Efficacy and Safety of Ultra-high Dose Methylcobalamin vs Placebo among Patients with Early-stage Amyotrophic Lateral Sclerosis (ALS): A Systematic Review

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ABSTRACT

Background

Amyotrophic lateral sclerosis is one of the neurodegenerative disorders with very limited treatment options owing to its progressive course and diverse pathophysiology. Majority of patients succumb to death within three to five years after the onset of symptoms, mostly due to respiratory failure. This study aimed to determine the efficacy and safety of ultra-high dose methylcobalamin versus placebo among patients with early-stage amyotrophic lateral sclerosis in terms of slowing down functional decline.

Methods

MEDLINE, CENTRAL, and Google Scholar databases were searched from inception up to September 23, 2023. The impact of treatments was measured by risk ratios with 95% confidence interval. The overall certainty of the evidence was evaluated using GRADE.

Results

No significant difference was detected for the outcome median change in the ALSFRS-R score for the whole cohort. Post-hoc analyses showed that ultra-high-dose methylcobalamin decreased ALSFRS-R scores (p=0.003 for 50 mg and p=0.01 for all methylcobalamin groups) in a dose-responsive manner. Mean difference was 1.97 in favor of methylcobalamin (95% CI, 0.44- 3.50; P = .01).

Conclusion

Ultra-high dose methylcobalamin can reduce ALSFRS-R scores of patients in its early stage but the scarcity of clinical trials makes it difficult to support a robust conclusion. Ultra-high dose methylcobalamin therapy remains to be investigational.

Keywords*: methylcobalamin, amyotrophic lateral sclerosis, systematic review*

Introduction

Amyotrophic lateral sclerosis (ALS) is one of the neurodegenerative disorders with very limited treatment options owing to its progressive course and diverse pathophysiology.[1] The involvement of both the upper and lower motor neurons, which control voluntary muscle movements, is the hallmark of this disease. Eventually, patients lose their ability to stand, walk, swallow or breathe. People aging from 55 to 75 years old are usually affected with slight male preponderance than female, however it can also occur in younger populations.[2] The incidence of amyotrophic lateral sclerosis (ALS) varies across continents. A higher incidence rate of 1.5 to 4.7 per 100,000 person-years (py) among white individuals in Europe and North America compared to non-white populations in East Asia and South Asia (0.89 per 100, 000 py and 0.79 per 100,000 py respectively) was evident.[2] Once a patient is suspected of having ALS, informing the family becomes a disconcerting task for

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physicians. The median survival rate is low from the time of diagnosis. Majority of patients succumb to death within three to five years after the onset of symptoms, with respiratory failure as the most frequent cause. Although chances of survival are thin, a longer survival rate is still possible.

Methylcobalamin, the active form of vitamin B12, is an essential water-soluble vitamin that plays an important role in the proper functioning of the nervous system. As a cofactor of methionine synthesis, it helps in the formation of S-adenosylmethionine (SAM) necessary for the production of myelin sheath and synthesis of neurotransmitters.[4] Without methylcobalamin, formation of methionine from homocysteine is not possible leading to elevation of toxic homocysteine levels. Because of its contribution to neuronal processes, various pieces of scientific evidence through clinical trials were propelled to support its neurologic benefits.

It is postulated that an increase in plasma homocysteine levels is associated with an increased risk of neuronal damage. In amyotrophic lateral sclerosis, homocysteine remains to be elevated.[4] It induces oxidative stress, excitotoxicity, inflammation and motor neuron death. A promising therapeutic target for ALS may be utilized if homocysteine levels could be reduced with methylcobalamin via its antioxidant and anti- inflammatory effects. Despite decades of clinical trials, current therapeutic options remain inadequate. There is no cure for amyotrophic lateral sclerosis (ALS) therefore innovations for its management has become the main focus of research. To date, the pathogenesis of this disease is multifactorial; hence no single drug could address its natural course. The advent of targeted novel interventions based on intricate mechanisms paved way for treatment possibilities of this incurable disease. [12] Example of which was a study conducted in 2020 on cerebral dopamine neurotropic factor (CDNF) that substantially attenuate ALS pathology in preclinical mouse models through modulation of endoplasmic reticulum stress. A trial on dextromethorphan combined with quinidine inhibit leaky conductance of mitochondria by decreasing oxygen consumption thereby increasing ATP production that is essential in stabilization of neuronal cells.[11] Considering the complex mechanisms of ALS, this systematic review aims to determine the efficacy and safety of ultra-high dose methylcobalamin versus placebo among patients with early-stage ALS in terms of slowing down patient functional decline and determine the safety of ultra-high dose methylcobalamin versus placebo among patients with early-stage ALS in terms of the proportion of adverse events.

METHODS

The review was written following the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement 2020 checklist for transparency and completeness.

Criteria for considering studies for this review:

Type of studies

The reviewers included randomized controlled trials (RCTs) comparing ultra-high dose methylcobalamin with placebo. The reviewers excluded other types of studies such as cluster RCTs and quasi-RCTs, controlled before-and-after studies, non- randomized studies of intervention (NRSI), or observational studies.

Type of participants

Studies involving adult patients aged 18 years or older diagnosed as having sporadic or familial ALS with definite, probable, or probable laboratory-supported categories using the updated Awaji criteria were included.

Type of intervention

The intervention of interest is defined as ultra-high dose (i.e., 50 mg) of methylcobalamin given intramuscularly twice weekly. The comparator of interest is placebo given intramuscularly twice weekly.

Type of outcome measures

Primary efficacy outcome

- mean change in ALSFRS-R total score from baseline Secondary outcomes
- Any adverse events

Search methods for identification of studies

Databases

The reviewers searched electronic databases including MEDLINE, CENTRAL, and Google Scholar without any language restrictions from database inception up to September 23, 2023. The reviewers also checked the reference lists of eligible studies and contacted researchers working in the field.

Unpublished and ongoing trials

For any unpublished and ongoing trials, the reviewers applied due diligence and contacted study authors and other researchers working on the topic or field of interest.

Reference lists

The reviewers also checked the reference lists of all eligible studies identified by the search strategy.

Data collection and analysis

Selection of studies

The reviewers independently assessed and applied the inclusion criteria to all the studies identified through the search. The reviewers also reviewed all reference citations and abstracts found through the search strategy to eliminate trials that clearly did not meet the inclusion criteria. If either of the review authors believed that a trial could potentially meet the inclusion criteria, they obtained the complete paper. Once they had access to the full reports of all potentially eligible studies, the reviewers evaluated them for inclusion in the review using a pre-established eligibility form based on the inclusion criteria. Any disagreements were resolved through discussions with a third party. Any studies that did not meet the inclusion criteria were excluded, and the reasons for their exclusion were documented in the 'Characteristics of excluded studies' table.

Data extraction and management

The reviewers independently collected the pre-specified data items using a pre- specified data extraction form. The data collected included details about the study design, methods, and characteristics of included patients, interventions, and outcomes. For dichotomous or binary data, the reviewers retrieved the proportion or count of specific events, the total number of individuals randomly assigned to each group, and the total number analyzed. For continuous data, the reviewers collected the total number of participants randomized per group, the number of patients analyzed, the distribution of participants across the compared groups, as well as the mean values and standard deviations for certain outcomes. To address any missing information or uncertainties, they reached out to the authors of the trials. In cases of discrepancies or uncertainties, a third party facilitated discussions to reach a resolution.

Assessment of risk of bias in included studies

The reviewers individually assessed the potential risk for bias in every study included in this review using the Cochrane Collaboration's tool for bias assessment as outlined by Higgins in 2011. When dealing with randomized controlled trials (RCTs), the reviewers assessed the randomization, allocation concealment, blinding (i.e., patient, caregiver, outcome assessors), incomplete outcome data, selective outcome reporting, and other potential sources of bias.

For each study trial considered, both reviewers independently assessed the procedures reported by the trial authors for each of these ROB domains. Subsequently, the reviewers determined the risk of bias for each domain by categorizing it as 'low risk' of

bias, 'high risk' of bias, or 'unclear risk' of bias.

In cases where essential information for assessing quality was missing, the trial authors were contacted for clarification. Any disagreements in the assessment were resolved through discussion and, when necessary, by consulting a third review author.

Measures of treatment effect

The reviewers used relative risk (RR) with 95% confidence interval (CI) for dichotomous outcomes including the occurrence and discontinuation of treatment due to adverse events. Change in ALSFRS-R total score from baseline was reported as a continuous variable; therefore the effect measure was expressed as mean difference (MD) with 95% (CI).

Unit of analysis issues

The reviewers excluded cluster-randomized controlled trials (cluster-RCTs) from the review; hence, the estimation of intra-cluster correlation coefficients (ICC) was not applicable.

Dealing with missing data

When dealing with missing data, to accurately report the trial outcomes, the reviewers reached out to the trial authors to obtain any missing data. The reviewers reported the trial results individually, utilizing an available-case analysis.

Assessment of heterogeneity

The reviewers assessed the presence of heterogeneity (as applicable) by conducting the Chi2 test for heterogeneity, setting a threshold at $P<0.10$, and utilizing the I2 statistic. An I2 value exceeding 50% indicated significant or substantial statistical heterogeneity.

Assessment of reporting bias

Due to the limited number of eligible trials, it was not feasible to statistically assess the potential for publication bias.

Data synthesis

The trials included in our study provided either binary or continuous data. Dichotomous outcomes were expressed as risk ratios (RR) with 95% (CI). Continuous outcomes were expressed as mean differences (or standardized mean differences if different scales are used) and the 95% CI. The results of our studies were not qualified to be synthesized in a quantitative manner; therefore these are presented as part of the qualitative review.

In assessing and grading the quality of evidence for primary outcomes, we employed the GRADE approach. The quality of evidence from various studies was categorized into four levels: high, moderate, low, or very low. Initially, randomized controlled trials (RCTs) were classified as high quality, but we had the option to downgrade this rating after considering five criteria: risk of bias, consistency, directness, imprecision, and potential publication bias, as outlined by Guyatt in 2008.

RESULTS

Description of the studies

The summary of the included studies is provided in the characteristics of included studies in Table 3.

Results of the search

The reviewers obtained 36 titles and abstracts after the removal of duplicates from the electronic databases; no additional articles were included after contacting researchers or hand searching the reference list. The reviewers judged three articles as potentially eligible after screening the abstract and retrieving the full-text articles for inclusion or exclusion. After reviewing the retrieved articles, one study was excluded since it was only an abstract of a conference proceeding.

Figure 1 shows the summary of the study selection process.

Figure 1. Study flow diagram

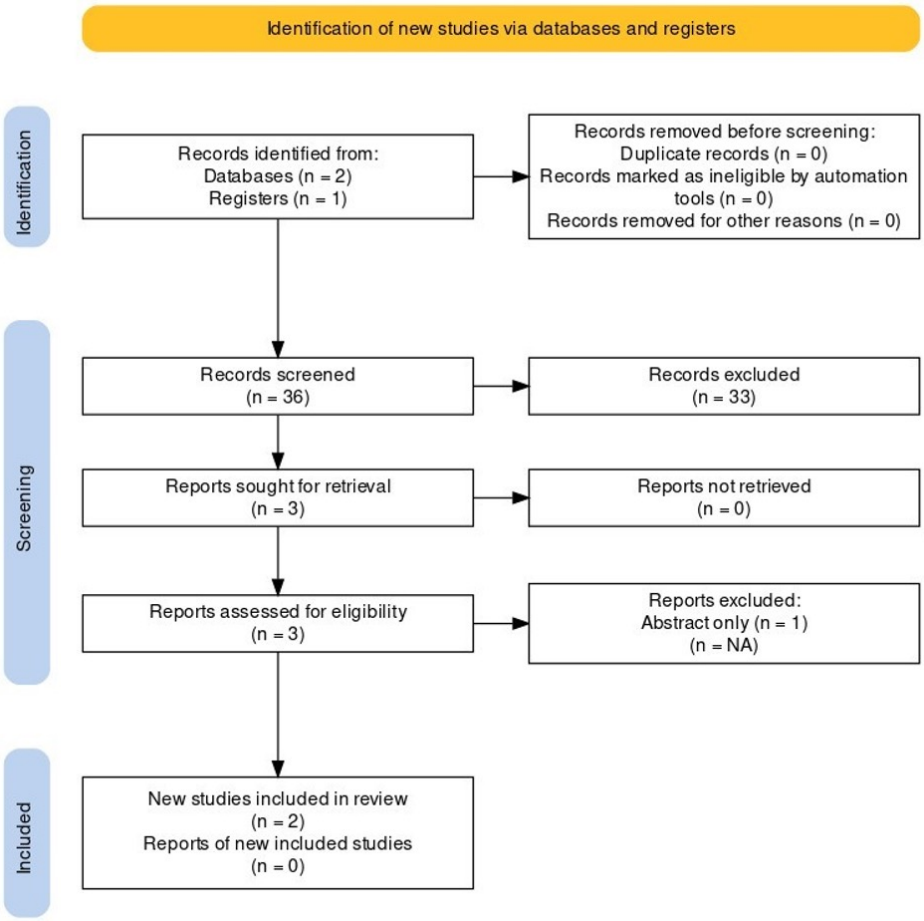


Figure 2. Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages

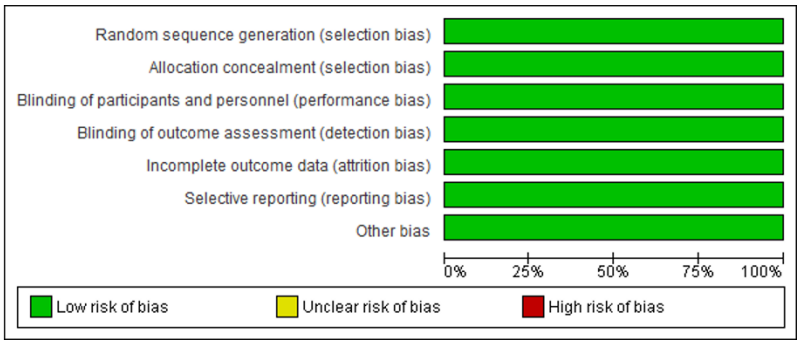
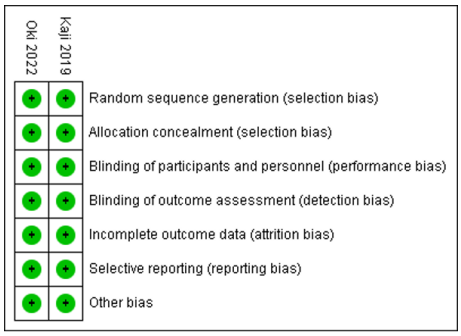


Figure 3. Risk of bias summary: review authors' judgements about each risk of bias item for each included



Included studies

Two studies involving 376 participants met the pre-specified inclusion criteria.

Type of intervention

Study by Kaji et al involved centrally randomizing patients to either receive placebo or 25 mg or 50 mg methylcobalamin groups. In the study by Oji et al, patients were randomly assigned in a 1:1 ratio to receive the investigational drug (either methylcobalamin 50 mg or placebo.)

Participants

Study by Kaji et al included patients satisfying the following inclusion criteria: outpatients aged 20 years or older; clinically definite, clinically probable, or clinically probable, laboratory-supported ALS diagnosis according to the revised El Escorial criteria (Airlie House criteria). In the study by Oji et al, patients included in the study were ambulatory patients 20 years or older who were diagnosed as having sporadic or familial ALS with definite, probable, or probable laboratory-supported categories using the updated Awaji criteria and within one year of symptom onset.

Setting

The two studies included in this review were conducted in an outpatient setting across clinics and sites in Japan.

Outcomes

The main outcome assessed in the study by Kaji et al included primary endpoints such as time to primary events and the change in ALSFRS-R score from baseline to week 182. For the study by Oki et al, the primary end point was the change in the ALSFRS-R total score from the allocation day (baseline) to week 16 of the treatment period.

Excluded studies

One study that was potentially eligible was excluded due to missing information as the article was only an abstract of a conference proceeding.

Risk of bias in included studies

The assessment of risk of bias by the reviewers is summarized in Figure 2 and 3.

Allocation

In the study by Kaji et al, the patients were centrally randomized to the either the placebo or 25 mg or 50 mg methylcobalamin groups using the order of registration with a minimization algorithm to balance the following factors: onset type (bulbar or upper or lower motor neuron onset), riluzole coadministration, ALSFRS-R score before study enrollment, and the change in this score during the observation period.

In the study by Oki et al, patients were randomly assigned in a 1:1 manner to receive either the investigational drug (either methylcobalamin 50 mg or placebo) with an electronic web-response randomization system on the basis of a complete randomization scheme prepared by the independent randomization expert.

Blinding

The blinding of the outcome assessors was adequate for both the included studies.

Incomplete outcome data

Both trials addressed incomplete outcome data adequately with intention-to-treat analysis employed for the full set analysis. There were clear numbers and reasons reported for those enrolled who withdrew from the studies.

Selective reporting

After the assessment of the reviewers, there was no clear indication of selective reporting in the included studies and all of the outcomes specified in the study methods sections were reported.

Other potential sources of bias

The assessment of the reviewers indicated that the included trials were free of other biases.

Effect of interventions

For the comparison of ultra-high dose (50 mg) methylcobalamin versus placebo in

the study by Kaji et al, there was no significant difference detected for the outcome median change in the ALSFRS-R score for the whole cohort. But in the post-hoc analyses of patients diagnosed early (²12 months after symptom onset), ultra-high-dose methylcobalamin prolongs time to death or ventilation support (HR [95% CI]: 0.64 [0.38 to 1.09] for 25 mg group and 0.50 [0.27 to 0.93] for 50 mg group; p=0.01 for placebo vs both methylcobalamin groups combined) and decreased ALSFRS-R scores (p=0.003 for 50 mg and p=0.01 for all methylcobalamin groups) in a dose-responsive manner. The incidence of treatment-related adverse events was similar and low in all groups.

For the comparison of ultra-high dose (50 mg) methylcobalamin versus placebo in the study by Oki et al, the mean (SD) least square mean difference in the ALSFRS-R total score at week 16 was −2.66 (0.61) in the methylcobalamin group and −4.63 (0.60) in the placebo group, and the difference was 1.97 in favor of methylcobalamin (95% CI, 0.44-3.50; P = .01). Adverse events were reported in 62% of patients (40 of 65) in the methylcobalamin group and in 66%of patients (42 of 64) in the placebo group.

DISCUSSION

Summary of main results

The authors included two randomized controlled trials in this review. These trials assessed the use of ultra-high dose methylcobalamin versus placebo to improve function among patients with amyotrophic lateral sclerosis. The evidence suggests that methylcobalamin did not show significant difference for the outcome median change in ALSFRS-R score for the whole cohort, but post hoc analyses had shown decreased ALSFRS-R scores (p=0.003 for 50 mg and p=0.01 for all methylcobalamin groups) in a dose-responsive manner, favoring the methylcobalamin group. The incidence of treatment-related adverse events was similar and low in all groups.

Comparison with previous studies

Our findings are consistent with a systematic review conducted by Vyloppilli et al in 2021 which concluded that methylcobalamin plays an essential role in the human body, whose deficiency, combined with other factors will cause neurological symptoms. A study by Ikeda et al in 2015 regarding the neuro-protective effect of ultra-high dose methylcobalamin in wobbler mouse model of amyotrophic lateral sclerosis indicated that mice treated with ultra-high dose (30mg/kg) significantly inhibited muscle weakness and contracture in the forelimbs and increased the weight of the bicep muscles and the number of musculocutaneous nerves. These findings concluded that treatment with methylcobalamin could delay progression of motor symptoms and neuropathological changes in motor neuron disease if very high doses are used. Preclinical trials like this had become the basis of the possible beneficial effects of ultra high-dose methylcobalamin in patients with early-stage amyotrophic lateral sclerosis.

Implications

Given the results, there may be significant implications for the treatment of amyotrophic lateral sclerosis, such as taking into consideration the possible beneficial effects of methylcobalamin when given within one year of diagnosis. This could potentially delay the need of ventilation support or improve the prognosis of ALS patients.

Limitations

However this review is not without limitations including the scarcity of randomized controlled trials of ultra high-dose methylcobalamin as a potential treatment of amyotrophic lateral sclerosis. The ALSFRS-R score, though widely used and validated, is dependent on the sample size required to detect a clinically meaningful change. The limitations in understanding the disease in genetics and molecular levels hindered the development of a more reliable objective and sensitive treatment researches.

Table 1. GRADE Evidence Profile

Certainty Assessment							Effect		Certainty		Importance
Number of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Ultra-high dose methycobalamin	Placebo	Relative (95% CI)	Absolute (95% CI)	
mean change from baseline in Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) total score											
1	randomised trials	not serious	not serious	not serious	not serious	publication bias strongly suspected[a]	For the comparison of ultra-high dose (50 mg) methylcobalamin versus placebo in the study by Oki et al, the mean (SD) least square mean difference in the ALSFRS-R total score at week 16 was −2.66 (0.61) in the methylcobalamin group and −4.63 (0.60) in the placebo group, and the difference was 1.97 in favor of methylcobalamin (95% CI, 0.44- 3.50; P = . 01).		□□□□ Moderate		
median change from baseline in Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) total score											
1	randomised trials	not serious	not serious	not serious	not serious	publication bias strongly suspected[a]	For the comparison of ultra-high dose (50 mg) methylcobalamin versus placebo in the study by Kaji et al, there was no significant difference detected for the outcome median change in the ALSFRS-R score for the whole cohort. But in the post-hoc analyses of patients diagnosed early (≤12 months after symptom onset), ultra-high-dose methylcobalamin decreased ALSFRS-R scores (p=0.003 for 50 mg and p=0.01 for all methylcobalamin groups) in a dose-responsive manner.		□□□□ Moderate		
Proportion of Adverse Events											
2	randomised trials	not serious	not serious	not serious	not serious	publication bias strongly suspected[a]	For the comparison of ultra-high dose (50 mg) methylcobalamin versus placebo in the study by Oki et al, adverse events were reported in 62% of patients (40 of 65) in the methylcobalamin group and in 66%of patients (42 of 64) in the placebo group. For the comparison of ultra-high dose (50 mg) methylcobalamin versus placebo in the study by Kaji et al, the incidence of treatment-related adverse events was similar and low in all groups.		□□□□ Moderate		

CI: confidence interval

a. Limited studies; possible unpublished studies exist

Recommendations for Future Research

Future research should focus on a larger sample size to confirm the effectiveness of methylcobalamin. Additional randomized controlled trials should be encouraged since current evidences are inconclusive.

Certainty of evidence (GRADE)

The reviewers assessed the quality or certainty of evidence using the GRADE approach and presented the findings in the evidence profile for the main comparison in Table 1. For patients given ultra-high doses of methylcobalamin for ALS, the reviewers assessed the certainty of evidence as moderate due to the risk of publication bias. However, the current evidence available is still limited to two RCTs done in Japan and additional or future trials would still be relevant and useful to improve the confidence in the review findings that can be generalized to other settings or populations.

Potential biases in the review process

The reviewers tried to minimize potential biases in the review process by adhering to the guidelines of Higgins 2011.

Agreements and disagreements with other studies or reviews

As of last search, there are no available systematic reviews on the use of ultra- high dose methylcobalamin versus placebo for patients with Amyotrophic Lateral Sclerosis.

AUTHORS' CONCLUSION

Ultra-high dose methylcobalamin reduced ALSFRS-R scores of patients with amyotrophic lateral sclerosis in its early stage, within one year of diagnosis. Although treatment-related adverse events were reported in two randomized controlled trials evaluated, no major adverse outcome like death was reported in both treatment and control groups. The scarcity of clinical trials makes it difficult to support a robust conclusion of benefit. Based on the results and existing evidence, the window of opportunities for more clinical trials including patients at the late onset of disease, is recommended. Ultra-high dose methylcobalamin therapy remains to be investigational.

REFERENCES

1. L. Tzeplaeff, S. Wilfling, MV. Requardt, M. Herdick (2023) Current State and Future Directions in the Therapy of ALS. doi: 10.3390/cells12111523
2. N. Maragakis, N. Galvez-Jimenez (2023). Epidemiology and pathogenesis of amyotrophic lateral sclerosis. Retrieved from: https://www.uptodate.com/contents/epidemiology-and-pathogenesis-of- amyotrophic-lateral-sclerosis
3. G. Logroscino, M. Piccininni (2019). Amyotrophic Lateral Sclerosis Descriptive Epidemiology: The Origin of Geographic Difference. Neuroepidemiology (2019) 52 (1-2): 93–103. https://doi.org/10.1159/000493386
4. S. Pai (2018). Peripheral Neuropathy. Integrative Medicine (Fourth Edition).Retrieved from: https://www.sciencedirect.com/topics/biochemistry-genetics-and- molecular-biology/methylcobalamin#:~:text=Methylcobalamin%20is%20the%20active%20form,autonomic%20symptoms%20compared%20to%20placebo
5. Batra G, Jain M, Singh RS, Sharma AR, Singh A, Prakash A, Medhi B. Novel therapeutic targets for amyotrophic lateral sclerosis. Indian J Pharmacol. 2019 Nov-Dec;51(6):418-425. doi: 10.4103/ijp.IJP_823_19. Epub 2020 Jan 16. PMID: 32029967; PMCID: PMC6984016.
6. S. Vyloppilli, A. Thangavelu, S. Vichattu and S. Sayd (2021). Safety and Efficacy of Methylcobalamin in the Treatment of Peripheral Nerve Injuries and Diabetic Neuropathies - A Systematic Review. Retrieved from: https://

actascientific.com/ ASPS/pdf/ ASPS-05-0769.pdf

7. Gil Mart'nez, V.; Avedillo Salas, A.; Santander Ballest'n, S. Vitamin Supplementation and Dementia: A Systematic Review. *Nutrients* 2022, *14*, 1033. https://doi.org/10.3390/nu14051033
8. Gršber U, Kisters K, Schmidt J. Neuroenhancement with vitamin B12-underestimated neurological significance. Nutrients. 2013 Dec 12;5(12):5031-45. doi: 10.3390/nu5125031. PMID: 24352086; PMCID: PMC3875920
9. Hu, N., Wang, X. The level of homocysteine in amyotrophic lateral sclerosis: a systematic review and meta-analysis. Neurol Sci 44, 1185–1192 (2023). https://doi.org/10.1007/s10072-022-06518-6
10. R. Kaji, S. Kuzuhara, Y. Iwasaki, K.Okamoto, M. Nakagawa, T. Imai, T. Takase, H. Shimizu, K. Tashiro (2015). Ultra-high dose methylcobalamin (E0302) prolongs survival of ALS: Report of 7 years' randomised double-blind, phase 3 clinical trial (ClinicalTrials.gov NCT00444613) (P7.060). Neurology Apr 2015, 84 (14 Supplement) P7.060
11. C. Wong, M. Stavrou, E. Elliott, J. Gregory, N. Leigh, A. Pinto, T. Williams, J. Chataway, R. Swingler, M. Parmar, N. Stallard, C. Weir, R.Parker, A. Chaouch, H. Hamdalla, J. Ealing, G. Gorrie, I. Morrison, C. Duncan, P. Connelly, F. Javier, Carod-Artal, R. Davenport, P. Garcia Reitboeck, A. Radunovic, V. Srinivasan, J. Preston, A. Mehta, D. Leighton, S. Glasmacher, E. Beswick, J. Williamson, A. Stenson, C. Weaver, J. Newton, D. Lyle, R. Dakin, M. Macleod, S. Pal, S. Chandran, Clinical trials in amyotrophic lateral sclerosis: a systematic review and perspective, *Brain Communications*, Volume 3, Issue 4, 2021, fcab242, https://doi.org/10.1093/braincomms/fcab242
12. Lauer, A.A.; Grimm, H.S.; Apel, B.; Golobrodska, N.; Kruse, L.; Ratanski, E.; Schulten, N.; Schwarze, L.; Slawik, T.; Sperlich, S.; et al. Mechanistic Link between Vitamin B12 and Alzheimer's Disease. *Biomolecules* 2022, *12*, 129. https://doi.org/10.3390/biom12010129
13. Zhang M, Han W, Hu S, Xu H. Methylcobalamin: a potential vitamin of pain killer. Neural Plast. 2013;2013:424651. doi: 10.1155/2013/424651. Epub 2013 Dec 26. PMID: 24455309; PMCID: PMC3888748.
14. Wolf, J., Safer, A., Wšhrle, J.C. *et al.* Factors predicting one-year mortality in amyotrophic lateral sclerosis patients - data from a population-based registry. *BMC Neurol* 14, 197 (2014). https://doi.org/10.1186/s12883-014-0197-9

Appendix

Table 2. Detailed search strategies

Element of interest	Free-text terms	Others
Population	Amyotrophic Lateral Sclerosis	
Intervention	methylcobalamin	vitamin B12
Comparator	placebo	
Outcomes	Slowing down of Functional decline efficacy safety	

MEDLINE and CENTRAL as of September 25, 2023

1	"methylcobalamin"[All Fields] OR "vitamin b12"[All Fields]	19,756
2	"amyotrophic"[All Fields] OR "amyotrophic lateral"[All Fields] OR "amyotrophic lateral sclerosis"[All Fields]	34,729
3	("methylcobalamin"[All Fields] OR "vitamin b12"[All Fields]) AND ("amyotrophic"[All Fields] OR "amyotrophic lateral"[All Fields] OR "amyotrophic lateral sclerosis"[All Fields])	36
4	(("methylcobalamin"[All Fields] OR "vitamin b12"[All Fields]) AND ("amyotrophic"[All Fields] OR "amyotrophic lateral"[All Fields] OR "amyotrophic lateral sclerosis"[All Fields])) AND (clinicaltrial[Filter])	4