

Therapy Trial Design in Vanishing White Matter

An Expert Consortium Opinion

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Abstract

Vanishing white matter (VWM) is a leukodystrophy caused by recessive variants in the genes *EIF2B1-EIF2B5*. It is characterized by chronic neurologic deterioration with superimposed stress-provoked episodes of rapid decline. Disease onset spans from the antenatal period through senescence. Age at onset predicts disease evolution for patients with early onset, whereas disease evolution is unpredictable for later onset; patients with infantile and early childhood onset consistently have severe disease with rapid neurologic decline and often early death, whereas patients with later onset have highly variable disease. VWM is rare, but likely underdiagnosed, particularly in adults. Apart from measures to prevent stressors that could provoke acute deteriorations, only symptomatic care is currently offered. With increased insight into VWM disease mechanisms, opportunities for treatment have emerged. *EIF2B1-EIF2B5* encode the 5-subunit eukaryotic initiation factor 2B complex, which is essential for translation of mRNAs into proteins and is a principal regulator of the integrated stress response (ISR). ISR deregulation is central to VWM pathology. Targeting components of the ISR has proven beneficial in mutant VWM mouse models, and several drugs are now in clinical development. However, clinical trials in VWM pose considerable challenges: low numbers of known patients with VWM, unpredictable disease course for patients with onset after early childhood, absence of intermediate biomarkers, and novel first-in-human molecular targets. Given these challenges and considering the critical need to offer therapies, we have formulated recommendations for enhanced diagnosis, drug trial setup, and patient selection, based on our expert evaluation of molecular, laboratory, and clinical data.

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Glossary

ATF4 = activating transcription factor 4; **CHOP** = C/EBP homologous protein; **DTI** = diffusion tensor imaging; **eIF2B** = eukaryotic initiation factor 2B; **ER** = endoplasmic reticulum; **FDA** = Food and Drug Administration; **FLAIR** = fluid-attenuated inversion recovery; **GADD34** = growth arrest and DNA damage protein 34; **GSK3 β** = glycogen synthase kinase 3 β ; **ISR** = integrated stress response; **mcDESPOT** = multicomponent driven-equilibrium single-pulse observation of T1/T2; **MLD** = metachromatic leukodystrophy; **MRS** = magnetic resonance spectroscopy; **MTR** = magnetization transfer ratio; **MWF** = myelin water fraction; **NODDI** = neurite orientation dispersion and density imaging; **OMIM** = Online Mendelian Inheritance in Men; **VWM** = vanishing white matter.

Vanishing white matter (VWM), or childhood ataxia with CNS hypomyelination, was recognized in the 1990s (Online Mendelian Inheritance of Men (OMIM) 603896).¹⁻³ Initially, VWM was described as an early childhood-onset devastating encephalopathy with chronic deterioration, superimposed episodes of stress-provoked decline, and early fatality.¹⁻³ Once the 5 genes associated with VWM were identified, it became apparent that VWM has a pleomorphic natural history, with disease onset ranging from the antenatal period through senescence and an extremely variable disease course ranging from rapid progression and early death to minimal involvement over decades.⁴

Considering the almost exclusive involvement of the CNS white matter in VWM, it was unexpected that the genetic cause resides in the ubiquitously expressed eukaryotic initiation factor 2B (eIF2B).⁵ eIF2B is essential for initiation of translation of mRNAs into proteins.⁶ It is also a central regulator of mRNA translation rate and principal component of the integrated stress response (ISR), a molecular pathway responsive to different types of stress.⁶ Deregulated ISR is central to the pathophysiology of VWM,⁷ and ISR modulation can reduce disease severity in VWM mouse models.⁷⁻⁹

These insights have opened an exciting new era of potential drug therapies for VWM. However, the constitutive biologic processes involved in VWM pathophysiology⁶ and inherent variability of its natural history⁴ make clinical development of therapies challenging. The need for unbiased assessment of safety, tolerability, and efficacy of several promising drugs is additionally challenged by the low numbers of known patients with VWM. Initiation of simultaneous competing drug trials may limit the ability to assess drug efficacy with sufficient power.

Consortium of Clinical Experts on VWM

Recognizing these challenges, an international independent consortium of 9 clinician-scientists, expert in VWM, was founded in 2019.¹⁰ They were selected for clinical and research expertise in leukodystrophies, including VWM, as measured by publications and clinical trials in leukodystrophies, and for geographic representation of countries that follow the International Council for Harmonization–Good Clinical Practice guidelines. Patient and family organizations on leukodystrophies from those countries were invited to form an advisory board. The Industry

Alliance Office of Amsterdam Neuroscience provided expertise on clinician-industry interactions and trial setup.

The mission of the VWM Consortium is to improve patient identification, enable biomarker studies, develop guidelines for clinical trials, and support development of therapeutics. This consensus statement aims to provide evidence-based guidelines for trial design and biomarker considerations to enable and accelerate therapeutic developments and address recruitment challenges.

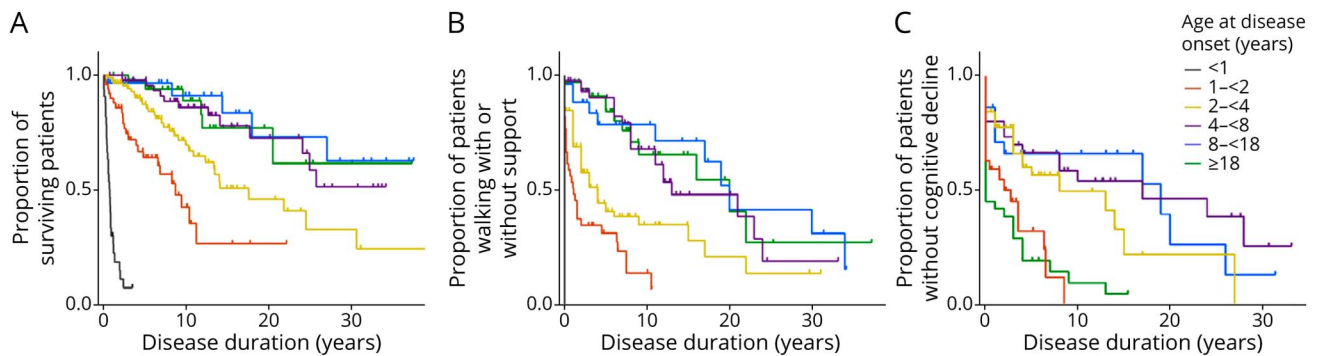
Background

VWM Natural History, MRI, and Genetic Etiology

Recently, an international natural history study described a wide range of phenotypes in 296 patients with VWM.⁴ In 90% of patients, age at onset is <18 years, and in 60%, it is <4 years. In 85%, the disease course is chronic with episodic deteriorations; in 15%, it is only chronic. The most common provoking factors are febrile and afebrile infections and head trauma. Rapid neurologic decline is often accompanied by lowered consciousness. As age at onset predicts prognosis in VWM,¹¹ 6 groups were defined: onset <12 months, between 12 and <24 months, 2 and <4 years, 4 and <8 years, 8 and <18 years, and \geq 18 years.⁴ For onset <4 years, the exact age at onset correlates with disease severity, earlier onset being associated with more rapid decline and higher mortality, whereas for onset \geq 4 years, the clinical course is generally milder, with a wide variation in severity, not correlating with exact age at onset (Figure 1). Episodic deteriorations are important predictors for more severe disease course (Figure 2), independent of age at onset.⁴

Details per onset category are given in Table 1. Antenatal or early infantile onset (<12 months) is invariably associated with rapid deterioration and early death.⁶ Patients often display severe generalized encephalopathy with irritability and intractable epilepsy. Involvement of organs outside the CNS is common.⁴ Patients with onset between 12 and <24 months show rapid decline with loss of ambulation within months to a few years and early death.⁶ In the classic phenotype, disease manifests between age 2 and 4 years.¹⁻⁴ A stress-provoked episode often marks disease onset. Ataxia and spasticity predominate, whereas cognition is relatively spared. Mild epilepsy is common. Clinical evolution is more variable, but typically associated with early severe disability, even when

Figure 1 Disease Progression in Relation to Age at Onset



Kaplan-Meier plots for survival (A), walking with or without support (B), and cognition (C) in relation to disease duration, per the age at onset group. In all plots, censored patients (alive at last follow-up, still ambulant, or absence of cognitive decline) are indicated by crosses. Modified from Figure 3, Hamilton et al., *Ann Neurol* 2018, with permission.

patients reach adulthood.⁴ Onset ≥ 4 years is associated with extremely variable disease evolution.⁴ Stress-provoked episodic deterioration is less prominent.⁴ Decline is often slow, but sudden deterioration and death may occur.⁴ Patients with adult onset typically present with dementia or psychiatric symptoms and often no or mild motor involvement.⁴ Patients with onset after infancy have exclusive CNS involvement, except for ovarian failure, which is common in females.^{3,4}

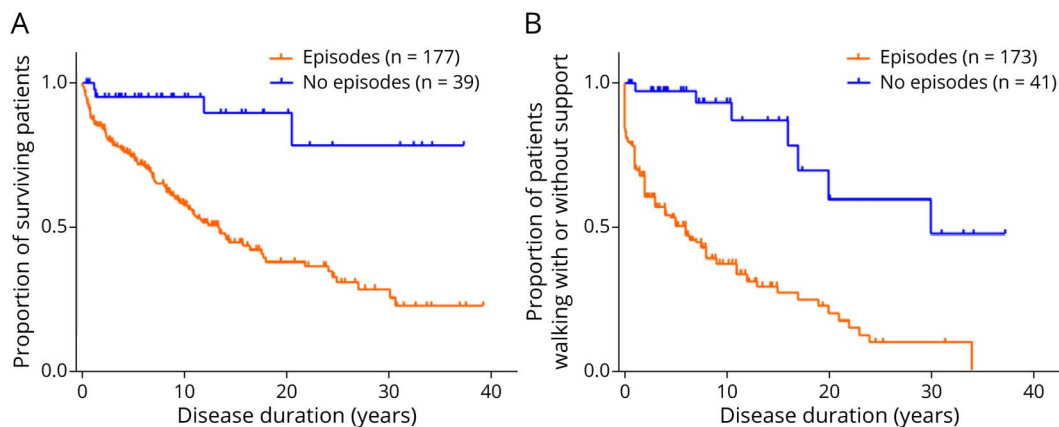
Brain MRI in affected individuals is often pathognomonic for VWM (Figure 3).^{3,12} T2 signal is increased throughout the cerebral white matter; the directly subcortical white matter may initially be spared.¹³ Fluid-attenuated inversion recovery (FLAIR) images show progressive rarefaction and cystic degeneration of the cerebral white matter. Radiating stripes are present, indicating better preserved white matter tissue strands.¹² The cerebral white matter abnormalities worsen over time and never reverse.¹⁴ Cerebellar white matter is

normal or less severely affected.¹² Gray matter structures mostly retain a normal appearance.¹²

MRI features are dependent on age at onset.^{12,14} White matter rarefaction is more rapid with earlier onset. In antenatal or early infantile onset, the cerebral white matter is initially T2 and FLAIR hyperintense and appears swollen and may disappear completely in a few months.¹⁴ The cystic white matter may collapse or retain a swollen appearance.¹⁴ With late infantile or early childhood onset, the cystic white matter may look highly swollen and be associated with increasing macrocephaly.¹⁴ In adolescents and adults, the abnormalities are often nonspecific. Only the periventricular cerebral white matter may be abnormal, with no or little rarefaction, but with pronounced atrophy.^{12,14}

VWM is caused by biallelic pathogenic variants in *EIF2B1-EIF2B5*, encoding the α - ϵ subunits of eIF2B.^{5,12} There is

Figure 2 Effect of Stress-Provoked Episodes



Survival (A) and walking with or without support (B) in relation to disease duration, grouped by disease course with and without episodic deterioration. In all plots, censored patients (alive at last follow-up or still ambulant) are indicated by crosses. Modified from Figure 3, Hamilton et al., *Ann Neurol* 2018, with permission.

Table 1 Clinical Characteristics

Age at onset groups	<12 mo	1–<2 y	2–<4 y	4–<8 y	8–<18 y	≥18 y
Disease onset provoked by trigger	43%	66%	72%	40%	54%	21%
Exacerbating disease course	84%	88%	93%	76%	68%	59%
Delayed early cognitive development	63%	21%	9%	9%	11%	3%
Achieved walking without support	0%	74%	100%	100%	100%	100%
Median age of loss of walking without support	n.a.	2 y	3 y	14 y	25 y	44 y
Median time to loss walking without support ^a	n.a.	2 mo	1 y	8 y	15 y	7 y
Median age of full wheelchair dependency	n.a.	3 y	7 y	18 y	33 y	56 y
Median time to full wheelchair dependency ^a	n.a.	1 y	4 y	13 y	20 y	20 y
Median time to cognitive decline ^a	n.a.	2y	8 y	17 y	19 y	0 y
Median age of death [quartiles]	9 [6–14] mo	4 [2–8] y	9 [6–15] y	13 [9–23] y	29 [16–34] y	37 [29–50] y
Median disease duration at death [quartiles]	7 [3–10] mo	2 [1–6] y	7 [3–13] y	6 [5–17] y	14 [4–22] y	10 [4–14] y

Abbreviations: mo = months; n.a. = not applicable; y = years.

The information in this table is obtained from Hamilton et al., *Ann Neurol* 2018; 84:274-288.

^a Calculated from disease onset.

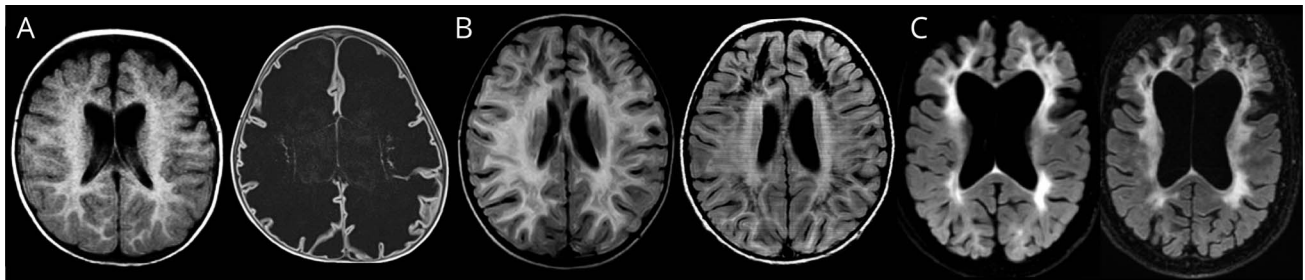
clear genotype-phenotype correlation, with mild and severe variants.^{4,11,15,16} If a mild and a severe variant are compound heterozygous, disease severity is intermediate.¹⁵ Although patients with the same variants tend to have a similar disease course, variation may be considerable, especially in onset ≥ 4 years, even within families.⁴

Disease Mechanisms

eIF2B is an enzyme with guanine exchange factor activity, required for translation of mRNAs into proteins,^{17,18} and central to regulation of mRNA translation rate by the ISR.¹⁹ The ISR (Figure 4) is an evolutionarily highly conserved homeostatic pathway, activated by different stressors, including fever, amino acid deprivation, viral infection, heme deficiency, reactive oxygen species, and endoplasmic reticulum (ER) overload with

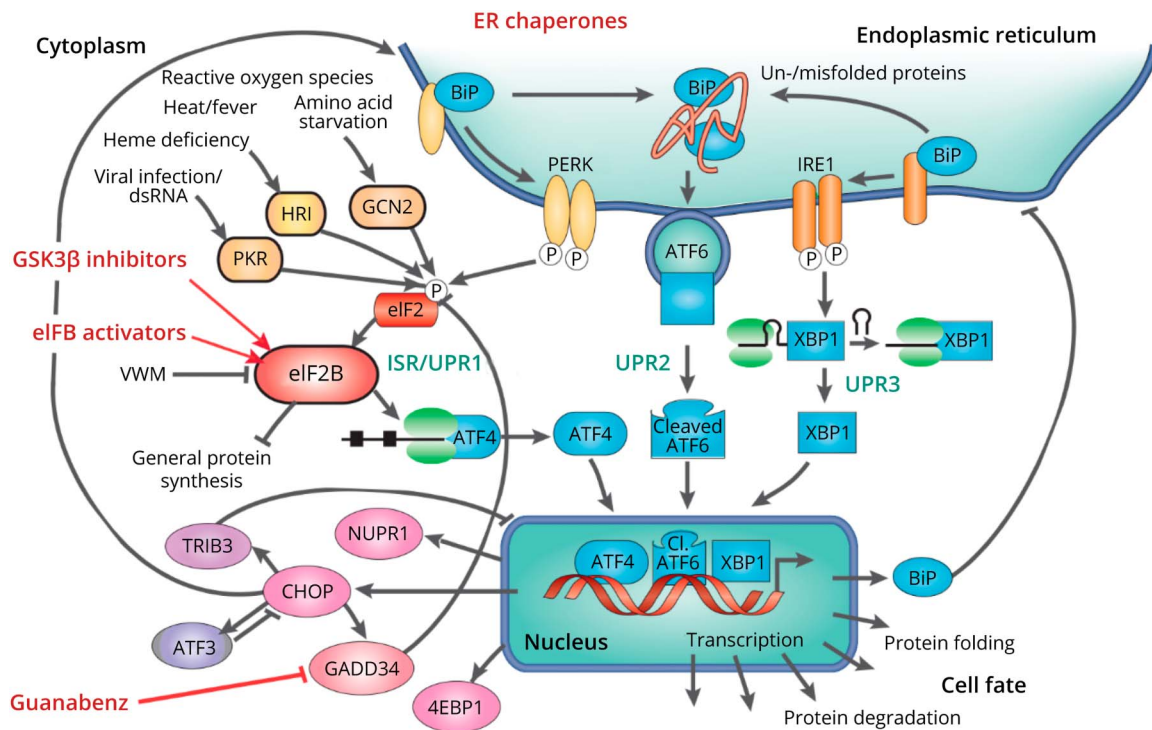
unfolded or misfolded proteins.²⁰ Stressors activate kinases, which phosphorylate eIF2. Phosphorylated eIF2 inhibits eIF2B, thereby reducing mRNA translation rate. Inhibition of eIF2B also activates activating transcription factor 4 (ATF4) transcription response, which has numerous downstream effects, including cell survival or apoptosis, cell cycle arrest or proliferation, differentiation, and maturation.²⁰ C/EBP homologous protein (CHOP), downstream effector of ATF4, is proapoptotic and through a feedback loop sensitizes the ER for further stress. Growth arrest and DNA damage protein 34 (GADD34), downstream effector of CHOP, dephosphorylates eIF2, thereby restoring eIF2B activity and abating the ISR.²⁰

VWM pathogenic variants reduce eIF2B activity,²¹ causing constitutive activation of the downstream ISR. Activation of the

Figure 3 MRI in Vanishing White Matter

FLAIR images in a patient at clinical presentation at 7 months and follow-up at 24 months (A) show that initially, the cerebral white matter is abnormal but not rarefied, whereas at 24 months, all cerebral white matter has vanished, but still looks swollen. FLAIR images in a patient at clinical presentation at 4½ years and follow-up at 6 years (B) show abnormal cerebral white matter that is increasingly rarefied and cystic. Note the radiating stripes representing better preserved tissue strands. FLAIR images in a patient at clinical presentation at 48 years and follow-up at 55 years (C) show that the cerebral white matter is abnormal, but the directly subcortical white matter is spared. There are minimal signs of rarefaction, and there is some cerebral atrophy. At follow-up, the atrophy has progressed.

Figure 4 Integrated Stress Response, Drug Targets, and Drugs



The ISR is activated by 4 kinases (PKR, HRI, GCN2, and PERK), each a sensor for a particular stress type. ER stress activates the unfolded protein response (UPR), which has 3 arms, mediated by PERK, ATF6, and IRE1. The PERK pathway is shared by the ISR and UPR. Activated kinases phosphorylate eIF2 α , which binds and inhibits eIF2B. Pathogenic variants in EIF2B1-EIF2B5 also decrease eIF2B activity. Decreased eIF2B activity lowers overall mRNA translation and activates the activating transcription factor 4 transcription response, which has major effects on cell fates as cell death, recovery, survival, proliferation, differentiation, and maturation. GADD34 dephosphorylates eIF2 α , thereby restoring eIF2B activity. C/EBP homologous protein sensitizes the ER for stress. eIF2B activators stabilize and activate eIF2B directly. Glycogen synthase kinase 3 β inhibitors decrease eIF2B phosphorylation, thereby increasing its activity. Guanabenz inhibits GADD34, thereby modulating eIF2 α phosphorylation and thus eIF2B activity. ER chaperones decrease ER stress. Modified from box 1, links.lww.com/NXG/A512, Schepers et al., Nat Rev 2007, with permission. eIF2B = eukaryotic initiation factor 2B; ER = endoplasmic reticulum; GADD34 = growth arrest and DNA damage protein 34; ISR = integrated stress response; UPR = unfolded protein response.

ATF4 transcription response is observed in VWM mouse and patient brain tissue.⁷ eIF2 phosphorylation is decreased, indicating activation of the GADD34 feedback loop, which is, however, insufficient to restore the downstream ISR to normal.⁷ Activation of conflicting cell fate drivers (proliferation, proapoptosis, and antiapoptosis) is seen in patient brain tissue.²² Modulation of the ISR at different levels affects disease severity in VWM mice and patients: ISR stressors, including fever and infections, exacerbate clinical deterioration in patients with VWM and negatively affect outcome (Figure 2),^{3,4} whereas drugs enhancing eIF2B activity or modulating the GADD34 feedback loop improve the disease in VWM mice.⁷⁻⁹

Deregulated ISR in astrocytes is a key cause of pathology. At autopsy, the cerebral white matter is rarefied and cystic and lacks reactive astrocytes.²³ Astrocytes have highly abnormal morphology with few, short, thick processes.²³ Premyelinating oligodendrocyte precursor cells are increased in number, but mature cells are lacking.²⁴ Oligodendrocytes and astrocytes fail in their mature functions, including formation and maintenance of myelin and astroglial scar, explaining the lack of myelin and cystic white matter degeneration.^{23,24} Coculture studies show that VWM astrocytes inhibit oligodendrocyte

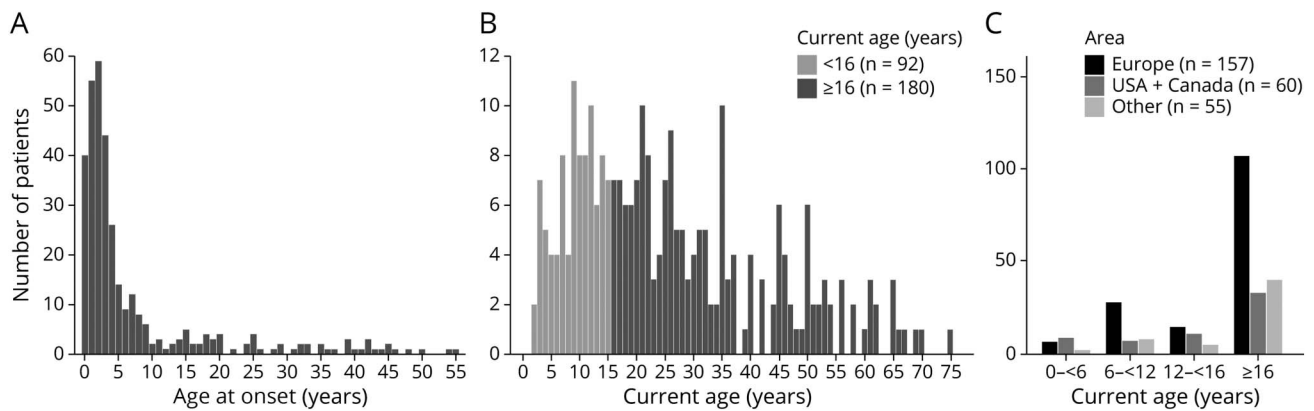
maturation and myelination by secreted factors, whereas VWM oligodendrocytes do not have an intrinsic defect in myelin formation.^{25,26} Strikingly, the ISR is almost exclusively activated in astrocytes.⁷

Therapy Targets and Compounds

Considering the central role of the ISR in the pathophysiology of VWM, drugs targeting the ISR are of high therapeutic interest. A novel compound called ISRIB (abbreviation of ISR inhibitor) enhances eIF2B activity.²⁷ ISRIB treatment of different VWM mice (*2bSho* and *2b4ho2b5he*) reduced expression of the ATF4 transcriptome, ameliorated white matter pathology, and improved motor skills, more so in the more severely affected *2b4ho2b5he* than in *2bSho* mice.⁷ ISRIB stabilizes the eIF2B complex through interaction with the eIF2B δ :eIF2B β interface and its effect may be influenced by individual gene variants.⁷ A derivative compound, 2BAct, has better pharmacologic properties and highly favorable results in *2bSho* mice.⁹ At present, several eIF2B activators are in preclinical development.

Glycogen synthase kinase 3 β (GSK3 β) phosphorylates eIF2B ϵ , thereby reducing eIF2B activity.²⁸ Antidepressant trazodone inhibits GSK3 β and thereby ISR activation.²⁹ Its

Figure 5 Numbers of Patients With Vanishing White Matter



(A) Histogram representing the number of patients per age at onset (years) for 291 patients with VWM. Modified from Figure 1, Hamilton et al., *Ann Neurol* 2018, with permission. (B) Histogram representing the number of patients per current age (years) for 272 living patients with VWM known to the consortium (236 living patients in the international VWM registry and 36 living patients outside the registry followed by consortium members). (C) Histogram representing the number of patients per age group and region of residency for 272 living patients with VWM known to the consortium, showing that most living patients are ≥ 16 years and diagnosed in Europe. VWM = vanishing white matter.

effects on VWM mice are being studied. Lithium also inhibits GSK3 β . In a zebrafish VWM model,³⁰ lithium effects were promising, but safe doses in VWM mice appeared ineffective (unpublished data).

GADD34 is the entry of the feedback loop to reduce eIF2 phosphorylation. Guanabenz and sephin1 inhibit GADD34.^{31,32} Guanabenz is an old, Food and Drug Administration (FDA)-approved $\alpha 2$ -adrenergic antihypertensive drug. Sephin1 has FDA orphan drug designation for Charcot-Marie-Tooth disease. As GADD34 inhibitors, they are expected to prolong ISR activation. Instead, for unknown reasons, both reduce ISR activation in mouse models of neurodegeneration.^{31,32} A recent trial shows some efficacy of guanabenz in patients with amyotrophic lateral sclerosis.³³ Guanabenz had beneficial effects in VWM mice on neuropathology⁸ and motor performance (unpublished data). The effects of sephin1 on VWM mice are under study.

ATF4 and components of the ATF4 transcription response are a third target. It is clear that their overexpression is central in the pathophysiology of VWM. Reducing the expression of ATF4 or components of the ATF4 transcriptome by antisense oligonucleotides is of potential therapeutic interest, and a study on the subject is set up.

A fourth target are stressors provoking the ISR. Although it is difficult to prove the effect of preventive measures to avoid stressors,¹² it is clear that occurrence of stress-provoked episodic decline is associated with poorer outcome.⁴ In addition, proteotoxic ER stress can potentially be reduced by compounds, like ursodiol and several other similar chaperone molecules.³⁴ Assessment of their potential in VWM mice is ongoing.

Mitochondrial dysfunction may play a role in VWM disease mechanisms³⁵ and could be favorably influenced by sigma1

receptor agonists.³⁵ Sigma1 receptors function at the ER-mitochondrial interface. Several sigma1 receptor agonists are available, and studies of their effect in VWM mice are ongoing.

During rapid neurologic decline, steroids have been applied with unclear benefit.³⁶ Steroids decrease levels of high-molecular-weight hyaluronan, which has been implicated in VWM pathomechanisms.²⁴ A study in VWM mice failed to show beneficial effects (unpublished data).

Currently, eIF2B activators and guanabenz are most promising, showing improved motor performance and brain pathology in VWM mice. They are ready for clinical development. Similar results are not yet available for other compounds. Multiple ISR modulating compounds are undergoing preclinical development. Sigma1 receptor agonists and steroids affect pathways further away from eIF2B, whereas ER chaperones only affect ER stress, leaving the other ISR activating stressors untouched. So far, only isolated effects of compounds are available; ongoing studies in VWM mice address the question whether a combination of drugs could prove more efficacious.

Patient Numbers

VWM is one of the more common leukodystrophies; US-based data from exome sequencing approaches indicate that VWM is fifth in relative incidence of leukodystrophies.³⁷ Assessment of large genomic databases suggests an incidence of 1 per 715,000.³⁸ Centralized diagnostics in the Netherlands for 2 decades yield an incidence of 1 per 80,000–100,000 live births and prevalence of 1.4 per 1,000,000 without evidence of founder effects.⁴ So, VWM is an ultra-rare disease, but clinical observations suggest a higher incidence than estimations based on databases.

Although disease incidence is highest at young ages, prevalence is mainly attributable to adolescent and adult patients.

Although two-thirds of the patients in the natural history study have an onset <6 years (Figure 5A),⁴ two-thirds of the known living patients are ≥16 years (Figure 5B). Epidemiology varies greatly for different geographic areas (Figure 5C). No important consanguinity or founder effects were observed, suggesting that the higher incidence observed in Europe may reflect the actual incidence and that VWM may be underdiagnosed outside Europe, especially in adults (Figure 5C).

Enhanced awareness of VWM and improved access to genetic testing will increase diagnosis. Although MRI diagnosis is typically straightforward in pediatric patients, it is more difficult in adults, in whom cerebral white matter abnormalities are often nonspecific: more limited in extent, inhomogeneous, and characterized by atrophy rather than cystic decay, leading to misdiagnosis as atypical multiple sclerosis and vascular dementia.¹⁴ In the experience of consortium members, adult patients are mostly referred from multiple sclerosis or dementia centers.

Biomarkers

Only a few biomarkers have been identified for VWM, including elevated CSF glycine and decreased CSF asialotransferrin.^{39,40} Their sensitivity and proportionality have not been assessed. In view of emerging trials, there is an urgent need for biologically relevant biomarkers for VWM.

Biomarkers reflecting VWM pathomechanisms may be relevant to monitor target engagement of therapies. The ATF4 transcriptome provides excellent candidates, which can be assessed at RNA level in blood.⁷ In addition, VWM patients and mice display consistent evidence of metabolic derangement with altered amino acids,^{7,39} making metabolomics of interest. Biomarkers may also reflect tissue damage. Excellent candidates in this respect are neurofilament light (marker of axonal degeneration) and glial fibrillary acidic protein (marker of astrocytic degeneration).⁴¹

Identifying biomarkers in VWM mouse and patient samples and testing their longitudinal proportionality with disease severity are critical for implementing therapeutic trials. VWM mice can be tested for sensitivity to target engagement. Banked and ongoing VWM patient sample collections can be used to assess correlation of biomarkers with disease presence or absence, disease progression rate including acute exacerbations, and current disease severity, thereby revealing proportionality.

MRI also supplies possible biomarkers. Techniques providing quantitative parameters of white matter microstructure are particularly valuable and include diffusion tensor imaging (DTI), magnetization transfer ratio (MTR), myelin water fraction (MWF), multicomponent driven-equilibrium single-pulse observation of T1/T2 (mcDESPOT), neurite orientation dispersion and density imaging (NODDI), magnetic resonance spectroscopy (MRS), and quantitative susceptibility mapping.⁴² Volumetry with assessment of normal, abnormal but present, and rarefied or cystic white matter is

another critical technique.¹⁴ MRI quantitative parameters are being assessed for their contribution in VWM.

Clinical Trials in VWM

Expert Consensus

The 9 consortium members had regular video meetings to discuss VWM pathophysiology, natural history, biomarkers, and clinical trial design. Recommendations were determined through rounds of voting. These votes are represented below by number of votes in favor (n) out of the 9 votes: consortium n/9. In addition, for recommendations regarding end points and scales for trials, a real-time Delphi, an expert-based consensus procedure,⁴³ was executed among the 9 consortium members. Expert opinion on trial design and statistics was provided by the nonclinical advisors of the consortium and discussed within the consortium. Patient and family organizations on leukodystrophies were invited for comments.

General Considerations and Recommendations

In ultra-rare diseases, clinical trials are challenging.⁴⁴ Very low patient numbers hamper meeting statistical standards for showing safety and efficacy of new drugs.⁴⁵ Here, we discuss topics specifically pertinent to VWM.

The extremely heterogeneous clinical presentation and disease course challenge trial development in VWM. Age at onset is the best predictor of clinical course,^{4,11} and trials should either select specific age at onset groups or stratify for onset, thus creating more homogeneous subgroups (consortium 9/9). Despite a consistent genotype-phenotype correlation, the genotype does not add extra predictive information¹⁶ and does not require further stratification. In early-onset cases, motor decline is predominant; in late-onset cases, cognitive decline is predominant.⁴ We recommend separate trials for early and late onset (consortium 9/9). Although incidence of VWM is highest in infants and children, its prevalence is mostly attributable to adolescent and adult patients. Trials targeting patients with early onset will largely depend on newly diagnosed cases. Considering the variable and largely unpredictable disease course in VWM,⁴ follow-up needs to be sufficiently long for a trial to be informative.

Traditionally, the ideal setup for therapy trials, preferred by the FDA, Health Canada, and European Medicines Agency, is randomized, double blind, and placebo controlled, although particularly for ultra-rare disorders, novel trial designs have been adopted.^{46,47} In the adolescent and adult VWM population, progression is generally slow, and given the absence of other treatment options, double-blind placebo-controlled trials are preferable (consortium 9/9). With early onset, the disease course is more predictable and generally fast. In untreated patients with onset of 1–<2 years, ~65% lose ambulation, and ~30% die within 4 years, whereas the numbers are ~50% and ~10%, respectively, for patients with onset of 2–<4 years.⁶ These figures explain why parents of patients

with early-onset VWM deem double-blind placebo-controlled trials unacceptable. The consortium (7/9) suggests that considering the predictable disease course of patients with early onset,⁴ existing natural history data may serve as control. Two consortium members would accept a placebo arm in trials for early-onset patients, on the condition that the double-blind study period would be less than 6 months, the treatment effect likely large, the number of patients receiving placebo small, and there would be an open-label extension.

Inclusion and Exclusion Criteria

The diagnosis of VWM needs to be genetically confirmed, and significant comorbidities, especially other genetic diseases, should be excluded (consortium 9/9). For trial participation, patients need to be clinically symptomatic (consortium 8/9). Presymptomatic patients, with genetically confirmed VWM but without neurologic signs, have unclear age at onset. Even for familial cases, considerable intrafamilial variation hampers prediction of onset.⁴ When suitable biomarkers have been developed that are proportionate to disease evolution, either biochemical or MRI parameter, inclusion of clinically presymptomatic patients can be considered.

Stage of disease progression is an important consideration in choosing pivotal trial populations. Multiple studies in other leukodystrophies have shown that therapies are effective only early in the disease.⁴⁸ As long as the general white matter structure is largely preserved, damage may be repairable, but cystic white matter cannot be repaired. Halting further clinical disease progression under therapy is measurable early in the disease, but in far advanced stages, arrest of further progression may not be discernible. For these reasons, trials should focus on patients early in the disease (consortium 8/9, see text below for specifics). One consortium member voted for inclusion of patients with more advanced disease.

Strict criteria are acceptable to enhance the chance of a definitive trial outcome (consortium 8/9), but leave numerous patients with VWM noneligible for efficacy trials. Compassionate use and expanded access programs should be considered for such patients and their data should be analyzed separately and used for additional refinement of treatment criteria (consortium 9/9).

Recommendations per Age at Onset

Patients with onset <1 year have a severe encephalopathy, often multiorgan involvement, and early death.⁴ Even with prenatal diagnosis, the brain is already severely affected at birth. Damage is thus likely irreparable at trial entry and hampers detection of treatment effects. The consortium (7/9) would advise to not include them in upcoming efficacy trials, but consider compassionate use or expanded access programs.

Patients with late infantile and early childhood onset have a rapid and rather predictable course.⁴ Relatively small and short trials may suffice to show efficacy of an intervention

(consortium 9/9; see below for statistical calculations). Results of the natural history study indicate that for early onset, ambulation and survival are sensitive parameters to assess efficacy (Figure 1).⁴ In analogy with trial design in late infantile metachromatic leukodystrophy (MLD),⁴⁹ ambulation without or with minimal support of one hand (10-step-walk test, item of the GMFM-88) is suitable as inclusion criterion (consortium 9/9).

For patients with onset after early childhood, disease course and life span are unpredictable.⁴ Therefore, double-blind placebo-controlled trials are highly preferable (consortium 9/9). Childhood presentation is predominantly motor, and adult presentation is predominantly behavioral and cognitive, whereas the presentation is variable or mixed for ages in between. Inclusion criteria and outcome parameters should reflect those issues. Patients should be included early, while ambulant and having relatively preserved cognitive functions (consortium 9/9).

Recommendation on Scales and End Points

Clinical end points must be meaningful, assessable in a standardized manner, preferably with established instruments, and should ideally be validated or at least previously used in VWM. The instruments should cover different functional domains, in particular motor, cognitive, emotional, and behavioral domains and should ideally be usable for different ages. Currently, no single instrument meets all criteria.

Optimal inclusion of the limited number of patients with VWM eligible for trials requires sharing of data, which necessitates open access to control data across trials and standardized core end points, which allow pooling of data from control arms and compare efficacy of therapies tested in different trials. Therefore, the 9 consortium members defined a core set of instruments for trials in VWM. To reach consensus, a modified real-time (rt) Delphi procedure was executed, using EDELPHI 2021 software.⁴³ This online software tool allows participants to give and revise their opinion within a predefined period. A systematic literature review on VWM and a recently performed Delphi procedure among experts on MLD served as input; consortium members could add scales and questionnaires, based on individual experience. In total, 24 clinical scales and questionnaires were reviewed. The 16 recommended instruments that comprehensively assess all relevant clinical domains are presented in Table 2 and supplementary file, links.lww.com/NXG/A512.

Currently, body fluid biomarkers, discussed above, and quantitative MRI parameters relevant to brain white matter integrity provide exploratory outcome measures. The standard MRI protocol to assess white matter integrity should comprise a high-resolution T1-weighted sequence (magnetization-prepared rapid gradient echo sequence) and T2-weighted and FLAIR sequences. All sequences should cover the entire intracranial cavity for segmentation and volumetry. To investigate white matter microstructure, a multimodal MRI protocol at higher

Table 2 Outcome Measures

	Validated for age range (y)	Specifically validated for leukodystrophies	Previously applied in leukodystrophies	Applied in VWM	Reported by	Clinically meaningful
QoL and level of functioning						
HUI3	1 scale, ≥1	No	Yes	Yes	P&P or clinician	Yes
PedsQL	4 scales, 2-18	No	Yes	Yes (unpublished)	P&P	Yes
EQ5D5L	3 scales, ≥4	No	No	Yes (unpublished)	P&P	Yes
Motor scales						
GMFM-88	1 scale, ≥½	No	Yes	Yes (unpublished)	Trained expert	Limited
10MWT/10SWT	≥2	No	Yes	Yes (unpublished)	Clinician	Yes
GMFC-MLD	1 scale, ≥1½	Yes	Yes	Yes (unpublished)	Clinician	Yes
GMFCS	1 scale, ≥1½	No	Yes	Yes (unpublished)	Clinician	Yes
(Mini-)MACS	2 scales, ≥1	No	Yes (unpublished)	Yes (unpublished)	Clinician	Yes
SARA	1 scale, ≥8	No	Yes (unpublished)	No	Clinician	Limited
BARS	1 scale, ≥4	No	No	No	Clinician	Limited
Eating and drinking						
EDACS	1 scale, ≥2		Yes (unpublished)	Yes (unpublished)	Clinician	Yes
Communication						
CFCS	1 scale, ≥2	No	No	Yes (unpublished)	Clinician	Yes
ELFC-MLD	1 scale, ≥1½	Yes	Yes	Yes (unpublished)	Clinician	Yes
Cognitive scales						
LIPS	≥2	No	No	Yes (unpublished)	Trained expert	Limited
Wechsler scales	≥2 ^a	No	Yes	No	Trained expert	Limited
Adaptive behavior scales						
Vineland-3	0-90	No	No	No	Parent, teacher, or partner	Yes

Abbreviations: 10MWT = 10 m walk test; 10SWT = 10-step-walk test; BARS = Brief Ataxia Rating Scale = EDACS = Eating and Drinking Ability Classification System; CFCS = Communication Function Classification System; ELFC-MLD = Expressive Language Function Classification for MLD; EQ5D5L = EuroQoL 5D/5L; GMFC-MLD = Gross Motor Function Classification for MLD; GMFCS = General Motor Classification System; GMFM = Gross Motor Function Measure; HUI3 = Health Utilities Index Mark 3; LIPS = Leiter International Performance Scale; MACS = Manual Ability Classification System; P&P = patients and/or proxies; PedsQL = Pediatric Quality of Life Inventory; QoL = quality of life; SARA = Scale for Assessment and Rating of Ataxia (SARA); VWM = vanishing white matter. References for all scales are presented in the supplementary file, links.lww.com/NXG/A512.

^a Different tests for different age ranges; a selection of subtests can be used focused on cognitive impairment in leukodystrophies.

field strength (3 T) is recommended, including sequences as DTI, MTR, NODDI, MWF, mcDESPOT, MRS, and quantitative susceptibility mapping.⁴²

Trial Safety Considerations

Given that the ISR is a protective response, an important question is whether eIF2B activity can still be adequately modulated in the context of stress. A reassuring finding is that cells are insensitive to ISRIB during acute ISR activation and that cytoprotective ISR effects remain intact.⁵⁰ Another concern may be that enhanced eIF2B activity stimulates

growth and may be associated with an increased long-term risk of cancer.^{51,52}

Innovative Trial Design

Given that VWM is ultra-rare and that potentially different drugs will need to be assessed in trials that overlap in time, trial strategies should be extremely well planned and maximize the contribution of the few eligible patients. We propose the use of (1) statistical methods that incorporate historical data in the assessment of efficacy and (2) platform trials or other trial designs using a common, shared core protocol.

In settings of general consensus that placebo-controlled trials are considered unethical, for instance because of high death rates without therapy and highly promising preclinical studies, single-arm trials should be considered. Data from natural history studies may serve as an indirect comparator for such single-arm trials by providing an external control group that received standard of care. Furthermore, historical data may augment control arm data in 2-arm trials, thereby allowing a lower number of patients randomized to the control arm. The latter is generally referred to as historical borrowing.⁵³ Historical control information may allow single-arm studies in patients with VWM with late infantile and early childhood onset. As soon as the first efficacious drug has been identified, subsequent trials can use this drug as active control arm. Data from the single-arm trial for the first efficacious drug could potentially be used as historical control data to enrich subsequent trials and increase power.

Platform trials use a single master protocol to simultaneously evaluate multiple experimental treatments.^{54,55} The master protocol defines the inclusion and exclusion criteria. On inclusion, a patient is randomized to the control arm or one of the experimental treatment arms. As the control arm can be used as a comparator for multiple experimental treatments, the number of participants that needs to be allocated to control treatment is substantially reduced compared with a standard setting, in which separate placebo-controlled trials are run for each experimental drug. Platform trials can be adaptive in nature, allowing treatments to be declared futile or efficacious at interim analyses. Platform trials using a single master protocol also provide a durable infrastructure for treatment evaluation, in which new experimental treatments can be evaluated as soon as they become available.

If separate trials are run for different drugs, sharing data of patients in control arms allows a larger proportion of patients to be randomized to experimental therapies. A limited core protocol can be designed, in which only the inclusion and exclusion criteria, a limited set of outcome measures and assessment intervals, are determined to allow sharing of control data. Outside the core protocol, each trial can decide on numbers and trial duration, prioritize and add outcome measures, and plan studies on biomarkers and pharmacokinetics. Different core protocols can be designed for patients with early- and late-onset VWM.

Sample Size and Statistics

In single-arm trials with historical control data, adequate matching or use of analyses adjusting for confounding variables is essential. Historical controls and trial participants should be matched for age at onset. Matching for genotype is not advisable because of the very high number of different pathogenic variants and because the genotype does not add predictive information in addition to age at onset. Depending on the number of patients in the natural history cohort satisfying the trial inclusion criteria, different methods can be considered to reduce bias in estimates of treatment effects. Appropriate methods include one-to-many matching, propensity score matching, or adjustment for age at

onset and other possible confounders in a regression framework. Statistical methods should take into consideration the nature of the matching, with weighted least squares analysis typically required when one-to-many matching is used.

Single-arm trials can be adequately powered for detection of large treatment effects. The current single-arm trial investigating the efficacy of guanabenz in ambulatory patients with VWM with age at onset <6 years (clinicaltrialsregister.eu/ctr-search/trial/2017-001438-25/NL) uses time until loss of ambulation as the primary efficacy outcome measure. To estimate the number of patients needed, we determined that in the absence of treatment, based on the VWM natural history study,⁴ the proportion of ambulatory patients 2 years after disease onset is estimated to be approximately 60%. For a power of 80% and a 2-sided significance of less than 5%, a single-arm trial with 34 patients and an equal number of historical controls allows detection of an absolute increase of 25% of ambulatory patients. This calculation assumed that both the accrual time and the minimum follow-up time are 2 years.

In the adolescent and adult VWM population, randomized controlled trials can likely be adequately powered for detection of large treatment effects. Based on data from the VWM natural history study,⁴ the average decline in HUI cognition score in patients with an onset ≥ 16 years in the 2 years after onset was estimated to be 0.12 points with an SD of 0.15. Under the assumption that the experimental treatment stops the cognitive decline, a sample size of 26 patients per arm would suffice to demonstrate a difference in mean 2-year cognitive decline relative to placebo, with 80% power assuming 2-sided testing at a 5% significance level. Use of repeatedly measured outcomes could increase power and allows detection of smaller effects.

Conclusions and Recommendations

VWM is a fatal disease with serious morbidities. Apart from measures to prevent stressors provoking acute deteriorations, only symptomatic care is available. This grim situation is shifting. Different molecular targets have been identified, and several drugs are in clinical development. Efficacy trials are, however, challenged by the low number of known patients with VWM, clinical variability, and unpredictable disease course for onsets after early childhood. Given these challenges and the critical need to offer therapies, we have formulated recommendations based on evaluation of available clinical, laboratory, and molecular data:

1. Increase the diagnostic rate of VWM. Undiagnosed patients have no access to promising new drugs and cannot contribute to their evaluation in trials.
2. Develop biomarkers that can be used in therapy trials. Both body fluid and MRI biomarkers are promising. Their responsiveness to intervention and proportionality should be explored in initial trials to provide evidentiary criteria for future interventional trials.
3. For patients with late infantile and early childhood disease, a single-arm open-label study is recommended until an effective

therapy is determined. In this group, disease course is predictable, and matching with historical controls is possible. After demonstrating the efficacy of the first drug, subsequent studies can be double blind and controlled, with the use of platform design or core protocols to maximize the number of patients treated with new drugs. Pivotal populations should consider disease stage; preserved ambulation or other motor function could serve as inclusion criterion. Stabilization or improvement of motor skills can serve as primary outcome measure.

4. For patients with onset after early childhood, disease course and life span are variable and unpredictable. Therefore, double-blind placebo-controlled trials are preferable and must be of sufficient duration. Inclusion criteria and outcome measures should reflect the variable age-dependent presentations. Patients should be included early in the disease, while ambulant and having relatively preserved cognitive function. Use of platform design or core protocols with shared controls for different drugs helps minimize the number of patients on placebo and maximize the patients on trial drugs, facilitating unbiased comparison of different drugs.
5. Data from all trials should be shared. Therefore, all trials should use a minimum common set of outcome measures (Table 2).
6. Compassionate use and expanded access programs for trial drugs should be considered for patients not fulfilling the inclusion criteria.

The opportunity to advance therapy for VWM will depend on collaboration of patients and families, clinicians, industries, and regulatory agencies, with the aim to coordinate and successfully execute trials, to ensure rapid drug assessments, and to deliver treatments to patients.

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Appendix (continued)

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