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Vitamin B12: Integrative Metabolism and Comparative Pharmacology of the Four Cobalamin Forms - Cyanocobalamin, Hydroxocobalamin, Methylcobalamin, and Adenosylcobalamin

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ABSTRACT

Background: Vitamin B12 deficiency is a global public health problem affecting 2.5 to 26% of the population depending on diagnostic criteria and the population studied, with the highest prevalence in elderly persons, vegans, patients on long-term proton-pump inhibitors or metformin, and those with digestive malabsorption. Four therapeutic cobalamin forms are available: cyanocobalamin (CNCbl), hydroxocobalamin (OHCbl), methylcobalamin (MeCbl), and adenosylcobalamin (AdoCbl). Despite their shared corrinoid core, their pharmacokinetic and clinical profiles differ substantially.

Methods: We systematically reviewed pharmacokinetic studies, randomized controlled trials (RCTs), meta-analyses, and mechanistic investigations comparing the four cobalamin forms with respect to absorption, tissue retention, biochemical efficacy, and clinical outcomes, incorporating animal-model and mechanistic data where human evidence is lacking.

Results: All four forms effectively correct biochemical markers of deficiency and reverse hematologic manifestations. OHCbl shows superior plasma retention (plasma half-life 9–10 days vs. approximately 6 days for CNCbl) and reduced urinary losses after intramuscular injection, supporting extended dosing intervals. High-dose oral CNCbl (≥1000 µg/day) is bioequivalent to intramuscular therapy when absorption capacity is preserved (Lacombe et al., 2024). MeCbl demonstrates meta-analytically proven superiority over CNCbl for nerve conduction velocity in diabetic peripheral neuropathy (SMD 0.72; 95% CI 0.42–1.02) and has shown a 43% slowing of functional decline in early-stage ALS at ultra-high dose (50 mg IM twice weekly) in the JETALS phase III trial (JAMA Neurology, 2022). OHCbl is the first-line treatment for inborn errors of cobalamin metabolism, particularly cblC defect, in which CNCbl is contraindicated.

Conclusions: No single cobalamin form is universally superior. Form selection should be guided by the underlying etiology, route of administration, neurologic involvement, renal function, and specific metabolic disorder. A rational, individualized

approach to cobalamin prescribing - moving beyond the reductive concept of interchangeable 'vitamin B12' - is warranted by the available evidence.

1. Introduction

Vitamin B12, or cobalamin, is structurally the most complex of all known vitamins and the only one to contain a metallic ion - cobalt - at its center. Identified in the 1920s as the curative factor in otherwise-fatal pernicious anemia, it was chemically isolated and characterized in 1948. Subsequent decades have substantially deepened understanding of its active forms, intracellular metabolism, and therapeutic applications.

Its deficiency constitutes a global public health problem, affecting between 2.5% and 26% of the population depending on diagnostic criteria and the population studied[1,2] with particularly high prevalence in elderly persons, vegans, patients receiving long-term proton-pump inhibitors or metformin[3] and those with digestive malabsorption (e.g., Biermer's disease, gastric bypass, ileal resection). Four therapeutic cobalamin forms are currently available.

Cyanocobalamin (CNCbl) is the oldest and most widely distributed pharmaceutical form, favored in France and many other countries for its exceptional chemical stability. Hydroxocobalamin (OHCbl) is a natural, long-acting form recommended as first-line parenteral therapy in the United Kingdom by the British National Formulary[2]. Methylcobalamin (MeCbl) is the cytosolic active coenzyme, extensively studied in neurology. Adenosylcobalamin (AdoCbl) is the mitochondrial coenzyme whose role is particularly recognized in disorders of methylmalonic acid metabolism. These four forms share a common corrinoid nucleus but differ in the upper axial ligand at the cobalt β-position, a structural distinction that determines their physicochemical stability, metabolic routing, and pharmacokinetic profiles.

The persistent conflation of these forms in clinical practice - routinely assimilated under the label 'vitamin B12' - leads to suboptimal therapeutic choices, particularly in neurological indications where the superiority of methylcobalamin over cyanocobalamin is now documented in published meta-analyses[4,5]. The objective of this review is to provide a structured, up-to-date synthesis of the integrative metabolism of vitamin B12, followed by a rigorous comparative analysis of the four available forms from pharmacokinetic, clinical, and toxicological perspectives, directed at clinicians who make these choices in everyday practice.

2. Methods

2.1. Search strategy and eligibility criteria

We conducted a narrative systematic review following the reporting principles of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) framework, adapted for a comparative pharmacological review. Searches were performed independently by two authors (E.A. and X.J.) in four electronic databases: MEDLINE/PubMed, Embase, the Cochrane Central Register of Controlled Trials (CENTRAL), and Scopus. The search was completed in January 2026 and encompassed publications from inception through December 2025, with no language restriction applied to the initial retrieval; non-English articles were included if an adequate English abstract was available and data could be reliably extracted.

The search strategy combined Medical Subject Headings (MeSH) terms and free-text keywords organized into three conceptual blocks:

- The intervention - "vitamin B12," "cobalamin," "cyanocobalamin," "hydroxocobalamin," "methylcobalamin," "mecobalamin," "adenosylcobalamin," "cobamamide";
- The clinical domain - "vitamin B12 deficiency," "cobalamin deficiency," "pernicious anemia," "food-cobalamin malabsorption," "peripheral neuropathy," "diabetic neuropathy," "subacute combined degeneration," "amyotrophic lateral sclerosis," "methylmalonic acidemia," "inborn errors of cobalamin metabolism," "cblC," "homocysteinemia"; and
- Study design terms - "randomized controlled trial," "meta-analysis," "systematic review," "pharmacokinetics," "bioavailability," "comparative study." Terms within each block were combined with Boolean OR operators, and the three blocks were intersected with AND. Reference lists of included systematic reviews and meta-analyses were hand-searched for additional eligible studies.

2.2. Study selection and data extraction

Titles and abstracts were screened independently by two reviewers (E.A. and N.L.-V.), with full-text review performed for all potentially eligible records. Disagreements were resolved by consensus, with arbitration by a third reviewer (T.V.) when required. Studies were included if they met all of the following criteria:

- They directly compared two or more cobalamin forms (CNCbl, OHCbl, MeCbl, or AdoCbl), or reported pharmacokinetic parameters of a single form in sufficient detail for cross-study comparison;
- The primary outcome was at least one of the following - serum cobalamin concentration, holotranscobalamin (HoloTC), methylmalonic acid (MMA), total homocysteine (tHcy), hematological indices (hemoglobin, mean corpuscular volume), nerve conduction velocity, functional neurological scores (e.g., ALSFRS-R), or clinical endpoint;
- The study population comprised human participants (of any age) with confirmed or at-risk vitamin B12 deficiency, or healthy volunteers in pharmacokinetic studies; and
- The study design was a randomized controlled trial (RCT), prospective or retrospective cohort study, systematic review, meta-analysis, or pharmacokinetic investigation.

Studies were excluded if they: reported only supplementation without a deficiency-relevant endpoint; were limited to dietary intake surveys without therapeutic intervention; enrolled exclusively patients with inborn errors whose metabolic phenotype precluded generalization to the common deficiency population (unless specifically analysed in a dedicated

subsection); or were available only as conference abstracts without peer-reviewed full-text publication. Animal and in vitro mechanistic studies were retained as a supplementary evidence tier to inform pathways for which human pharmacokinetic data are absent or insufficient, and are identified as such throughout the text.

Data extraction was performed using a standardized form capturing: study design and setting, population characteristics (sample size, age, sex, clinical indication), cobalamin form(s) and comparators, route and dose of administration, duration of treatment and follow-up, primary and secondary outcomes with measures of central tendency and dispersion, and adverse events. For pharmacokinetic studies, extracted parameters included peak plasma concentration (Cmax), area under the plasma concentration-time curve (AUC), plasma half-life ($t_{½}$), volume of distribution (Vd), urinary excretion fraction, tissue distribution data, and protein binding characteristics. When individual patient data were not available, aggregate data were extracted from published figures using validated digital extraction software.

2.3. Quality assessment and evidence grading

Risk of bias in individual RCTs was assessed with the Cochrane Risk of Bias tool (RoB 2.0), evaluating five domains: randomization process, deviations from intended intervention, missing outcome data, measurement of the outcome, and selection of the reported result. Observational studies and pharmacokinetic investigations were assessed with the Newcastle-Ottawa Scale (NOS). Included systematic reviews and meta-analyses were appraised with the AMSTAR-2 tool.

The overall certainty of evidence for each clinically relevant comparison was graded using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) framework, producing four levels: high, moderate, low, and very low. Downgrading factors considered included risk of bias, inconsistency across studies (statistical heterogeneity I^2 >50% or substantial clinical heterogeneity), indirectness of evidence (surrogate outcomes, divergent populations), imprecision (wide confidence intervals crossing the threshold of no effect or clinically important difference), and suspected publication bias (assessed by funnel-plot asymmetry when ≥10 studies were available for a given comparison). Upgrading factors included large effect size (OR or RR >2 or <0.5), a dose-response gradient, or residual confounding that would be expected to attenuate rather than inflate the observed effect. Evidence levels cited in the clinical sections and summary tables are reported using the Oxford Centre for Evidence-Based Medicine (CEBM) 2011 grading (1a–5) for compatibility with the referenced meta-analyses, with GRADE certainty noted in parentheses where available.

2.4. Outcome definitions and analytical approach

Outcomes were classified as pharmacokinetic, biochemical, hematological, or clinical. Pharmacokinetic outcomes encompassed parameters characterizing absorption, distribution, metabolism, and elimination, as described above. Biochemical outcomes included changes in serum cobalamin (total and in the active HoloTC fraction), plasma MMA, and total homocysteine - the three functional biomarkers currently considered most informative for the assessment of true tissue-level B12 status.

Hematological outcomes encompassed hemoglobin concentration, mean corpuscular volume (MCV), reticulocyte count, and time to normalization of the blood count. Clinical outcomes included neurological scores (nerve conduction velocities for motor and sensory fibres; validated scales such as the Michigan Neuropathy Screening Instrument [MNSI], the Total Neuropathy Score [TNS], and the ALSFRS-R in the ALS subgroup), rates of complete and partial biochemical or clinical response, and patient-reported outcomes where available. Safety outcomes included all adverse events and serious adverse events regardless of causality attribution.

Given the substantial heterogeneity in study designs, populations, routes of administration, doses, and follow-up durations across the retrieved literature, a formal pooled meta-analysis of the primary data was not performed by the present review. Instead, we adopted a structured narrative synthesis that groups evidence by clinical domain (hematological, neurological, rare metabolic disorders), highlights the best available comparative data within each domain, and incorporates the quantitative summaries of previously published meta-analyses - particularly those of Abdelwahab, et al.[6] Wang, et al.[4] Sawangjit, et al.[5] and Arhip, et al.[7] - as the highest available tier of synthesised evidence for the respective comparisons.

2.5. Role of animal and mechanistic evidence

For several clinically important aspects of cobalamin pharmacology - including subcellular trafficking, intracellular interconversion kinetics, blood-brain barrier penetration, and the neuroprotective mechanisms of MeCbl - controlled human studies are either absent or ethically impracticable. In these domains, we incorporated evidence from three supplementary evidence tiers, listed in descending order of priority:

- Non-human primate studies reporting tissue distribution after administration of labelled cobalamin forms;
- Rodent deficiency-repletion models in which biochemical and functional endpoints were compared across cobalamin forms; and
- *In vitro* studies in human neuronal cell lines or primary cultures elucidating molecular targets of MeCbl (methionine synthase activation, axonal Erk1/2 and Akt signaling, NF-κB-mediated neuroinflammatory suppression). All findings derived from these non-human models are explicitly identified in the text and interpreted with appropriate caution regarding the limits of translational extrapolation.

2.6. Scope and pre-specified limitations

This review is explicitly scoped to the four pharmaceutical cobalamin forms available in clinical practice. It does not address dietary sources of vitamin B12 in isolation, nor the epidemiology of deficiency beyond what is necessary to contextualize therapeutic choice. The scope encompasses parenteral (intramuscular, intravenous, subcutaneous), oral, and sublingual routes of administration; nasal formulations were excluded owing to an absence of comparative data against the principal routes. Pediatric pharmacokinetics are addressed only in the context of inborn errors of metabolism, where the available evidence is specific to that population.

Pre-specified limitations of the evidence base are acknowledged throughout and are discussed systematically in the final Discussion section. These include: the predominance of short-term surrogate-endpoint trials over long-term clinical-

endpoint trials; the paucity of head-to-head RCTs specifically designed to compare cobalamin forms rather than to compare a cobalamin form against placebo or standard of care; the under-representation of women, elderly persons, and ethnically diverse populations in pharmacokinetic studies; and the absence of standardized, internationally harmonized diagnostic thresholds for vitamin B12 deficiency, which creates heterogeneity in the populations enrolled across published trials.

3. Integrative Metabolism of Vitamin B12

3.1. Chemical structure and natural forms

Cobalamin possesses a tetrapyrrolic corrin nucleus - analogous to the porphyrin ring of haem but containing a central trivalent cobalt ion (Co^{3+}). Four of the six cobalt coordination positions are occupied by the nitrogen atoms of the pyrrole groups. The fifth is bound to a 5,6-dimethylbenzimidazole (DMB) moiety forming the common lower axial base of all natural cobalamins. The sixth position - designated β or upper - is occupied by the variable ligand: a cyanide group (CN) in CNCbl, hydroxyl (OH) in OHCbl, methyl (CH^+) in MeCbl, and the 5′-deoxyadenosyl group in AdoCbl **(Figure 1)**[8].

AdoCbl is the predominant form in the liver and in tissues with high mitochondrial activity, whereas MeCbl predominates in plasma and nervous tissue. CNCbl is a synthetic form not found in the body under physiological conditions; it is produced industrially by bacterial fermentation followed by potassium cyanide treatment. Its exceptional thermal and photochemical stability makes it the reference form for long-term storage and low-cost oral formulations. OHCbl is a natural intermediate produced by many bacteria and represents the predominant form in human breast milk. MeCbl and AdoCbl are the only two biologically active coenzyme forms in the human body, serving respectively as cofactors for cytosolic methionine synthase and mitochondrial methylmalonyl-CoA mutase[9].

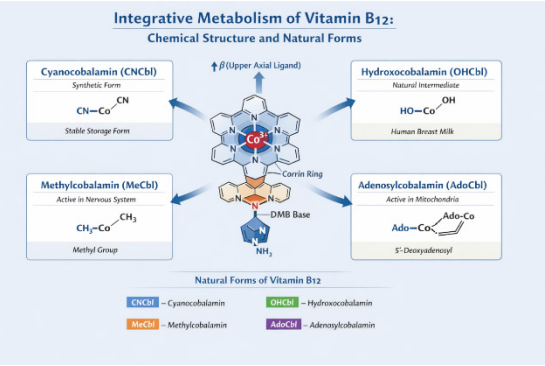

Figure 1: Comparative Structure of the Four Cobalamin Forms.

3.2. Gastric processing, Salivary transport, and Intrinsic factor

The absorption cascade for dietary cobalamin is remarkable in its complexity and vulnerability at multiple levels. Cobalamin is supplied exclusively by animal-source foods, in which it is tightly bound to food proteins - a bond requiring the combined action of gastric hydrochloric acid and pepsin for release. During the salivary phase, free cobalamin binds preferentially to haptocorrin (HC or R-binder), a glycoprotein secreted by salivary glands that protects cobalamin against the acidic gastric environment. In the duodenum and proximal jejunum, pancreatic proteases - primarily trypsin and chymotrypsin - hydrolyse HC and release cobalamin again, whereupon it binds to intrinsic factor (IF), a 44-kDa glycoprotein of exceptional specificity secreted exclusively by the oxyntic (parietal) cells of the gastric fundic mucosa. The affinity of IF for cobalamin is exceptionally high (dissociation constant Kd ~10^{-10} mol/L) **(Figure 2)**.

The IF-cobalamin complex resists intestinal proteases and migrates to the terminal ileum, where it is internalized by receptor-mediated endocytosis via the cubilin-amnionless (CUBAM) receptor expressed exclusively on ileal enterocytes. This pathway is saturable, permitting absorption of only approximately 1.5 to 2 µg per meal regardless of the ingested cobalamin form[2].

3.3. Plasma transport: The role of transcobalamins

Once released from enterocyte lysosomes following IF degradation, cobalamin is transferred to the portal circulation by binding to transcobalamin II (TC-II), a 43-kDa transport protein synthesized by enterocytes, hepatocytes, and endothelial cells. In plasma, two carrier proteins exist: haptocorrin (TC-I), which represents approximately 70–80% of total plasma cobalamin but whose bound fraction is not directly accessible to peripheral cells; and TC-II, which represents only 20–30% of total plasma cobalamin but constitutes the sole biologically available fraction for tissues - the holotranscobalamin (HoloTC)[10,11].

This distinction is fundamental in clinical biochemistry: holotranscobalamin is considered the earliest and most specific marker of vitamin B12 depletion, reflecting the fraction actually available to cells well before total serum cobalamin falls below established reference thresholds. Its measurement, together with methylmalonic acid (MMA) and total homocysteine (tHcy), constitutes the most sensitive diagnostic approach to functional B12 deficiency[10].

3.4. Intracellular metabolism and enzymatic pathways

Cellular uptake of cobalamin occurs via the TC-II receptor (CD320), ubiquitously expressed on all nucleated cells. Endocytosis of the HoloTC/CD320 complex is followed by lysosomal degradation of TC-II and release of free cobalamin, which then undergoes enzymatic reduction from Co^{3+} to Co^{2+} and subsequently to Co^{1+} (cob(I)alamin). This highly reductive form constitutes the common precursor to both active coenzymes, whose synthesis then diverges along two distinct compartmental pathways[12].

In the cytosolic compartment, cob(I)alamin is methylated by methionine synthase reductase (MTRR) to form MeCbl, which serves as an immediate cofactor for methionine synthase (MTR). This enzyme catalyzes the transfer of the methyl group from 5-methyltetrahydrofolate (5-MTHF) to homocysteine, simultaneously generating methionine and tetrahydrofolate (THF). Methionine is subsequently converted to S-adenosylmethionine (SAM), the principal universal methyl donor involved in DNA methylation, histone modification, neurotransmitter synthesis, phospholipid biosynthesis, and myelin maintenance. In the mitochondrial compartment, cob(I)alamin is adenosylated by cobalamin adenosyltransferase (MMAB) to form AdoCbl, the indispensable cofactor of methylmalonyl-CoA mutase (MUT), which catalyzes isomerisation of L-methylmalonyl-CoA to succinyl-CoA, enabling entry of branched-chain amino acids and odd-chain fatty acids into the Krebs cycle[8,9].

3.5. The methionine cycle, Methyl-folate trap, and clinical implications

The role of MeCbl at the crossroads of the methionine

cycle and folate cycle explains the pathophysiological link between B12 deficiency and neurological, hematological, and cardiovascular disease. In MeCbl deficiency, methionine synthase is non-functional, producing:

- Accumulation of homocysteine - neurotoxic at elevated concentrations and an independent cardiovascular risk factor;
- Accumulation of 5-MTHF in an unusable form, creating functional folate deficiency despite potentially normal serum levels - the 'methyl-folate trap' - inducing megaloblastic hematopoiesis common to both B12 and folate deficiencies; and
- Depletion of SAM, compromising myelin methylation and contributing to the white-matter lesions of subacute combined degeneration of the spinal cord[1,8].

Concurrently, AdoCbl deficiency disrupts mitochondrial catabolism of odd-chain fatty acids and branched-chain amino acids, causing accumulation of methylmalonic acid (MMA) whose neuronal, renal, and myocardial toxicity is well established[9].

3.6. Hepatic storage and enterohepatic circulation

The liver is the principal storage organ for cobalamin, concentrating 50-90% of total body reserves - approximately 2-5 mg in a well-nourished adult. This exceptional storage capacity, combined with active enterohepatic circulation - approximately 0.5-9 µg of cobalamin is secreted in bile daily, of which 80-90% are reabsorbed in the ileum via IF - explains the long latency of clinical deficiency: in the complete absence of exogenous supply (strict vegan diet), several years may elapse before clinical signs appear. This enterohepatic circulation is compromised in patients with ileal resection or total gastrectomy, considerably accelerating reserve depletion[1].

3.7. Renal conservation mechanisms

The kidney participates, though more limitedly than the liver, in cobalamin homeostasis through highly efficient glomerular filtration and proximal tubular reabsorption mechanisms mediated by the megalin-cubilin receptor system. In healthy subjects, more than 95-99% of filtered cobalamin is recovered in the proximal tubule, limiting daily losses to minute quantities (generally <0.1 µg/day). When parenteral high-dose therapy saturates plasma binding capacity, the unbound fraction increases and urinary excretion becomes significant - accounting for the rapid renal clearance of excess cobalamin observed after intramuscular injection of CNCbl compared with OHCbl.

4. Comparative Pharmacology of the Four Cobalamin Forms

4.1. Cyanocobalamin (CNCbl)

Cyanocobalamin is the oldest synthetic therapeutic form and the most widely distributed worldwide, serving as the default form in France and many other countries. Its remarkable thermochemical and photochemical stability confers a shelf life of up to five years at ambient temperature, making it the reference form for low-cost oral formulations. After oral administration at low (physiological) doses (≤10 µg), its absorption is entirely IF-dependent, with a bioavailability of approximately 50%. At high pharmacological doses (≥500 µg), passive non-saturable diffusion accounts for approximately 1-2% of the administered dose - quantitatively significant at the doses used clinically[13,14].

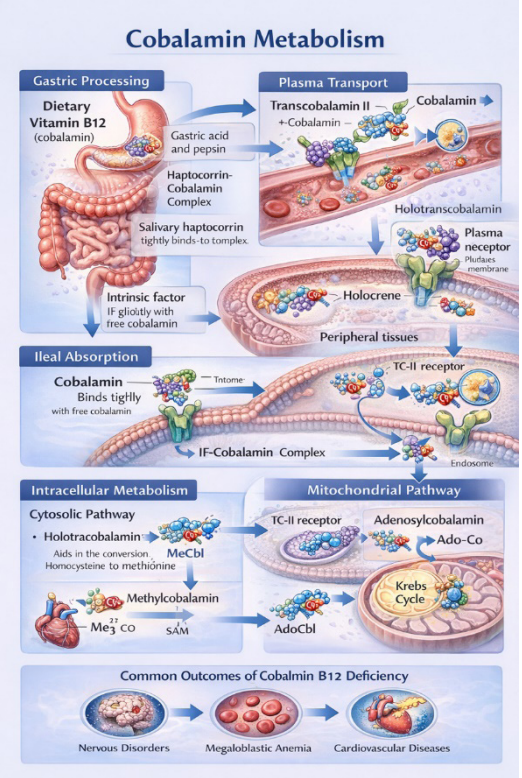

Figure 2: Metabolism of the Cobalamin.

CNCbl is a prodrug that must be converted to active forms by the organism. This conversion requires a prior enzymatic decyanation step (catalyzed by cytosolic cob(I)alamin reductase), releasing a cyanide ion (CN^+) excreted in urine and generating hydroxocobalamin or cob(II)alamin, which then enters normal metabolic pathways. The amount of cyanide released is clinically insignificant at standard therapeutic doses (well below the toxic threshold) but may become problematic in severe renal failure, chronic occupational cyanide exposure, Leber hereditary optic neuropathy, or specific inborn errors of metabolism. Its plasma half-life is approximately 6 days, benefiting from enterohepatic recycling. Urinary excretion after intramuscular injection is greater than for the other forms, explaining the characteristic yellow discoloration of urine[1].

4.2. Hydroxocobalamin (OHCbl)

Hydroxocobalamin is a semi-natural form, an intermediate in the cobalamin metabolic pathway, requiring no decyanation step. It exhibits the longest plasma half-life of the four forms - estimated at 9 to 10 days after intramuscular injection - due to its high affinity for albumin and plasma haptocorrin[15]. This prolonged retention allows more widely spaced injection intervals in maintenance therapy (up to one injection every three months) and superior overall tissue retention compared with CNCbl. These pharmacokinetic advantages have led the British National Formulary to recommend OHCbl as first-line parenteral therapy in the United Kingdom, where intramuscular CNCbl has been classified as 'less suitable for prescribing[15]'.

Beyond supplementation, OHCbl has a unique indication: antidote to cyanide poisoning. By binding with high affinity to free cyanide to form excretable cyanocobalamin, it is administered at very high intravenous dose (5 g, Cyanokit®) in cyanide and carbon monoxide poisoning from fires - making it the only cobalamin form that simultaneously serves as a nutritional supplement, a neurological therapeutic, and a

toxicological antidote. OHCbl parenteral therapy is also the reference treatment for inborn errors of intracellular cobalamin metabolism, notably cblC defect (MMACHC mutations), where it is administered urgently at high dose to prevent acute metabolic decompensation[7].

4.4. Methylcobalamin (MeCbl)

Methylcobalamin is one of the two biologically active coenzyme forms in the human body, predominating in plasma and nervous tissue. Unlike CNCbl and OHCbl, it requires no enzymatic activation and can be directly utilized by cytosolic methionine synthase. Its oral bioavailability is good, and the sublingual route is particularly attractive because buccal mucosal absorption partially bypasses IF-dependence, conferring an advantage in IF-deficiency states[16]. A 2023 study published in the Indian Journal of Neurosciences confirmed non-inferiority of the subcutaneous versus intramuscular route for MeCbl 1500 µg with similar pharmacokinetic profiles and better local tolerability[16].

MeCbl is distinguished by markedly superior cerebral penetration compared with the other forms, attributable to its high affinity for nervous tissue membrane transporters. Its preferential accumulation in the cerebral cortex, dorsal root ganglia, and peripheral nerves confers specific neuroprotective and neuro-reparative properties, including: direct activation of methionine synthase and restoration of SAM-mediated myelin methylation; promotion of axonal regeneration via Erk1/2 and Akt signaling pathways; modulation of neuroinflammation through regulation of T-lymphocytes and NK cells; and an indirect antagonist effect at NMDA receptors reducing glutamatergic excitotoxicity[17]. Its plasma half-life is shorter (3–4 days) than that of OHCbl, necessitating more frequent administration, and its light sensitivity requires particular storage precautions.

4.5. Adenosylcobalamin (AdoCbl)

Adenosylcobalamin is the active coenzyme form in the mitochondrial compartment, predominating in the liver, skeletal muscle, and myocardium - tissues with high oxidative metabolic activity. Directly active without prior conversion, it serves as cofactor for methylmalonyl-CoA mutase (MUT), catalyzing isomerisation of L-methylmalonyl-CoA to succinyl-CoA, enabling mitochondrial oxidation of odd-chain fatty acids, branched-chain amino acids (valine, isoleucine, methionine, threonine), and thymine. Its deficiency leads to accumulation of methylmalonic acid (MMA) and propionyl-CoA, molecules whose toxicity for neurons, renal tubules, and myocardium is well established[9].

AdoCbl is extremely photosensitive, degrading rapidly under ambient light, necessitating strict storage and handling conditions. Its plasma half-life is the shortest of the four forms (2-3 days), though intracellular mitochondrial storage is extensive and prolonged, partially compensating for this rapid elimination. Commercial availability is more limited than the other three forms, particularly in injectable formulations, restricting clinical use to situations in which AdoCbl is specifically irreplaceable - principally cobalamin-sensitive methylmalonic acidemias and specific MUT mitochondrial defects **(Table 1)**.

Table 1: Comparative Pharmacokinetic Parameters of the Four Therapeutic Cobalamin Forms.

PK Parameter	Cyanocobalamin (CNCbl)	Hydroxocobalamin (OHCbl)	Methylcobalamin (MeCbl)	Adenosylcobalamin (AdoCbl)
Upper axial ligand (β-position)	Cyano (CN^-)	Hydroxyl (OH^-)	Methyl (CH_3)	5′-Deoxyadenosyl
Biologically active form	No (prodrug)	No (precursor)	Yes - cytosol	Yes - mitochondria
Oral bioavailability (IF-mediated)	~50% (IF-dependent)	Good	Good + sublingual	Variable; limited data
Transcobalamin binding (TC-I / TC-II)	~80% / 20%	~85% / 15%	~75% / 25%	~75% / 25%
Plasma t½ (intramuscular)	~6 days	~9–10 days	~3–4 days	~2–3 days
CNS / nerve penetration	Low	Moderate	Excellent +++	Moderate
Preferred storage compartment	Liver (general)	Liver +++	CNS / peripheral nerves	Mitochondria
Urinary excretion	High (>50%)	Moderate	Moderate	Moderate
Light stability	Very stable	Stable	Photosensitive (+)	Very photosensitive (++)

Abbreviations: CNCbl = cyanocobalamin; OHCbl = hydroxocobalamin; MeCbl = methylcobalamin; AdoCbl = adenosylcobalamin; IF = intrinsic factor; TC-I/TC-II = transcobalamin I and II; subl. = sublingual; CNS = central nervous system; IM = intramuscular; t½ = plasma half-life. Data from published pharmacokinetic studies; parameters for MeCbl and AdoCbl are less well characterized than for CNCbl and OHCbl.

5. Comparative Clinical Efficacy: Meta-Analytic Evidence

5.1. Correction of deficiency and hematological restoration

For hematological outcomes, available randomized controlled trials and meta-analyses demonstrate functional equivalence of all four cobalamin forms in correcting megaloblastic anemia and normalizing erythrocyte indices, provided adequate dose and appropriate route are selected. The network meta-analysis by Abdelwahab et al. (2024), encompassing 4,275 patients from 13 comparative studies, compared three routes of administration (oral, intramuscular, sublingual)[6]. Intramuscular administration was associated with the largest increase in plasma cobalamin levels (mean difference: +94.09 pg/mL vs. oral), followed by sublingual (+43.31 pg/mL), though these differences did not reach statistical significance owing to the limited number of available studies - confirming that high-dose oral supplementation (1000–2000 µg/day) constitutes a valid alternative to intramuscular injection for deficiency correction except in severe cases requiring rapid repletion.

The earlier work of Andrès and collaborators on food-cobalamin malabsorption (FCM) - an entity corresponding to the inability to release cobalamin from dietary proteins despite preserved IF secretion - represented a pivotal step in understanding 'subtle' forms of vitamin B12 deficiency in elderly persons. The Strasbourg group demonstrated that this situation, frequently associated with gastric atrophy, Helicobacter pylori infection, or prolonged proton-pump inhibitor and biguanide (metformine) use, could be effectively treated with high-dose oral cyanocobalamin (≥1000 µg/day), normalizing serum cobalamin, homocysteine, and MMA[13,14,18].

More recently, Lacombe, et al., prospectively evaluated oral CNCbl (1000 µg/day) in 26 consecutive patients with confirmed pernicious anemia (anti-IF and/or anti-parietal cell antibodies, immunological gastritis). After one year of follow-up, 88.5% of patients had normalized their vitamin B12 status, with significant reductions in homocysteinemia and plasma MMA[19]. This result is remarkable in that it challenges the widely held belief that patients with IF deficiency cannot effectively absorb cobalamin by the oral route: at pharmacological doses of 1000 µg, IF-independent passive ileal diffusion (representing approximately 1% of dose) is sufficient to compensate for the absorption deficit.

5.2. Peripheral neuropathy: The superiority of methylcobalamin

It is in the neurological domain that differences between cobalamin forms manifest with the greatest clinical clarity. The meta-analysis by Wang, et al., encompassing 1,248 patients from 14 randomized controlled trials evaluating treatment of diabetic peripheral neuropathy, demonstrated significant superiority of MeCbl over CNCbl for improvement of motor and sensory nerve conduction velocities (standardized mean difference [SMD]: 0.72; 95% CI: 0.42–1.02)[4]. These improvements are consistent with the neurobiological mechanism specific to MeCbl: its direct utilization by neuronal methionine synthase without a conversion step, superior penetration of the blood-brain barrier, and capacity to stimulate axonal regeneration via Erk1/2 and Akt signaling pathways.

The meta-analysis by Sawangjit, et al. systematically evaluated MeCbl efficacy in peripheral neuropathy of all etiologies (diabetic, post-herpetic, uremic) including 26 randomized trials[5]. Results indicate that MeCbl monotherapy significantly improves global clinical therapeutic efficacy relative to active control (RR = 1.17; 95% CI: 1.03-1.33) and that MeCbl in combination is even more effective (RR = 1.32; 95% CI: 1.21-1.45) for nerve conduction parameters. Neither MeCbl alone nor in combination demonstrated statistically significant efficacy on pain scores or subjective neuropathic symptoms, underscoring the need for a multimodal approach in chronic neuropathic pain management.

A recent review by Ramadhani et al. of the molecular pharmacology of MeCbl in chronic peripheral neuropathic pain specified anti-inflammatory mechanisms involved: regulation of pro-inflammatory cytokine secretion (IL-6, TNF-α), modulation of regulatory T-lymphocytes, and inhibition of neuroinflammation via the NF-κB pathway[17]. These data open therapeutic perspectives beyond simple substitutive treatment, positioning MeCbl as a neuromodulatory agent in its own right.

Most recently, the meta-analysis by Deng et al. evaluated the combination of dapagliflozin with MeCbl in type 2 diabetic peripheral neuropathy across trials published through September 2024[20]. Results suggest that the combination demonstrates superior efficacy over MeCbl alone on electrophysiological markers and symptom scores, reinforcing interest in combined strategies in this indication where vascular and metabolic components are predominant.

5.3. Ultra-high-dose methylcobalamin in amyotrophic lateral sclerosis

One of the most significant advances of recent years in the clinical use of MeCbl concerns amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disease for which therapeutic options remain extremely limited. The JETALS trial (Japan Early-Stage Trial of Ultrahigh-Dose Methylcobalamin for ALS), a multicenter, randomized, double-blind, placebo-controlled phase III trial, evaluated intramuscular MeCbl 50 mg twice weekly for 16 weeks in 130 patients with early-stage ALS (symptom onset within 12 months) and moderate progression[21].

The primary endpoint - change in ALSFRS-R score (Revised ALS Functional Rating Scale) - demonstrated a significant slowing of functional decline in the MeCbl group compared with placebo (2.66 vs. 4.63 points over 16 weeks; $P<0.05$), representing a 43% reduction in the rate of functional progression. The safety profile was excellent, with no difference in adverse events between groups. These results follow the phase II/III trial by Kaji et al., which evaluated 373 ALS patients over 3.5 years, finding no significant effect across the entire cohort but a positive trend in the subgroup treated early (within 12 months of symptom onset)[22]. On the basis of combined JETALS data, Eisai submitted a marketing authorization application in Japan in 2024 for ultra-high-dose MeCbl in ALS, where it has held orphan drug status since May 2022. These data position MeCbl as the first cobalaminic agent to demonstrate efficacy in a major neurodegenerative disease beyond its classical substitutive indications.

5.4. Hydroxocobalamin in inborn errors of cobalamin metabolism

Parenteral OHCbl constitutes the reference treatment for inborn errors of intracellular cobalamin metabolism, particularly cblC defect (MMACHC mutations) - the most common such disorder. The systematic review by Arhip et al. encompassing 240 patients with late-onset cblC disease, showed that OHCbl (117 patients, predominantly IV or IM) demonstrated superior efficacy in normalizing MMA and total homocysteine compared with CNCbl (42 patients) or other forms[7]. This advantage arises from OHCbl's capacity to bypass the deficient decyanation step (MMACHC protein is precisely the enzyme defective in cblC) and directly supply the biosynthetic pathway for both active coenzymes. CNCbl, requiring MMACHC protein for its activation, is contraindicated in cblC defect.

5.5. Routes of administration: Oral, sublingual versus parenteral

A meta-analysis published in Frontiers in Pharmacology (2025), including 25 studies with a total of 6,098 participants, evaluated comparative efficacy of sublingual and oral routes versus intramuscular administration[23]. For high-dose oral forms (1000-2000 µg/day), the increase in serum cobalamin is

comparable to that obtained by intramuscular route after one to four months of treatment. The sublingual route, particularly for MeCbl, offers intermediate bioavailability and represents an attractive alternative for patients reluctant to undergo injection. These data, consistent with current American Family Physician recommendations (2022)[24], support the development of high-dose oral supplementation as a valid alternative to intramuscular injection in most non-urgent clinical situations, with the exception of severe malabsorption states or documented IF deficiency requiring very high doses to ensure adequate passive absorption.

5.6. Safety and drug interactions

The overall safety profile of vitamin B12 in all its forms is excellent; cobalamin is considered one of the least toxic known therapeutic substances. No tolerable upper intake level (UL) has been defined by the European Food Safety Authority (EFSA) for cobalamin, owing to an absence of toxicity data at usual therapeutic doses. Nonetheless, differences in safety profiles exist among the four forms, justifying specific precautions according to clinical context.

The principal safety concern for CNCbl relates to its cyanide content. At standard therapeutic doses (up to 1000 µg/day oral or monthly IM), the amount of CN^- released (approximately 50 µg per 1000 µg injection) carries no clinical consequence in subjects with preserved renal clearance. However, in advanced renal failure (eGFR <30 mL/min/1.73 m²), Leber hereditary optic neuropathy, or chronic occupational cyanide exposure, OHCbl or MeCbl are preferred. OHCbl and MeCbl, devoid of cyanide, present superior safety profiles in these specific contexts. OHCbl may produce transient skin pigmentation and red-brown urinary discoloration after injection, without clinical significance. AdoCbl, being extremely photosensitive, carries a risk of rapid degradation if poorly stored, potentially resulting in administration of an inactive product.

Drug interactions common to all four forms include metformin, which reduces intestinal cobalamin absorption by interfering with the ileal cubilin-amnionless receptor (CUBAM) via a calcium-dependent mechanism, explaining the high prevalence (up to 30%) of B12 deficiency in diabetic patients on long-term metformin therapy[21]. Proton-pump inhibitors reduce B12 absorption by decreasing the gastric acidity required to release cobalamin from dietary proteins. Colchicine, hydroxychloroquine, and certain anticonvulsants may also interfere with cobalamin absorption or metabolism. These interactions argue for regular biochemical monitoring of B12 status - ideally by measurement of holotranscobalamin and/or methylmalonic acid - in patients receiving these medications long-term **(Table 2)**.

Table 2: Comparative Safety Profiles of the Four Therapeutic Cobalamin Forms.

Safety Parameter	Cyanocobalamin (CNCbl)	Hydroxocobalamin (OHCbl)	Methylcobalamin (MeCbl)	Adenosylcobalamin (AdoCbl)
Cyanide moiety	Yes (small)	No	No	No
Safe in severe renal failure	Use with caution	Safe	Safe	Safe
Pregnancy / breastfeeding	Acceptable	Recommended	Preferred	Limited data
cblC defect (MMACHC)	Contraindicated	First-line	Adjunct possible	Adjunct (AdoCbl)
Allergic reactions	<1%	<1%	Very rare	Very rare
Urine / skin discoloration	Yellow urine	Red-brown urine/skin	None	None

Abbreviations: CNCbl = cyanocobalamin; OHCbl = hydroxocobalamin; MeCbl = methylcobalamin; AdoCbl = adenosylcobalamin; eGFR = estimated glomerular filtration rate; cblC = MMACHC defect. EFSA has defined no tolerable upper limit for any cobalamin form.

6. Therapeutic Positioning and Clinical Decision Framework

6.1. Cyanocobalamin: Economic reference for simple nutritional deficiency

CNCbl remains the form of choice for treatment of isolated nutritional deficiency - principally arising from strict vegan diet or food-cobalamin malabsorption - and for systematic supplementation of at-risk populations (elderly persons, patients on long-term proton-pump inhibitors or metformin, chronic H. pylori infection). Its exceptional stability, low cost, and availability in multiple formulations make it the most practical form for high-dose oral supplementation. At doses of 1000 µg/day, passive IF-independent absorption is sufficient to correct deficiency even in the absence of functional IF, as confirmed by Lacombe, et al. in pernicious anaemia[19]. It is contraindicated in inborn errors of cobalamin metabolism (notably cblC defect) and should be avoided in advanced renal failure or Leber hereditary optic neuropathy.

6.2. Hydroxocobalamin: Parenteral reference and metabolic emergencies

OHCbl is the form of choice for parenteral treatment of all severe deficiencies requiring rapid correction: pernicious anemia, subacute combined degeneration of the spinal cord requiring rapid intramuscular loading, and all severe malabsorption states. Its long plasma half-life (9-10 days) permits less frequent maintenance injections (one injection every three months), improving compliance and reducing patient burden. It is the first-line treatment for inborn errors of cobalamin metabolism (cblC, cblD defects) and for acute cyanide poisoning (Cyanokit®, 5 g IV). In the United Kingdom, it is the only form currently recommended for parenteral prescription within the NHS[15].

6.3. Methylcobalamin: first-line for neurological indications

MeCbl is the form of choice in all indications involving the nervous system - diabetic or other peripheral neuropathy, demyelinating polyneuropathy, chronic neuropathic pain, and cognitive decline associated with hyperhomocysteinemia. Its superiority over CNCbl in diabetic neuropathy is now documented by meta-analyses graded A/B by GRADE criteria[4,5]. In early-stage ALS, the JETALS trial has opened a

major potential new indication at ultra-high dose (MeCbl 50 mg twice weekly IM), currently under regulatory evaluation in Japan[21]. The sublingual form is particularly interesting for IF-deficient patients reluctant to undergo injection, and represents a pharmacokinetically documented alternative to intramuscular administration[16]. MeCbl is also recommended in preference during pregnancy and breastfeeding for optimal fetal and neonatal neurological development, given the absence of cyanide and its direct bioavailability.

6.4. Adenosylcobalamin: Irreplaceable in mitochondrial disease

AdoCbl retains an irreplaceable therapeutic niche in pathologies specifically linked to mitochondrial dysfunction of methylmalonyl-CoA mutase: cobalamin-sensitive methylmalonic acidemia (mut and cblA/cblB subtypes) and management of cblD variant 2 defects. In these indications, AdoCbl (or OHCbl, convertible to AdoCbl by the organism) is administered in combination with a diet low in propionate precursors (limited branched-chain amino acids) **(Table 3)**. The rarity of these conditions and the limited commercial availability of isolated AdoCbl explain why OHCbl remains in practice the preferred parenteral substitute in these situations, the organism converting it to AdoCbl according to mitochondrial demand[12].

Table 3: Therapeutic Positioning of Cobalamin Forms by Clinical Indication.

Clinical Indication	First-line Form	Alternative	Level of Evidence	Key Reference(s)
Nutritional deficiency / food-cobalamin malabsorption	CNCbl oral ≥1000 µg/day	MeCbl oral	A (meta-analyses)	13
Pernicious anemia (IF deficiency)	OHCbl IM	CNCbl oral or IM	A (RCTs, Cochrane)	19,25
Diabetic peripheral neuropathy	MeCbl oral or IM	OHCbl IM	A (meta-analysis, n=1248)	4,5
Subacute combined degeneration	OHCbl IM high-dose	MeCbl IM	B (case series, consensus)	2
Hyperhomocysteinemia	MeCbl + folate	CNCbl + folate	A (RCTs)	1
Early-stage ALS (moderate progression)	MeCbl 50 mg IM 2×/week	— (currently off-label outside Japan)	B (Phase III RCT)	21
Cyanide poisoning	OHCbl IV 5 g (Cyanokit®)	Sodium thiosulfate	A (toxicology RCTs)	Borron et al. (2007)
cblC defect (MMACHC)	OHCbl IM high-dose	MeCbl oral (adjunct)	B (pediatric series)	7
Methylmalonic acidemia (mut$^-$)	OHCbl IM + adapted diet	AdoCbl if available	B (registries, cases)	12
Pregnancy / breastfeeding	MeCbl oral	OHCbl IM if severe deficiency	C (expert consensus)	26

Abbreviations: CNCbl = cyanocobalamin; OHCbl = hydroxocobalamin; MeCbl = methylcobalamin; AdoCbl = adenosylcobalamin; IF = intrinsic factor; IM = intramuscular; RCT = randomized controlled trial; ALS = amyotrophic lateral sclerosis; cblC/cblD = MMACHC/MMADHC defect; Cyanokit® = hydroxocobalamin 5 g IV for cyanide poisoning. Evidence levels per Oxford CEBM 2011 grading.

7. Discussion

The present synthesis illuminates a persistent paradox in current medical practice: although the pharmacokinetic and clinical differences among the four cobalamin forms are now well-documented in the indexed scientific literature, prescribing in many countries continues to treat these forms as interchangeable therapeutic equivalents. This default choice - often favoring CNCbl for economic reasons, or OHCbl for regulatory reasons in Anglo-Saxon countries - is not always optimal, particularly in neurological indications where MeCbl offers a clinically significant and mechanistically rational advantage.

The publication of the JETALS trial in JAMA Neurology marks a turning point in the history of MeCbl, elevating it from the status of a common nutritional supplement to that of a potentially disease-modifying neurological therapeutic in early-stage ALS - a disease for which only two approved drugs exist (riluzole and edaravone) with modest effects[21]. While legitimate reservations persist regarding generalization of these results - principally related to the question of possible unblinding by the characteristic urinary discoloration of MeCbl and to the highly specific patient selection (recent onset, moderate progression) - the marketing authorization application submitted by Eisai to Japanese authorities in 2024 illustrates the clinical maturity of this new therapeutic application.

The question of cobalamin form selection must also integrate consideration of pharmacogenomic polymorphisms that may influence therapeutic response. Variants in the MTHFR gene (particularly C677T, homozygous in 10-15% of European populations), MTR (A2756G), MTRR (A66G), and TCN2 (C776G, encoding transcobalamin II) modify cobalamin requirements and preference for one or other active form[26]. These pharmacogenomic considerations, still insufficiently integrated into clinical practice, open the path to precision medicine in cobalamin deficiency management, where genotyping of the vitamin B12 metabolic pathways would allow individualization of form and dose.

Finally, the question of biochemical monitoring merits emphasis. The value of total serum B12 as a sole marker of status is recognized as insufficiently sensitive and specific; established lower reference limits (generally 200 pg/mL) may miss significant functional deficits, particularly in patients with low-normal values. Holotranscobalamin (HoloTC, the biologically available fraction), methylmalonic acid, and total homocysteine constitute the most robust complementary biomarkers for early diagnosis and monitoring of therapeutic correction[10]. Integration of these markers into follow-up protocols - particularly for at-risk populations - would significantly improve detection and management of subclinical deficits.

8. Conclusions and Perspectives

Vitamin B12 is a molecule of remarkable biological

complexity whose four clinically available forms - cyanocobalamin (CNCbl), hydroxocobalamin (OHCbl), methylcobalamin (MeCbl), and adenosylcobalamin (AdoCbl) - differ in pharmacokinetic, metabolic, and clinical properties sufficiently to justify a reasoned, indication-driven therapeutic choice rather than the reflexive prescription of a single interchangeable entity. The evidence reviewed herein supports a coherent, tiered positioning of each form: CNCbl as the economic first-line agent for nutritional deficiency and food-cobalamin malabsorption when the oral route is appropriate; OHCbl as the parenteral reference for all acute or severe indications and for inborn errors of cobalamin metabolism; MeCbl as the preferred form whenever the nervous system is the primary therapeutic target; and AdoCbl as an irreplaceable cofactor in the specific mitochondrial disorders for which it was, in essence, discovered. What this synthesis equally reveals, however, is that the evidentiary scaffolding supporting these choices is uneven - robust for some comparisons, suggestive but incomplete for others, and frankly absent in several areas of high clinical importance. The following perspectives outline the most pressing scientific and clinical research needs.

8.1. Perspective 1 - Head-to-head randomized trials in neurological indications

The meta-analytic evidence favoring MeCbl over CNCbl in diabetic peripheral neuropathy is statistically convincing at the level of nerve conduction velocity but does not yet extend to hard clinical endpoints such as validated functional disability scores, quality of life, or prevention of progression to foot ulceration or amputation. The trials included in the Wang et al. and Sawangjit et al. meta-analyses were predominantly short (8–24 weeks), enrolled heterogeneous populations with varying degrees of neuropathic severity, and were conducted almost exclusively in Asian populations - raising legitimate concerns about generalizability to European, North American, and African patient cohorts in whom metabolic, dietary, and genetic backgrounds differ substantially.

An international, multi-center, double-blind RCT directly comparing MeCbl to OHCbl in patients with confirmed B12-deficiency neuropathy - using pre-specified primary endpoints of nerve conduction velocity, validated neuropathy severity scale, and patient-reported neuropathic pain at 12 months - would substantially advance the evidence base and should be prioritized. Such a trial should be adequately powered for clinically important differences (not merely statistically significant differences in electrophysiological surrogates), stratified by baseline severity and vitamin B12 deficit etiology, and include patients with both type 2 diabetes and non-diabetic neuropathies to allow subgroup analysis. A similar trial design is needed for subacute combined degeneration of the spinal cord, where the current clinical consensus in favor of high-dose parenteral OHCbl is based on expert opinion and case series rather than randomized comparative data.

8.2. Perspective 2 - Ultra-high-dose methylcobalamin beyond ALS: A new therapeutic frontier in neurodegeneration

The JETALS trial has demonstrated, for the first time in a phase III randomized design, that pharmacological doses of a cobalamin form can meaningfully slow the progression of a major neurodegenerative disease. This finding - a 43% reduction in the rate of functional decline in early-stage ALS at MeCbl 50 mg twice weekly - is scientifically significant beyond its immediate clinical application, because it validates in humans the mechanistic hypothesis that supraphysiological MeCbl concentrations can promote neuronal survival and axonal regeneration through pathways distinct from simple cofactor repletion. The pending Japanese marketing authorization will be a pivotal regulatory event; should it be approved; it will create the first disease-modifying indication for any cobalamin form and will likely stimulate regulatory submissions in the European Union and the United States.

The mechanistic rationale for exploring ultra-high-dose MeCbl in other neurodegenerative conditions is scientifically compelling. Alzheimer's disease and other dementias associated with hyperhomocysteinemia represent a logical next step: MeCbl, by directly restoring methionine synthase activity, lowers homocysteine, raises SAM, and sustains the methylation reactions required for DNA repair, histone modification, and the synthesis of phosphatidylcholine - a key phospholipid in myelin and synaptic membranes. Two prospective registration trials examining high-dose B12-folate-B6 combinations in patients with elevated homocysteine and mild cognitive impairment (the VITACOG trial and its extensions) have demonstrated attenuation of brain atrophy on MRI; future trials should test whether MeCbl monotherapy at higher doses replicates or exceeds these findings. Parkinson's disease, multiple system atrophy, and hereditary spastic paraplegia - all conditions in which axonal integrity and mitochondrial function are compromised - represent additional candidate indications warranting exploratory phase II trials.

8.3. Perspective 3 - Pharmacogenomics and precision cobalamin therapy

The current paradigm of cobalamin therapy is population-level and dose-empirical. It treats all patients with B12 deficiency as a homogeneous group differentiated only by etiology and severity, ignoring the substantial inter-individual variation in cobalamin absorption, transport, intracellular processing, and coenzyme conversion that is encoded in the genome. The key pharmacogenomic variants relevant to cobalamin metabolism include: MTHFR C677T and A1298C (affecting methylation capacity and indirectly influencing the demand for MeCbl); MTR A2756G (methionine synthase); MTRR A66G (methionine synthase reductase, directly involved in MeCbl regeneration); TCN1 and TCN2 polymorphisms (affecting haptocorrin and transcobalamin II levels and affinity); CD320 variants (transcobalamin receptor, governing cellular uptake); and the CUBN/AMBN genes encoding cubilin and amnionless (modulating ileal absorption efficiency).

Individuals homozygous for MTHFR C677T, for example, have a thermolabile enzyme with approximately 70% reduced activity, resulting in blunted 5-methyltetrahydrofolate generation and consequently increased reliance on MeCbl to sustain methionine synthase function. The hypothesis that such individuals respond preferentially to directly bioavailable MeCbl rather than to prodrug CNCbl is biochemically rational but has not been formally tested in a prospective pharmacogenomic-stratified trial. Similarly, MTRR A66G homozygotes may have impaired regeneration of the methionine synthase-bound MeCbl, suggesting that higher or more frequent MeCbl dosing - or combination with riboflavin, which supports MTRR activity - might be warranted. Future research should incorporate routine

pharmacogenomic genotyping into cobalamin supplementation trials, with prospectively defined subgroup analyses by genotype. The long-term goal is a genomically informed prescribing algorithm that matches cobalamin form, dose, and monitoring frequency to individual metabolic risk profiles - a genuinely precision-medicine approach to a common deficiency.

8.4. Perspective 4 - Towards an integrated biomarker panel for diagnosis and therapeutic monitoring

The persistent reliance on total serum cobalamin as the primary diagnostic and monitoring biomarker represents a significant limitation of current clinical practice. Total serum B12 reflects predominantly TC-I-bound cobalamin - a biologically inert reservoir - and is insensitive to the early functional depletion of the active TC-II-bound fraction. As a result, patients with serum B12 levels in the low-normal range (200–350 pg/mL) may harbor significant functional deficiency with elevated MMA and homocysteine yet remain undetected and untreated. The holotranscobalamin (HoloTC) assay, now commercially available in most reference laboratories, measures the biologically available fraction directly and is the single most sensitive early marker of tissue-level B12 depletion. Its integration as the primary screening tool - with total serum B12 as a complementary rather than primary test - should be the subject of a formal international consensus update.

Beyond HoloTC, a four-biomarker panel - total serum B12, HoloTC, MMA, and tHcy - provides complementary information across different diagnostic dimensions: HoloTC for early store depletion, MMA for functional deficiency in the mitochondrial AdoCbl pathway, and tHcy for functional deficiency in the cytosolic MeCbl pathway. The "combined B12 indicator" (cB12), a composite score integrating all four markers, has demonstrated superior diagnostic sensitivity and specificity compared with any single marker in prospective cohort studies and warrants wider adoption in clinical guidelines. Future research should evaluate the utility of this composite biomarker panel as a treatment response metric - specifically, whether differential normalization of MMA versus tHcy at a given time point after initiation of a specific cobalamin form provides actionable information about metabolic routing and adequacy of dosing. Furthermore, the development of point-of-care testing for HoloTC, which would enable rapid assessment in primary care settings without laboratory referral, represents a major unmet need particularly relevant to high-prevalence populations such as the elderly, vegans, and metformin-treated diabetic patients.

8.5. Perspective 5 - Expanding the evidence base for non-injectable routes across all cobalamin forms

The evidence supporting high-dose oral CNCbl as an alternative to intramuscular injection - including in pernicious anemia - is now robust, resting on the Kuzminski RCT, the Cochrane systematic review, and most recently the Lacombe et al. (2024) prospective cohort. This evidence, however, exists almost exclusively for CNCbl. The pharmacokinetics of oral and sublingual MeCbl and OHCbl at pharmacological doses, their comparative ability to raise HoloTC and normalize MMA and tHcy, and their relative efficacy in patients with varying degrees of gastric pathology and intrinsic factor deficit remain incompletely characterized. The 2025 Frontiers in Pharmacology meta-analysis adds evidence for sublingual MeCbl as an intermediate-bioavailability option, but the underlying trials are heterogeneous in dose, formulation, and endpoint.

Rigorously designed dose-escalation pharmacokinetic studies for oral and sublingual MeCbl and OHCbl - with HoloTC and MMA as primary endpoints and blinded comparison to standard intramuscular OHCbl as the reference arm - are a research priority. These studies should specifically enroll patients with documented pernicious anemia (to assess passive absorption independently of IF function), food-cobalamin malabsorption (to isolate the gastric-liberation deficit), and post-bariatric surgery patients (to characterize absorption across a surgically altered intestinal anatomy). The clinical and economic implications of establishing non-injectable equivalents for MeCbl and OHCbl are considerable: they would reduce healthcare burden associated with injection scheduling, improve patient acceptability, and expand access to preferred neurological formulations in primary care settings where intramuscular injection infrastructure is limited.

8.6. Perspective 6 - Special populations: Pregnancy, elderly persons, and renal failure

Pregnant and breastfeeding women represent a population in whom cobalamin form selection has potential consequences for fetal and neonatal neurodevelopment, yet the comparative pharmacokinetics and placental transfer efficiency of the four forms have not been systematically studied. MeCbl is currently recommended by many experts on the basis of its direct bioavailability and absence of cyanide load, but this recommendation rests on mechanistic inference rather than prospective clinical data. Dedicated pharmacokinetic studies in pregnancy - measuring maternal and cord-blood HoloTC, MMA, and tHcy across forms and routes - are needed to provide an evidence-grounded basis for this clinically consequential choice.

Elderly persons with multimorbidity and polypharmacy constitute the largest single group at risk for B12 deficiency in developed countries, yet they are systematically under-represented in pharmacokinetic trials that tend to enroll young, healthy, and predominantly male volunteers. Age-related changes in gastric acid secretion, CUBAM receptor expression, TC-II synthesis, and renal tubular reabsorption efficiency are expected to alter the pharmacokinetics of all four cobalamin forms, but the magnitude and clinical relevance of these alterations are not quantified. Studies in patients with chronic kidney disease merit particular attention: the safety concern regarding CNCbl's cyanide moiety in advanced renal failure is biologically plausible but rests on pharmacological inference rather than clinical adverse-event data; conversely, the pharmacokinetics of OHCbl in the setting of impaired renal tubular handling are incompletely characterized. Dedicated studies in patients with eGFR below 30 mL/min/1.73 m^2 - comparing OHCbl and MeCbl as the candidate safe alternatives - would translate directly into actionable prescribing guidance.

8.7. Perspective 7 - Novel formulations and drug-delivery strategies

The photosensitivity of MeCbl and AdoCbl has historically constrained their pharmaceutical development - precluding standard liquid oral formulations and requiring amber-glass or foil-packaged parenteral presentations. Advances in pharmaceutical technology now offer realistic solutions. Light-

protective microencapsulation of MeCbl in lipid-based or polymer matrices has been demonstrated at laboratory scale to extend photostability by several orders of magnitude without impairing in vitro dissolution; transfer to clinical-grade manufacturing represents a tractable near-term objective. For ultra-high-dose intramuscular MeCbl (as in the JETALS protocol), development of a concentrated, light-protected, ready-to-inject formulation in a prefilled syringe format would substantially reduce preparation time and error risk in neurological units.

Nasal delivery of cobalamin - bypassing both intestinal absorption barriers and hepatic first-pass handling — is an underexplored route with particular appeal in patients with severe malabsorption who are also unable or unwilling to accept intramuscular injections. A nasal gel formulation of CNCbl has received regulatory approval in the United States (Nascobal®) and demonstrated effectiveness in pernicious anemia, but comparative nasal pharmacokinetic data for OHCbl and MeCbl are absent. Transdermal patch delivery, already used for certain water-soluble vitamins, and cobalamin-loaded nanoparticle systems targeting ileal CUBAM receptors via active ligand-directed uptake represent longer-horizon drug-delivery strategies whose feasibility is supported by early-phase preclinical evidence. Finally, the possibility of engineering modified cobalamin analogues with extended plasma half-life (through PEGylation or albumin fusion) - analogous to strategies used for other protein-based or small-molecule therapeutics - is a speculative but scientifically intriguing avenue for achieving sustained therapeutic concentrations from monthly or even quarterly dosing regimens.

8.8. Final synthesis: From empirical supplementation to rational cobalamin therapy

The intellectual trajectory of vitamin B12 therapeutics over the past century has followed a remarkable arc: from the empirical administration of raw liver extracts to a precisely characterized molecular pharmacology encompassing four structurally distinct coenzyme forms, each with its own absorption pathway, intracellular routing, tissue tropism, and clinical niche. What remains to be completed is the translation of this molecular sophistication into equivalently sophisticated prescribing practice. Too often, the clinician's therapeutic vocabulary for B12 deficiency still does not distinguish between CNCbl and MeCbl, between intramuscular and oral OHCbl, between the renal patient and the pregnant woman, between the dietary-deficient vegan and the patient with pernicious anemia and progressive myelopathy.

The perspectives outlined above converge on a single research agenda: replacing the population-level, one-size-fits-all paradigm of "vitamin B12 replacement" with an individualized, indication-driven, and ultimately genomically informed approach to cobalamin therapy. This agenda is not merely academic - B12 deficiency affects hundreds of millions of patients worldwide, its neurological sequelae are frequently irreversible when treatment is delayed or suboptimal, and the cost differential between the available forms is no longer an insurmountable barrier to rational prescribing in most healthcare systems. The scientific infrastructure - validated biomarkers, existing pharmacogenomic tools, proven formulations, and a growing body of comparative clinical evidence - is in place. What is required is the clinical will to use it, sustained by the kind of rigorous, head-to-head, adequately powered comparative trials that this field has so far produced only in insufficient number. The patients whose neurological recovery depends on whether their clinician prescribes CNCbl or MeCbl - and whether they prescribe it in time - deserve nothing less.

9. Disclosures

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