PLL Therapeutics

PLL THERAPEUTICS: DRIVING A NEW APPROACH TO ALS DIAGNOSIS AND THERAPY

PLL Therapeutics is developing a new paradigm for the early diagnosis and treatment of patients with amyotrophic lateral sclerosis.

Our pioneering therapy, currently undergoing a phase I/II study, is based on emerging insights into the role of the gut-brain axis in the pathophysiology of the condition, while our investigational diagnostic identifies patients on the basis of a unique serum antibody signature.

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Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease, characterized by injury to and loss of motor neurons in the brain and spinal cord. Initial symptoms can include muscle weakness and cramping or difficulties in swallowing or speaking. As these gain in severity and spread to voluntary muscles throughout the body, patients lose the ability to perform everyday activities, experience impaired mobility, and eventually develop almost total paralysis. The condition, although highly heterogeneous, has a poor prognosis and a rapid course. Patients typically die from respiratory failure within two to five years of their diagnosis[1].

Clinical manifestations of the disease vary from patient to patient, but most exhibit one of two distinct types of onset. Spinal or limb onset ALS, which accounts for about 70% of cases, starts with muscle weakness or stiffness, often affecting a hand, arm or leg. Bulbar-onset ALS, so-called because of the involvement of the bulbar region of the brainstem, starts with slurred speech or problems with swallowing. It usually follows a more aggressive course. About 15% of ALS patients also develop frontotemporal dementia (FTD)[2], while up to 60% of patients exhibit less severe cognitive and behavioural changes.

Rising prevalence in US and EU

ALS is an Orphan disease. Its prevalence varies across different populations. In the U.S., the estimated prevalence in 2022 was 9.9 cases per 100,000; this is projected to rise to 10.5 cases per 100,000 by 2030[3]. About 500,000 people are currently living with the condition – and this number is expected to grow by 6% annually over the next fifteen years. In Europe and the U.S. combined, about 30,000 new cases are diagnosed every year.

Most cases are sporadic, having no known cause, but about 10% are inherited. Mutations in over thirty genes have been linked with ALS[4], but a small fraction of these – notably hexanucleotide expansions in chromosome 9 open reading frame 72 (C9orf72) and mutations in the genes encoding superoxide dismutase 1 (SOD1), TAR DNA-binding protein 43 (TARDBP), fused in sarcoma (FUS) and TANK-binding kinase 1 (TBK1) – account for a majority of familial cases. Epidemiological studies suggest a range of environmental risk factors may also influence an individual's susceptibility, including exposure to pesticides, metals, air pollution, neurotoxins, infectious agents, trauma, diet, and electromagnetic fields[5]. In addition, occupational and lifestyle factors, may also play a role.

Despite extensive efforts, progress in developing effective therapies for ALS has been minimal. Riluzole, an inhibitor of the neurotransmitter glutamate, gained approval in 1995. It slows disease progression and may offer a modest survival benefit[6]. Radicava (edaravone), an antioxidant which gained FDA approval in 2017, slows the loss of physical function[7]. Qalsody (tofersen), an antisense oligonucleotide that causes degradation of SOD1 mRNA, gained FDA approval in 2023 for treating patients with SOD1-mutated familial ALS. It slows disease progression and extends survival[8].

Dysbiosis implicated in ALS

The pathophysiology of ALS is complex. Inflammation, mitochondrial dysfunction, DNA repair defects, toxic protein aggregation, and impaired axonal transport within neurons have all been implicated in disease initiation or progression[9]. So, too, has blood-brain barrier dysfunction[10]. In addition, there is a growing body of evidence linking dysbiosis – or perturbations in the gut microbiome – with ALS. Preliminary evidence from a study in transgenic mice expressing multiple copies of a mutated human SOD1 gene suggests that certain bacterial species in the microbiome can improve ALS symptoms, whereas others exacerbate them.

Moreover, by manipulating the composition of the microbiome, the study authors were able to vary the severity of the disease[11]. A separate study in C9orf72-mutant mice suggests that the C9ORF72 gene product suppresses inflammation induced by gut bacteria, which suggests a direct link between dysbiosis and the most common form of familial ALS[12]. Additional evidence comes from an analysis of the microbiomes of ALS patients – they contain lower levels of several species of butyrate-producing bacteria than do those of healthy controls[13].

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Microbiome changes: causative rather than consequential role

Critically, these microbiome changes and gastrointestinal symptoms often precede motor symptoms, suggesting a potentially causative rather than a merely consequential role[14-15]. The relationship appears bidirectional: dysbiosis impairs intestinal barrier integrity leading to endotoxemia and systemic inflammation, which damages the blood-brain barrier and allows lipopolysaccharide (LPS) and inflammatory cells to enter the central nervous system (CNS). Conversely, ALS progression itself worsens dysbiosis, creating a vicious cycle of neuroinflammation and neurodegeneration[16-18].

PLL Therapeutics is at the forefront of efforts to further evaluate the role of dysbiosis in ALS in patients, by conducting clinical trials of a candidate therapy, **PLL001**, which is designed to halt systemic and neural inflammation and restore gut health. Under normal physiological conditions, the gut microbiome – a highly diverse bacterial community, which varies greatly from person to person but which typically comprises about 10^{14} bacterial cells – helps to maintain the integrity of the intestinal barrier.

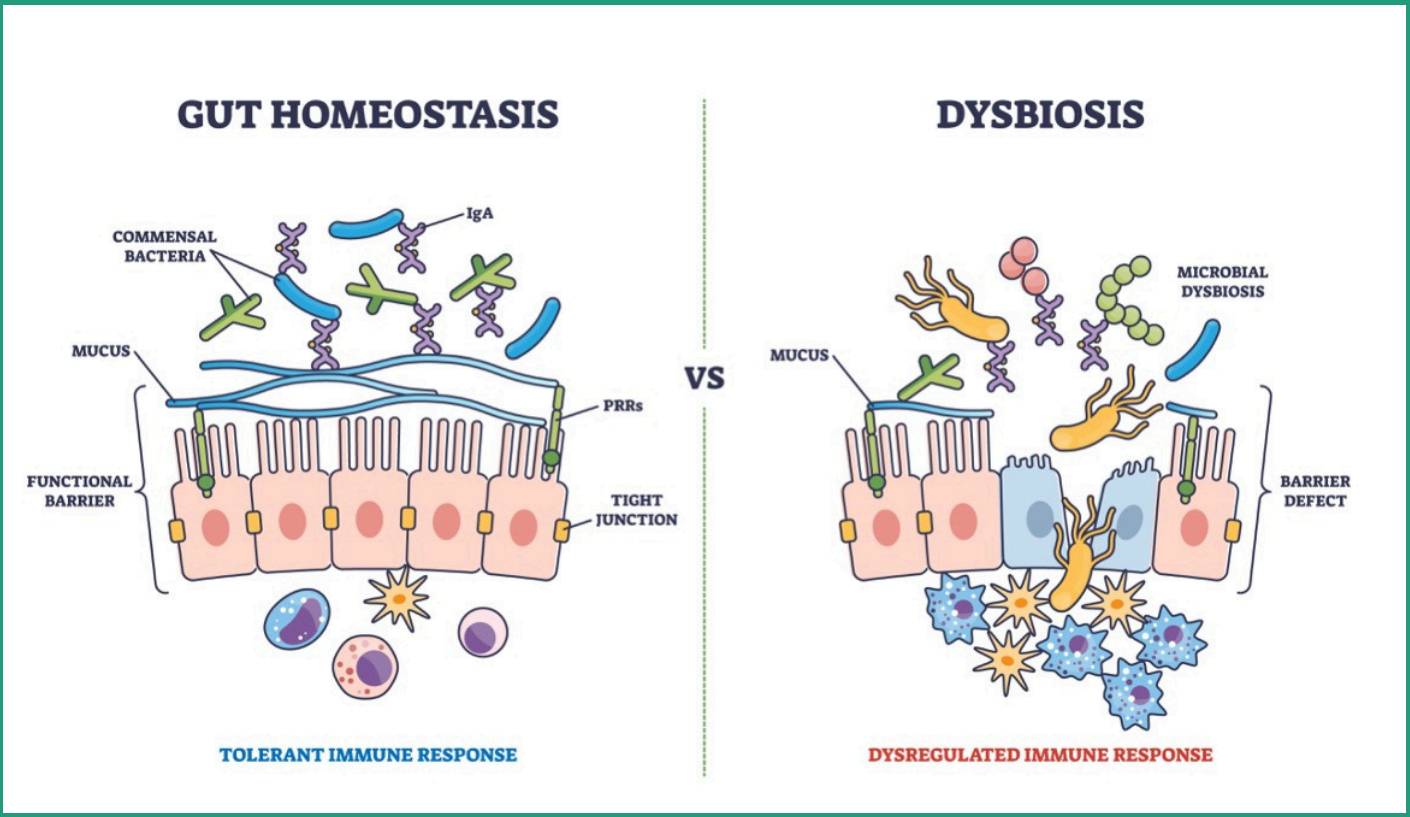


Figure 1 illustrates the two states of the microbiota in the intestinal lumen. In the first case, known as the physiologically healthy state, the microbiota and the intestinal barrier interact in a functional partnership without loss of function or the passage of harmful elements through the barrier. In contrast, in the second case, dysbiosis—caused by a change in the microbiota in a pathological state—creates an inflammatory environment leading to a loss of barrier function and the passage of bacterial elements into the bloodstream.

Resident microbes stimulate endogenous expression of the proteins that form tight junctions, which limit the passage of macromolecules between epithelial cells of the gut lining. They also influence the production of mucus, which maintains a physical barrier between the epithelial cells and the bacteria present in the gut. And they also promote immune homeostasis throughout the gastrointestinal tract.

Pleiotropic mechanism

Short chain fatty acids (SCFAs) play a key role in the maintenance of gut health. Derived from microbial digestion of dietary fibre, they act as the principal energy source for the epithelial colon cells that form the intestinal barrier, including colonocytes, which absorb nutrients, moisture, and electrolytes, mucus-producing goblet cells, hormone-secreting enteroendocrine cells, and the stem cells that constantly replenish the gut lining, which turns over every five to seven days. They also act as signalling molecules, through free fatty acid receptors (FFARs), to suppress innate and adaptive immune responses and broadly influence gut physiology[19]. Their effects are not limited to the gut.

In microglial cells within the CNS, SCFAs influence epigenetic regulation of gene expression by inhibiting histone deacetylase enzymes, leading to the downregulation of pro-inflammatory cytokines interleukin-1 beta (IL-1b), IL-6 and tumor necrosis factor alpha (TNF-a) and upregulation of anti-inflammatory cytokines transforming growth factor beta (TGFb) and IL-4. They also exert anti-inflammatory effects on peripheral blood mononuclear cells, by inhibiting activation of nuclear factor kappa B (NF-kB)[20].

Bacteroides and Firmicutes bacterial species are the microbiome's main sources of SCFAs. Declines in their activity can lead to a reduction in SCFA availability and an increase in gut permeability, immune activation, and chronic systemic and CNS inflammation, as antigens and toxins normally held within the gut lumen spill out into the circulation and cross the blood-brain barrier. PLL001 alleviates this shortage directly. It comprises four active drug substances, the SCFAs acetate, butyrate, lactate and propionate, each conjugated to a poly-L-lysine (PLys) carrier to improve cellular uptake. Each PLys comprises a linear peptide containing 70 lysine residues – to which an average of seven individual SCFA molecules are attached by an amidation reaction. These enter cells by endocytosis, and the SCFA moieties are released within the cytoplasm following deamidation. They then undergo metabolism or exert their pharmacological effects, depending on the cellular context.

Given that it comprises four distinct active drug substances, PLL001 has multiple activities – in the CNS as well as in the gut. In a range of different animal models, butyrate, the main source of energy for epithelial colon cells, restores blood-brain barrier integrity, reverses neuroinflammation and blocks neuronal apoptosis[21-22].

Previous clinical history

PLL's patent-protected PLys, the first polylysine to be produced under Good Manufacturing Practice (GMP) conditions, is structurally distinct from food-grade polylysine. It has a linear rather than a branched structure and, unlike e-polylysine, it is not cytotoxic. A prior entity previously tested a different formulation of PLys in two trials, which recruited patients with rheumatoid arthritis and secondary progressive multiple sclerosis, respectively. The conjugated active pharmaceutical ingredients employed also differed from those of PLL001, but each study confirmed the safety and tolerability of the constructs, while also showing preliminary signs of clinical efficacy.

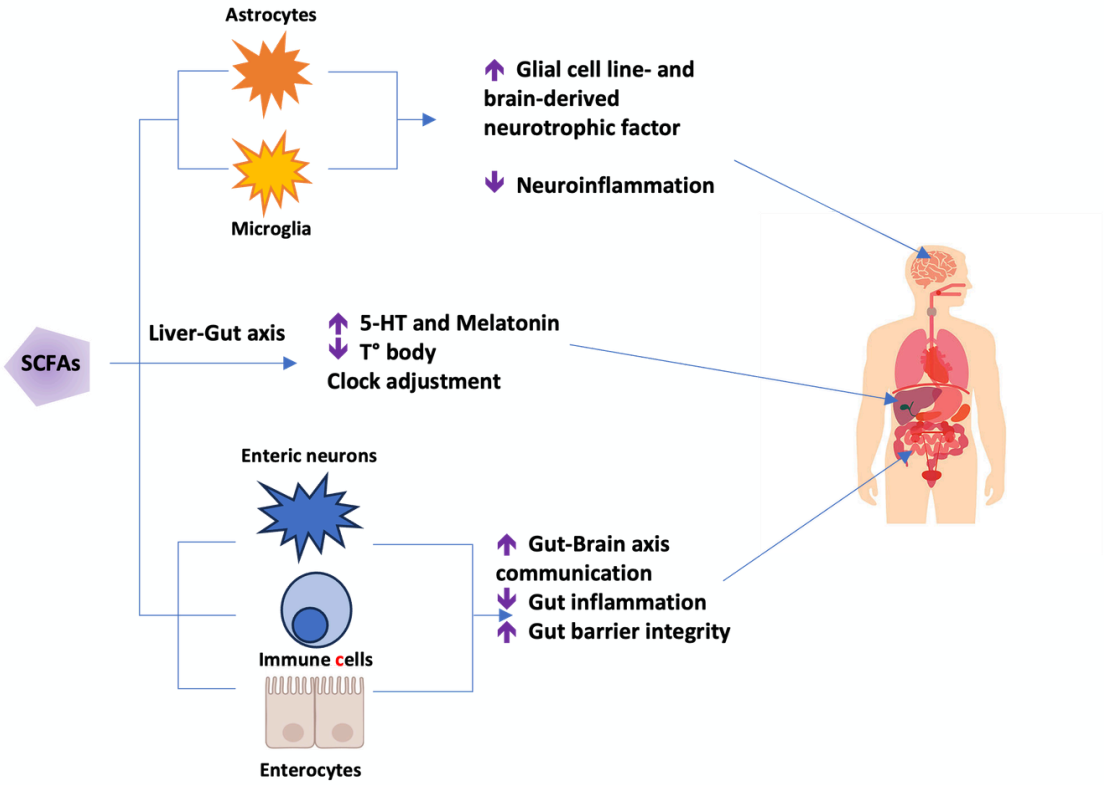
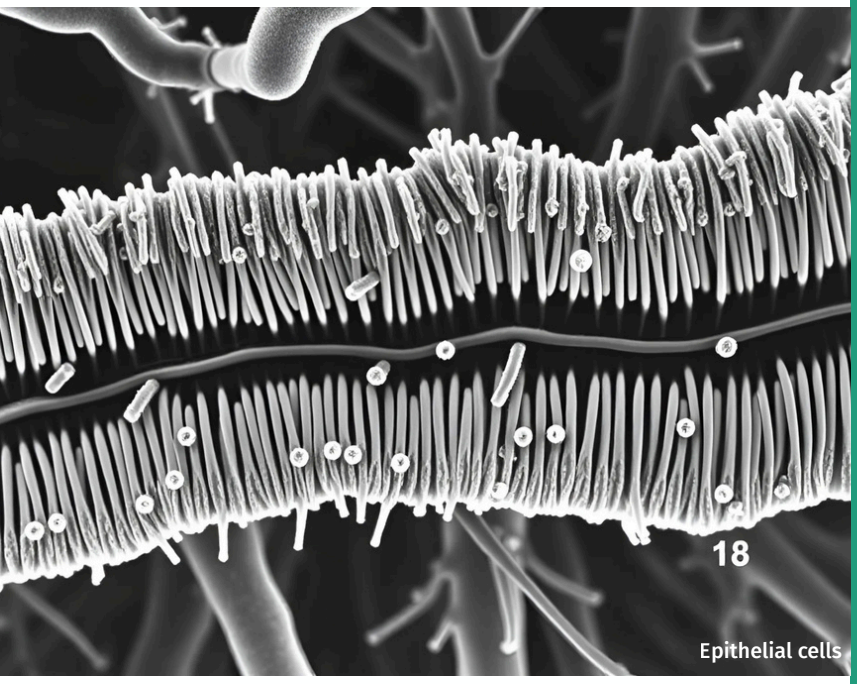


Figure 2 illustrates SCFAs, produced by gut microbiota, impacting three key areas: the intestine (homeostasis, inflammation, barrier integrity, gut-brain communication), the liver (hormonal regulation, circadian rhythm, sleep), and the brain (anti-inflammatory effects, neurogenesis, blood-brain barrier integrity). Their effects span digestion, metabolism, and neural health.

In the widely used SOD1-G93A transgenic mouse model of ALS, butyrate supplementation delayed the onset of ALS symptoms, slowed disease progression, and improved survival[23]. Propionate has anti-oxidant, neuroprotective and neuroregenerative effects[24], and it also helps to maintain the integrity of the blood-brain barrier[25].

Lactate has both signaling and metabolic actions in the brain, with effects on energy availability, neuronal excitability and neuronal plasticity[26]. Acetate attenuates neuroinflammation, by regulating the activity of the CNS-resident innate immune cells microglia and astrocytes[27].

In silico modelling studies indicate that the hydrophilic PLys carrier ensures efficient systemic transport of the molecules, while the lipophilic SCFAs then facilitate attachment to and transport across cell membranes. A third-party study illustrates the efficiency of the approach – a similar poly-L-lysine carrier boosted cellular uptake of a conjugated enzyme 900-fold as compared with uptake of the naked enzyme[28]. A different form of polylysine, e-polylysine, has a long history of human use as a food preservative in Japan and Korea, due to its anti-microbial properties. It has had GRAS (generally regarded as safe) designation in the U.S. for over two decades[29].



Building an evidence base

Human data on the therapeutic potential of SCFAs in ALS are lacking. Most of the evidence the field has generated to date is based on experiments conducted in animal models, particularly in the SOD1-G93A transgenic mouse model. No model captures all of the features of the human disease, however, and the biological relevance of animal data may often be limited to a small minority of patients with the associated genetic mutation.

PLL Therapeutics has, therefore, prioritised clinical studies in ALS patients, in order to build a more substantial evidence base relevant to the broad patient population.

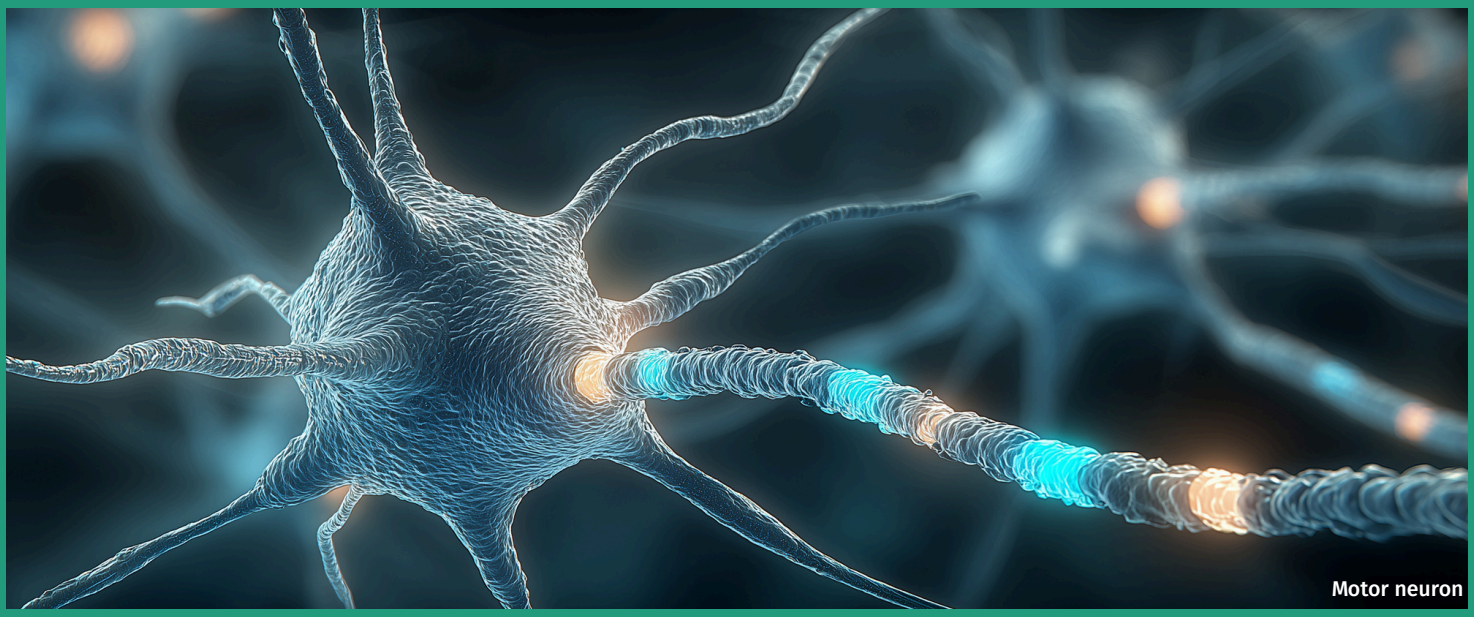
Before opening that study, however, it commissioned over a dozen in vitro cytotoxicity and in vivo toxicology studies to establish the preclinical safety of PLL001. The in vitro studies included standard transepithelial electrical resistance (TEER) measurements of gut barrier integrity, which showed that PLL001 had a positive effect on this physiological parameter.

In a SOD1 mouse model, PLL001 demonstrated improvements in mobility and in behaviour. These effects, moreover, were positively correlated with greater abundance of Lachnospiraceae bacterial species, which are important butyrate producers, and negatively correlated with the presence of Desulfovibrionaceae and Prevotellaceae bacteria.

These findings constitute preliminary in vivo proof of concept.
Since then, PLL Therapeutics has initiated a multicentre phase I/II randomised, double-blinded, placebo-controlled clinical trial of PLL001 in patients in Australia and New Zealand (NCT06513546).

It has already completed part 1 of the trial, a single-ascending dose study, in which twelve patients were assigned to one of three dose cohorts. In each cohort, participants were randomised, in a 3:1, ratio to receive a single subcutaneous dose of PLL011 or placebo.

"The therapy was safe and well tolerated – no serious adverse events and no treatment-related adverse events resulting in study discontinuation were reported. "



Motor neuron

Preparation for Phase 2

Part 2 of the study will recruit 140 patients, who will receive a daily injection of either **PLL011** or placebo over a six-month treatment period.

The primary efficacy endpoint comprises a change from baseline in participants' Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised (ALSFRS-R) scores, which will be measured every two months during part 2, as well as blood, stool, urine and, for some of them, cerebrospinal fluid to obtain a comprehensive picture of how the biomarker changes over time and of the therapy's efficacy.

The effect of **PLL011** on survival will also be evaluated. In addition, the effect of the therapy on patients' quality of life, respiratory function, upper and lower limb muscle strength will also be followed, and patients and physicians will also complete questionnaires designed to capture their subjective assessments of **PLL011**'s effects.

Patients' microbiota and serum antibody profiles will also be assessed during the treatment period. At the conclusion of Part 2, all study participants will be invited to enrol in Part 3 of the trial, an open-label extension study, which will involve an additional six months of therapy.

Early-stage diagnosis of ALS

Management of ALS is complicated by the protracted nature of the diagnostic process. Because of a lack of reliable, disease-specific biomarkers, it typically takes a year or more to arrive at a definitive diagnosis[30].

In parallel with our therapeutic development efforts, PLL Therapeutics is developing a companion diagnostic that could substantially reduce the time required to diagnose ALS accurately and also provide ongoing insights into the progression of the condition in each patient.

It is based on the same underlying principle that is guiding our drug development efforts – that dysbiosis is a significant driver of ALS. As well as offering opportunities for therapeutic intervention, perturbation of the gut microbiome and the consequent impairment of intestinal barrier integrity also opens up diagnostic possibilities, as a 'leaky' intestine introduces bacterial components and metabolites to the circulation.

Outside the gut's tolerogenic milieu[31] this results in both innate and adaptive immune responses.

Our investigational diagnostic captures the resulting crosstalk between the gut microbiome and the systemic immune system. It comprises a panel of antibodies that detects specific antibody isotypes, which recognise small-molecule haptens – bound to endogenous proteins – that are associated with the ALS pathology.

Promising biomarker data

Unpublished data from screens against sera from two unrelated patient cohorts indicate that several of these candidate biomarkers can distinguish with a high degree of accuracy between female patients with bulbar-onset ALS and female patients with either Alzheimer's disease (AD) or Parkinson's disease (PD).

In this particular patient segment, the area under the receiver operating characteristic (ROC) curve – a statistical method for analyzing the relationship between true and false positives – was 0.9 (a score of 1 implies a perfect test). The same metric was ≥ 0.85 for the same biomarkers in distinguishing female patients with spinal onset ALS from female patients with either AD or PD and in distinguishing male patients with spinal or bulbar onset ALS from male patients with either AD or PD. Further refinement of the panel is now underway in order to improve its utility.

Conclusion

There is an urgent need for more timely diagnosis of ALS and for therapies that can slow or even halt the condition's devastating loss of neuronal function and muscle control. The two agendas are interdependent. Achieving progress in drug development will remain challenging under the current diagnostic paradigm, as it precludes the possibility of early intervention to arrest the overwhelming pathological cascade that characterises disease progression. And it is essential that newly diagnosed patients have access to a wider range of therapeutic options than is currently available.

That is why PLL Therapeutics is committed to a dual strategy of developing a novel therapy and a companion diagnostic. Because they are based on the same underlying biological principle, they have the potential to work in tandem: the diagnostic may select for those patients most likely to benefit from PLL001, although this has to be demonstrated prospectively.

Elsewhere, genetics studies have galvanized research into the pathophysiology of ALS, opening up new drug targets and new therapeutic possibilities. Not all of these insights will be clinically actionable, however, and those that are may not necessarily apply to the majority of patients with sporadic disease. In any case, PLL001, if it can be shown to be safe and effective, has the potential to become an ideal combination partner for any targeted therapy that may emerge over the next decade. And PLL's diagnostic could revolutionise the diagnosis and management of this intractable condition.

References

01 Ann. Clin. Transl. Neurol. 10: 757–764 (2023)

02 Hum. Mol. Genet. 26 (R2): R105–R113 (2017)

03 Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration 26: 290–295 (2025)

04 J. Hum. Genet. 68: 131–152 (2023)

05 Nat. Rev. Neurol. 19: 617–634 (2023)

06 N. Eng. J. of Med. 330: 585–591 (1994)

07 Lancet Neurol. 16: 505–512 (2017)

08 JAMA Neurol. epub. Dec. 22, 2025 (doi:10.1001/jamaneurol.2025.4946)

09 Lancet Neurol. 21: 465–479 (2022)

10 J. Cereb. Blood Flow Metab. 43: 642–654 (2023)

11 Nature 572: 474–480 (2019)

12 Nature 582: 89–94 (2020)

13 Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration 22: 186–194 (2021)

14 Int. J. Mol. Sci. 26: 8419 (2025)

15 Neuromolecular Med. 27: 50 (2025)

References

16 Front. Neurol. 14: 1133546 (2023)

17 Cells 11: 4038 (2022)

18 Pharmacol. Ther. 231: 107988 (2022)

19 Nat. Rev. Microbiol. 23: 635–651 (2025)

20 BMC Medicine 19: 13 (2021)

21 Neurochem. Int. 176: 105745 (2024)

22 J. Cereb. Blood Flow Metab. 41: 267–281 (2020)

23 Clin. Ther. 39: 322–336 (2017)

24 Proc. Natl. Acad. Sci. USA 120: e2216941120 (2023)

25 Microbiome 6: 55 (2018)

26 Nat. Rev. Neurosci. 19: 235–249 (2018)

27 J. Neuroinflammation 21: 101 (2024)

28 J. Cell Physiol. 113: 167–178 (1982)

29 Regul. Toxicol. Pharmacol. 37: 328–340 (2003)

30 Lancet Neurol. 21: 480–493 (2022)

31 Nat. Immunol. 26: 1645–1659 (2025)

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