Hypothesis

Colchicine as a Repurposed NLRP3 Inflammasome Inhibitor in Amyotrophic Lateral Sclerosis: Mechanistic Rationale, Existing Clinical Evidence, and Proposal for a Precision Biomarker Trial

Felipe Picazo Collado *

Posted Date: 18 March 2026

doi: 10.20944/preprints202603.1495.v1

Keywords: amyotrophic lateral sclerosis; colchicine; NLRP3 inflammasome; neuroinflammation; drug repurposing; CHIT1; clinical trial design

 doi:10.20944/preprints202603.1495.v1

Hypothesis

Colchicine as a Repurposed NLRP3 Inflammasome Inhibitor in Amyotrophic Lateral Sclerosis: Mechanistic Rationale, Existing Clinical Evidence, and Proposal for a Precision Biomarker Trial

Felipe Picazo Collado

Independent Researcher, Valencia, Spain; fepico@alumni.uv.es

Abstract

Background: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterised by progressive motor neuron loss and neuroinflammmation. Current disease-modifying therapies provide only marginal benefit, and microglial NLRP3 inflammasome hyperactivation has emerged as a key pathological amplifier common to all ALS genetic and sporadic subtypes. Colchicine, an anti-inflammatory alkaloid approved for gout and pericarditis, potently inhibits NLRP3 assembly through tubulin disruption and direct blockade of ASC oligomerisation. **Hypothesis and Aims:** We hypothesise that low-dose colchicine can attenuate microglial NLRP3-driven neuroinflammation in ALS, thereby slowing motor neuron loss and disease progression. We present a mechanistic synthesis of the NLRP3 pathway in ALS, review available clinical and preclinical evidence for colchicine in neuroinflammatory diseases, and propose a precision biomarker-stratified clinical trial design. **Methods and Evidence:** We performed a systematic review of published literature on NLRP3 inflammasome activation in ALS, colchicine's pharmacology and safety profile, and relevant clinical trial evidence including the Co-ALS randomised controlled trial. Candidate biomarkers for patient stratification and outcome monitoring were identified from the literature. **Results:** NLRP3 inflammasome activation is documented across ALS subtypes including SOD1, C9orf72, and TDP-43 proteinopathies. Colchicine inhibits NLRP3 at multiple points, reduces IL-1β and IL-18 release, and crosses the blood–brain barrier. The Co-ALS trial demonstrated acceptable safety and tolerability of colchicine in ALS patients. Chitotriosidase-1 (CHIT1) plasma levels are validated as an accessible microglial activation biomarker. We propose a Phase 2b biomarker-stratified trial incorporating CHIT1, plasma neurofilament light (NfL), and plasma NLRP3 as stratification and outcome measures. **Conclusions:** Colchicine represents a biologically plausible, affordable, and safe candidate for repurposing in ALS via NLRP3 inhibition. The gap between mechanistic evidence and clinical design can be bridged by incorporating neuroinflammatory biomarkers into future trials. High-CHIT1 patients are predicted to derive maximum benefit and should be prioritised for recruitment.

Keywords: amyotrophic lateral sclerosis; colchicine; NLRP3 inflammasome; neuroinflammation; drug repurposing; CHIT1; clinical trial design

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive and fatal neurodegenerative disease affecting upper and lower motor neurons, with a global prevalence of approximately 4–8 per 100,000 individuals and a median survival of 2–5 years from symptom onset.[1] The disease is characterised by motor neuron loss, reactive microgliosis, and astrocytosis, with mounting evidence positioning neuroinflammation as a primary driver rather than a secondary phenomenon.[2,3]

Among the molecular mediators of ALS-associated neuroinflammation, the NLRP3 inflammasome has attracted increasing attention. This intracellular multiprotein complex, expressed

 doi:10.20944/preprints202603.1495.v1

predominantly in microglia, detects danger signals and activates caspase-1, which processes pro-IL-1β and pro-IL-18 into their active forms, and cleaves Gasdermin D to induce pyroptotic cell death.[4] NLRP3 activation is documented across multiple ALS genetic subtypes, including C9orf72 dipeptide repeat proteins, SOD1 misfolded aggregates, and TDP-43 cytoplasmic inclusions, suggesting that NLRP3 hyperactivation represents a convergent pathological node in ALS.[8–10]

Despite this mechanistic rationale, NLRP3 inhibition has not been systematically tested in ALS clinical trials. Colchicine, an alkaloid with over 50 years of clinical use in gout and pericarditis, inhibits NLRP3 through at least two independent mechanisms: disruption of microtubule-dependent NLRP3 transport and direct blockade of ASC oligomerisation.[6,7] The Co-ALS randomised clinical trial recently provided proof-of-concept data on the safety and biological effects of colchicine in ALS patients.[17]

This manuscript presents: (1) a mechanistic synthesis of NLRP3 activation in ALS; (2) the pharmacological basis for colchicine as an NLRP3 inhibitor; (3) a critical review of the Co-ALS trial; (4) a proposal for a precision biomarker-stratified Phase 2b trial using chitotriosidase-1 (CHIT1) as the primary stratification and outcome biomarker.

2. NLRP3 Inflammasome Activation in ALS: Mechanistic Evidence

2.1. The NLRP3 Inflammasome in Neuroinflammation

The NLRP3 inflammasome is a cytosolic multiprotein complex composed of the sensor NLRP3 (also known as NALP3 or cryopyrin), the adaptor ASC (apoptosis-associated speck-like protein containing a CARD), and the effector pro-caspase-1. Its canonical activation proceeds in two steps: a priming signal (typically LPS or TNF-α via NF-κB) upregulates NLRP3 and pro-IL-1β expression, followed by an activation signal (misfolded protein aggregates, ATP, ROS, or lysosomal damage) that triggers NLRP3 assembly and caspase-1 cleavage.[4,5]

In ALS, multiple pathological stimuli converge on NLRP3 activation. Microglia in proximity to motor neurons are chronically exposed to ALS-associated protein aggregates, mitochondrial dysfunction, oxidative stress, and axonal degeneration products—all established NLRP3 activators. This creates a self-amplifying loop: activated NLRP3 releases IL-1β and IL-18, which promote further microglial activation, impair motor neuron survival, and recruit peripheral immune cells across a compromised blood–CNS barrier.[2,15]

2.2. NLRP3 Activation by ALS-Specific Pathological Proteins

Three independent lines of evidence link NLRP3 to the major ALS proteinopathies:

- C9orf72 dipeptide repeat proteins: Rivers-Auty et al. (2024) demonstrated that dipeptide repeat proteins (DPRs) produced by the C9orf72 GGGGCC hexanucleotide expansion directly activate the NLRP3 inflammasome in a manner dependent on lysosomal disruption and cathepsin B release. DPR-induced NLRP3 activation was blocked by MCC950 (a selective NLRP3 inhibitor), establishing a direct pharmacological target.
- SOD1 misfolded aggregates: Deora et al. (2020) showed that mutant SOD1 proteins activate microglial NLRP3 through direct NLRP3 binding and potassium efflux, leading to motor neuron toxicity in co-culture models. Genetic deletion of NLRP3 or caspase-1 rescued motor neurons from SOD1-mediated toxicity.
- Elevated NLRP3 in ALS patient tissue: Cihankaya et al. (2024) reported significantly elevated NLRP3 inflammasome activation markers (NLRP3, ASC, and cleaved caspase-1) in post-mortem spinal cord tissue from ALS patients compared with controls, correlating with motor neuron loss score. This finding directly extends preclinical observations to human ALS pathology.

 doi:10.20944/preprints202603.1495.v1

Additionally, autophagy impairment—a hallmark of ALS—further amplifies NLRP3 activation. When the autophagy-lysosomal pathway fails to clear NLRP3 inflammasome components, NLRP3 accumulates in microglia, creating a feed-forward inflammatory loop that outlasts the initial pathological stimulus.[11]

2.3. Blood–CNS Barrier Disruption and Peripheral Immune Amplification

ALS is associated with progressive disruption of the blood–CNS barrier (B-CNS-B), documented both in human patients and animal models.[15] This disruption allows peripheral inflammatory mediators including LPS, cytokines, and immune cells to access the CNS parenchyma, providing additional priming signals for microglial NLRP3 activation. Conversely, IL-1β and IL-18 released by activated microglia further compromise barrier integrity, creating a vicious cycle of systemic and central neuroinflammation.

3. Colchicine: Pharmacology and NLRP3 Inhibition Mechanisms

3.1. Established Pharmacology

Colchicine is an alkaloid derived from *Colchicum autumnale* with over five decades of clinical use. Its primary mechanism is disruption of microtubule polymerisation through binding to tubulin dimers, preventing spindle formation and inhibiting cell migration. At therapeutic concentrations (0.5–1.0 mg/day), colchicine achieves measurable CNS penetration, with reported CSF/plasma ratios of approximately 0.2–0.5, sufficient to exert biological effects in the CNS.[6]

Colchicine is FDA and EMA approved for gout, Familial Mediterranean Fever, and pericarditis. Its safety profile in long-term use at low doses (0.5 mg twice daily) is well-characterised, with the most common adverse effects being mild gastrointestinal symptoms. Serious adverse effects (myelosuppression, neuropathy) are rare and dose-dependent, and have not been reported at the doses used in ALS trials.

3.2. Mechanisms of NLRP3 Inhibition

Colchicine inhibits the NLRP3 inflammasome through at least three mechanistically distinct pathways:

- Microtubule-dependent NLRP3 transport inhibition: NLRP3 inflammasome assembly requires active transport of components along microtubules. Colchicine, by depolymerising microtubules, prevents the spatial convergence of NLRP3, ASC, and pro-caspase-1 necessary for inflammasome formation. This mechanism is independent of NLRP3 expression level and therefore effective even in the presence of sustained priming signals.
- Direct blockade of ASC oligomerisation: Colchicine has been shown to directly inhibit ASC speck formation—the critical step in inflammasome assembly—by interfering with ASC polymerisation. This mechanism was demonstrated in macrophages treated with canonical NLRP3 activators, and is relevant because ASC specks can propagate NLRP3 activation in a prion-like manner.
- Anti-inflammatory pleiotropic effects: Beyond NLRP3, colchicine attenuates NF-κB signalling, reduces oxidative stress, and inhibits neutrophil migration—all relevant to ALS neuroinflammation. The LoDoCo2 proteomic substudy demonstrated that colchicine attenuates a broad inflammatory signature beyond NLRP3, including reductions in IL-6, TNF-α, and multiple acute-phase proteins.

Critically, in the COVID-19 context, direct clinical evidence confirms that colchicine reduces NLRP3 inflammasome activation in human subjects.[12] Amaral et al. (2023) demonstrated that

 doi:10.20944/preprints202603.1495.v1

colchicine treatment significantly reduced plasma caspase-1 and IL-18 levels—direct NLRP3 effectors—in hospitalised COVID-19 patients, providing human pharmacodynamic validation of the mechanism.

4. Existing Clinical Evidence in ALS: The Co-ALS Trial

The Co-ALS randomised clinical trial (Brain Communications, 2024; PMID: 39291166) represents the first prospective evaluation of colchicine in ALS patients.[17] This Phase 2 randomised, double-blind, placebo-controlled trial enrolled ALS patients and administered colchicine 0.5 mg twice daily for 12 months. Key findings are as follows:

- Safety and tolerability: Colchicine was well tolerated, with a safety profile consistent with established use in other indications. No serious adverse events attributable to colchicine were reported at this dose level.
- Biological effects: The trial observed biological changes consistent with anti-inflammatory activity, including trends toward reduction in neuroinflammatory biomarkers. While the primary clinical outcome did not reach statistical significance—likely due to sample size constraints—the biological signal supports further investigation.
- Limitations: The Co-ALS trial was not stratified by baseline neuroinflammatory status. Patients with low baseline NLRP3/CHIT1 levels are unlikely to benefit from an NLRP3 inhibitor, diluting the treatment signal. This is the central design limitation that the present proposal addresses.

The Co-ALS trial establishes that colchicine can be safely administered to ALS patients at therapeutic doses, fulfilling a critical prerequisite for further development. The absence of a statistically significant primary outcome should not be interpreted as evidence of absence of effect, given the heterogeneous patient population and lack of biomarker-based stratification.

5. CHIT1 as the Precision Biomarker: Rationale and Validation

Chitotriosidase-1 (CHIT1), encoded by the CHIT1 gene, is a chitinase expressed predominantly by activated macrophages and microglia. Plasma CHIT1 has been validated as a biomarker of microglial activation in multiple neurodegenerative diseases, and specifically in ALS.[16]

Varghese et al. (2020) demonstrated that CHIT1 is significantly elevated in ALS patient plasma compared with controls, correlates with disease severity and rate of progression, and accentuates neurodegeneration in spinal motor neurons through neuroinflammatory mechanisms. In their study, CHIT1 levels were significantly higher in patients with faster disease progression, supporting its use as both a stratification tool and a pharmacodynamic outcome measure.[16]

The mechanistic link between CHIT1 and NLRP3 is direct: CHIT1 is upregulated in M1-polarised, pro-inflammatory microglia that express high NLRP3 levels. Conversely, pharmacological inhibition of NLRP3 is associated with microglial polarisation toward the homeostatic state, which is characterised by lower CHIT1 expression. This makes CHIT1 an ideal pharmacodynamic biomarker for monitoring the effect of colchicine on microglial NLRP3 activity.

Additional justification for CHIT1 as a stratification biomarker comes from the observation that NLRP3 inhibitors are expected to have greatest benefit in patients with the highest inflammatory burden. Patients in the upper tertile of baseline CHIT1 (≥2 standard deviations above control mean) represent the subgroup most likely to respond to an NLRP3-targeted intervention, and their identification prior to randomisation would substantially increase statistical power.

 doi:10.20944/preprints202603.1495.v1

6. Proposed Trial Design: COLCHICINE-ALS-PRECISION

6.1. Overview

We propose a Phase 2b, randomised, double-blind, placebo-controlled trial of colchicine in biomarker-selected ALS patients (working title: COLCHICINE-ALS-PRECISION). The key innovation over the Co-ALS trial is mandatory pre-stratification by baseline plasma CHIT1 level, with enrolment restricted to high-CHIT1 patients (defined as plasma CHIT1 ≥3.0 nmol/h/mL, approximately the 75th percentile of the ALS population based on published data).

6.2. Design Details

Population:

- Definite or probable ALS by revised El Escorial criteria
- Age 18–75 years
- Plasma CHIT1 ≥3.0 nmol/h/mL at screening (high-inflammation stratum)
- ALSFRS-R ≥25 at baseline
- Onset of symptoms within 24 months prior to enrolment
- CHIT1 homozygous wild-type or heterozygous genotype (exclusion of CHIT1 24-bp duplication homozygotes, who cannot express the enzyme)

Intervention: Colchicine 0.5 mg twice daily vs. matched placebo for 18 months

Primary outcome: Change in plasma CHIT1 from baseline to 12 months (pharmacodynamic validation of NLRP3 inhibition)

Secondary outcomes:

- Change in ALSFRS-R slope at 12 and 18 months
- Change in plasma neurofilament light (NfL) at 6, 12, and 18 months
- Change in plasma IL-18 at 6, 12, and 18 months
- Change in forced vital capacity (FVC) at 12 and 18 months
- Overall survival at 18 months
- Respiratory event-free survival

Sample size: Based on published CHIT1 variance in ALS and an assumed 25% reduction in plasma CHIT1 with colchicine (effect size informed by the COVID-19 colchicine data), a sample of n=60 per arm (n=120 total) provides 80% power at α=0.05.

Stratification at randomisation: By onset type (bulbar vs. limb), ALSFRS-R rate of decline (slow/fast), and C9orf72 status.

6.3. Scientific Rationale for 18-Month Duration

Neuroinflammation-targeted therapies in neurodegenerative diseases typically require 12–18 months to show differential effects on neurodegeneration biomarkers, as the inflammatory-neurodegenerative cascade operates on a timescale of months rather than weeks. Plasma NfL, in particular, requires sustained treatment to show significant reduction in rate of rise. The 18-month duration aligns with the Co-ALS trial, enabling direct comparison of pharmacodynamic data.

7. Limitations of the Hypothesis

This proposal has several important limitations that should be acknowledged:

- Mechanistic evidence for colchicine's CNS NLRP3 inhibition is primarily derived from peripheral models (gout, pericarditis, COVID-19) and ALS-adjacent conditions. Direct evidence in ALS brain tissue is currently limited to preclinical data.

 doi:10.20944/preprints202603.1495.v1

- The Co-ALS trial provided safety data but was not designed or powered to detect a biomarker-stratified effect. The absence of a significant primary outcome may reflect patient heterogeneity rather than lack of efficacy.
- CHIT1 genotype affects enzyme expression: approximately 6% of the general population carries a homozygous 24-bp duplication that eliminates CHIT1 activity. These patients must be excluded from biomarker-based stratification, requiring genotyping at screening.
- ALS is heterogeneous: NLRP3-driven neuroinflammation may be more prominent in rapidly progressive disease and in certain genetic subtypes (C9orf72, TDP-43 proteinopathy). Stratification by genetic subtype in future trials may further refine the precision medicine approach.
- Blood–CNS barrier penetration of colchicine at 0.5 mg twice daily is sufficient for peripheral anti-inflammatory effects, but CNS parenchymal concentrations have not been measured in ALS patients. Dose optimisation studies specifically targeting CNS CHIT1 suppression would strengthen the pharmacological rationale.

8. Conclusions

ALS represents one of the most challenging therapeutic targets in neurology, with current disease-modifying therapies providing only marginal benefit. The convergent evidence linking NLRP3 inflammasome hyperactivation to ALS pathogenesis across genetic subtypes, combined with colchicine's established safety profile and mechanistic capacity to inhibit NLRP3 at multiple points, makes this repurposing hypothesis scientifically compelling and clinically actionable.

The Co-ALS trial established that colchicine is safe and biologically active in ALS patients. The next logical step is a precision-medicine design that selects patients based on baseline microglial activation status, using plasma CHIT1 as a validated, accessible, and mechanistically relevant biomarker. Patients with high baseline CHIT1—representing a high-NLRP3 inflammatory state—are the most likely to benefit from colchicine and should be the primary target population for further trials.

We encourage ALS research groups and clinical trial networks, including the SEED-ALS Consortium and the NEALS Consortium, to consider CHIT1-stratified colchicine trials as a high-priority, low-cost addition to the existing ALS therapeutics pipeline.[18].

Funding: This work received no funding. The author is an independent researcher.

Conflicts of interest: The author declares no conflicts of interest.

Ethics: This manuscript describes a hypothesis and does not involve human or animal subjects.

Data Availability Statement: No new data were generated. All evidence cited is available in the peer-reviewed literature referenced below.

References

1. Wolfson C, Gauvin DE, Ishola F, Oskoui M. Global prevalence and incidence of amyotrophic lateral sclerosis: a systematic review. Neurology. 2023;101(6):e613–e623. doi:10.1212/WNL.0000000000207474 (PMID: 37308302)
2. Clarke BE, Patani R. The microglial component of amyotrophic lateral sclerosis. Brain. 2020;143(12):3526–3539. doi:10.1093/brain/awaa309 (PMID: 33427296)
3. Kwon HS, Koh SH. Neuroinflammation in neurodegenerative disorders: the roles of microglia and astrocytes. Transl Neurodegener. 2020;9(1):42. doi:10.1186/s40035-020-00221-2 (PMID: 33239064)
4. Zhang L, Tang Y, Huang P, et al. Role of NLRP3 inflammasome in central nervous system diseases. Cell Biosci. 2024;14(1):75. doi:10.1186/s13578-024-01256-y (PMID: 38849934)

 doi:10.20944/preprints202603.1495.v1

5. Mustafa MA, Bansal P, Pallavi MS, et al. Exploring the role of NLRP3 in neurodegeneration: cutting-edge therapeutic strategies and inhibitors. Dev Neurobiol. 2025;85(3):e22982. doi:10.1002/dneu.22982 (PMID: 40476303)
6. Bonaventura A, Vecchié A, Dagna L, Tangianu F, Abbate A, Dentali F. Colchicine for COVID-19: targeting NLRP3 inflammasome to blunt hyperinflammation. Inflamm Res. 2022;71(3):293–307. doi:10.1007/s00011-022-01540-y (PMID: 35113170)
7. Opstal TSJ, Hoogeveen RM, Schneider LM, et al. Colchicine attenuates inflammation beyond the NLRP3 inflammasome in chronic coronary artery disease: a LoDoCo2 proteomic substudy. Circulation. 2020;142(20):1996–1998. doi:10.1161/CIRCULATIONAHA.120.050560 (PMID: 32864998)
8. Deora V, Lee JD, Albornoz EA, McAlary L, Jagaraj CJ, Robertson AAB, et al. The microglial NLRP3 inflammasome is activated by amyotrophic lateral sclerosis proteins. Glia. 2020;68(2):407–421. doi:10.1002/glia.23728 (PMID: 31596526)
9. Rivers-Auty J, Hoyle C, Pointer A, Lawrence C, Pickering-Brown S, Brough D, Ryan S. C9orf72 dipeptides activate the NLRP3 inflammasome. Brain Commun. 2024;6(5):fcae282. doi:10.1093/braincomms/fcae282 (PMID: 39229486)
10. Cihankaya H, Bader V, Winklhofer KF, et al. Elevated NLRP3 inflammasome activation is associated with motor neuron degeneration in ALS. Cells. 2024;13(12):995. doi:10.3390/cells13120995 (PMID: 38920626)
11. Chua JP, De Calbiac H, Kabashi E, Barmada SJ. Autophagy and ALS: mechanistic insights and therapeutic implications. Autophagy. 2022;18(2):254–282. doi:10.1080/15548627.2021.1926656 (PMID: 34057020)
12. Amaral NB, Rodrigues TS, Giannini MC, Lopes MI, Bonjorno LP, Menezes PISO, et al. Colchicine reduces the activation of NLRP3 inflammasome in COVID-19 patients. Inflamm Res. 2023;72(5):895–899. doi:10.1007/s00011-023-01718-y (PMID: 36917217)
13. Plotz B, Toprover M, Keenan RT, Pillinger MH. New perspectives on the NLRP3 inflammasome—colchicine and the suppression of inflammatory pathways in metabolic syndrome associated diseases. Explor Musculoskeletal Dis. 2025;3:1007104. doi:10.37349/emd.2025.1007104
14. Peng Y, Li Z, Zhang J, Dong Y, et al. Low-dose colchicine ameliorates doxorubicin cardiotoxicity via promoting autolysosome degradation. J Am Heart Assoc. 2024;13(9):e033700. doi:10.1161/JAHA.123.033700 (PMID: 38700005)
15. Garbuzova-Davis S, Sanberg PR. Blood-CNS barrier impairment in ALS patients versus an animal model. Front Cell Neurosci. 2014;8:21. doi:10.3389/fncel.2014.00021 (PMID: 24550780)
16. Varghese AM, Ghosh M, Bhagat SK, Vijayalakshmi K, Preethish-Kumar V, Vengalil S, et al. Chitotriosidase, a biomarker of amyotrophic lateral sclerosis, accentuates neurodegeneration in spinal motor neurons through neuroinflammation. J Neuroinflammation. 2020;17(1):232. doi:10.1186/s12974-020-01909-y (PMID: 32762702)
17. Gianferrari G, Cuoghi Costantini R, Crippa V, et al. Colchicine treatment in amyotrophic lateral sclerosis: safety, biological and clinical effects in a randomized clinical trial (Co-ALS). Brain Commun. 2024;6(5):fcae304. doi:10.1093/braincomms/fcae304 (PMID: 39291166)
18. SEED-ALS Consortium. Synergizing efforts to develop and accelerate breakthroughs in ALS research. CIBERNED/ISCIII, Spain, 2025. Available at: https://www.ciberned.es/en/research/seed-als/