulation of pathologic TDP-43 to prevent downstream neuronal dysfunction and improve motor and behavioural deficits in ALS and FTD.

> Disease-causing mutations in Cyclin F cause hyper-ubiquitylation of TDP-43 and neuronal death.

> Targeting Cyclin F represents a compelling strategy to clear the formation of disease-causing TDP43 aggregates in ALS and FTD.

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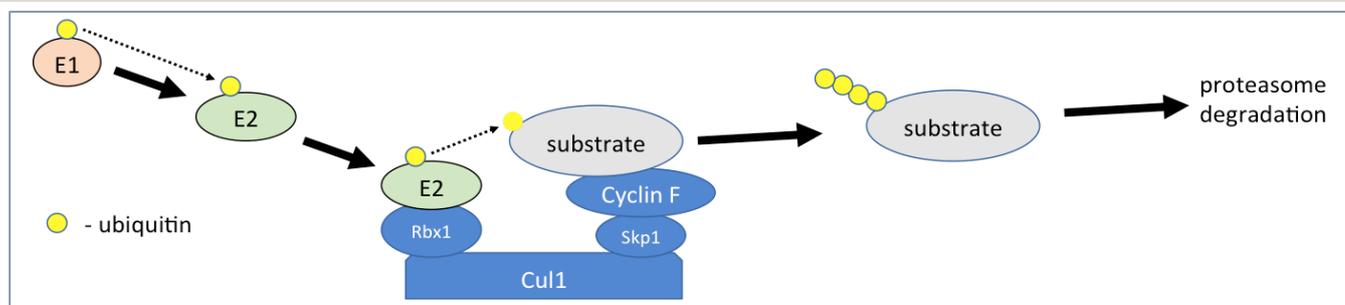


Figure 2. Overview of how Cyclin F ubiquitylates TDP-43 to regulate its clearance

Stage of development:

- Full validation of the discovery was achieved through biochemical, proteomic and *in vitro* cell culture models.
- AAV-delivery system has been produced and validated that it efficiently delivers CCNF gene therapy to neurons in wildtype mice, resulting in reduction in TDP-43 levels.
- Pre-clinical proof-of-concept trial is ongoing, using AAV-CCNF gene therapy (delivering the Cyclin F variant that localises in cytoplasm) in transgenic TDP-43 mice (supported by a large competitive non-diluting grant).

Complementary strategies:

Complementary strategies that utilise Cyclin F to regulate TDP-43 levels have been discovered. One involves the control of endogenous Cyclin F expression levels and would be particularly suitable for sporadic ALS and FTD patients i.e the majority. Accordingly, we have identified FDA approved drugs that increase Cyclin F levels in cells, which lowers (but not completely removes) TDP-43 levels in cells. This represents an opportunity to repurpose these drugs for treatment of ALS and FTD. Further information can be shared under CDA.

IS GENE THERAPY FOR ALS A POSSIBILITY? YES – SPINAL MUSCULAR ATROPHY

Spinal Muscular Atrophy (SMA) is one of the most common genetic causes of infant death. Motor neurons that innervate muscles are destroyed, causing progressive loss of motor function and death through respiratory failure generally before 4 years of age. **SMA is considered a childhood form of ALS.** The disease is caused by a dominantly inherited mutation in the *SMN1* gene, which causes loss of expression of functional SMN protein, directly leading to motor neuron degeneration and disease pathogenesis. Humans also have a nearly identical gene called *SMN2*, which produces about 10-20% of the normal level of functional SMN protein and about 80-90% constitutes the truncated non-functional protein.

In 2016, the first FDA-approved treatment for SMA reached the market – Nusinersen (Spinraza®), which is an antisense oligonucleotide therapy that blocks the alternative splicing of *SMN2* gene, hence producing a more functional SMN protein. It has halted disease progression in many SMA infants, with many also showing significant improvement in motor function. Nusinersen therapy involves 5 injections in the first year, and 3 injections per year in subsequent years.

There are phase 3 clinical trials currently underway to evaluate AAV9-mediated delivery of the *SMN1* gene in SMA infants, with promising results reported to date. This approach involves a single injection of the AAV-9 therapy. Current trials are underway by AveXis Inc (acquired by Novartis).



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RESEARCH EXPERTISE AND CAPABILITIES

A multidisciplinary team includes experts in neurogenetics of rare diseases, biochemistry and molecular biology, proteomics, *in vivo* live-imaging and advanced imaging techniques. Furthermore, the group has long-standing expertise with biochemical and proteomic assays and *in vitro* and *in vivo* experimental models of neurodegeneration (transgenic zebra fish and TDP-43 transgenic mouse).

We are the largest research centre in Australia solely focused on discovering the molecular origins of ALS (funded by one of only 6 national Dementia Teams Grants), co-located with Australia's largest ALS Clinic within the Macquarie University Hospital precinct. We have access to patients and an extensive longitudinal ALS biobank.

INTELLECTUAL PROPERTY PORTFOLIO

- PCT Publication WO2018081878, *Modulation of protein accumulation and uses therefor*, covers the target and therapeutic modalities
- Significant undisclosed know-how
- Future strategic IP portfolio may include combination therapies based on deep understanding of Cyclin F biology

PARTNERSHIP OPPORTUNITY

We are seeking an industry partner for further development and commercialisation of this unique and promising therapeutic approach through optimal partnership models. Furthermore, we are seeking collaboration opportunities in the area of neurodegenerative diseases, where we have significant expertise, capabilities, unique animal models and access to the Australia's largest ALS Biobank and Clinic, all in one location.

SELECTED PUBLICATIONS

Lee et al (2018) Pathogenic mutation in the ALS/FTD gene, CCNF, causes elevated Lys48-linked ubiquitylation and defective autophagy in neuronal cell lines. *Cellular & Molecular Life Sciences* 75 (2) pp 335 [2017 Impact Factor – 6.721]; **Lee et al (2017)** Casein Kinase II phosphorylation of cyclin F at Serine 621 regulates the Lys48-ubiquitylation E3 ligase activity of the SCF (cyclin F) complex. *Open Biology* 7 (10) [IF – 3.286]; **Svahn et al (2018)** Nucleo-cytoplasmic transport of TDP-43 studied in real time: impaired microglia function leads to axonal spreading of TDP-43 in degenerating motor neurons. *Acta Neuropathologica*. 136 (3) pp 445 [IF – 15.872]; **Hogan et al (2017)** Expression of ALS/FTD-linked mutant CCNF in zebrafish leads to increased cell death in the spinal cord and an aberrant motor phenotype. *Human Molecular Genetics* 26 (14) pp 2616 [IF – 4.902]; **Williams, et al. (2016)** CCNF mutations in amyotrophic lateral sclerosis and frontotemporal dementia. *Nature Communications*, Vol. 7, 11253, p. 1-8. [IF – 12.353];

References

[1] Rademakers et al, *Nat Rev Neurol*, 2012; [2] Buratti, *Adv Genet*, 2015; [3] Ke et al, *Acta Neuropath*, 2015; [4] Walker et al, *Acta Neuropath*, 2015; [5] Deloitte Access Economics report Economic Analysis of MND in Australia, 2015; [6] National Centre for Social and Economic Modelling NATSEM (2016) Economic Cost of Dementia in Australia 2016-2056; [7] Scotter et al, *Neurotherapeutics* 2015; [8] Williams et al, *Nat Comm* 2016.

INTERESTED TO LEARN MORE?

Gorjana Mitic, PhD; Commercialisation and Innovation Manager (Life Sciences)
+61-2-9850-4542; gorjana.mitic@mq.edu.au