Received: 5 July 2021 | Revised: 9 September 2021 | Accepted: 11 September 2021

DOI: 10.1111/bcp.15090

THEMED ISSUE REVIEW

Translational and clinical pharmacology considerations in drug repurposing for X-linked adrenoleukodystrophy—A rare peroxisomal disorder

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X-linked adrenoleukodystrophy (X-ALD) is an inherited, neurodegenerative rare disease that can result in devastating symptoms of blindness, gait disturbances and spastic quadriparesis due to progressive demyelination. Typically, the disease progresses rapidly, causing death within the first decade of life. With limited treatments available, efforts to determine an effective therapy that can alter disease progression or mitigate symptoms have been undertaken for many years, particularly through drug repurposing. Repurposing has generally been guided through clinical experience and small trials. At this time, none of the drug candidates have been approved for use, which may be due, in part, to the lack of pharmacokinetic/pharmacodynamic information on the repurposed medications in the target patient population. Greater consideration for the disease pathophysiology, drug pharmacology and potential drug–target interactions, specifically at the site of action, would improve drug repurposing and facilitate drug development. Incorporating advanced translational and clinical pharmacological approaches in preclinical studies and early-stage clinical trials will improve the success of repurposed drugs for X-ALD as well as other rare diseases.

KEYWORDS
clinical pharmacology, drug repositioning, drug repurposing, neurodegenerative diseases, rare diseases, translational pharmacology, X-linked adrenoleukodystrophy

1 | INTRODUCTION

X-linked adrenoleukodystrophy (X-ALD) is an inherited, peroxisomal disease characterized by the accumulation of saturated, very long-chain fatty acids (VLCFA), specifically C26:0. When VLCFAs accumulate in the central nervous system (CNS), this can lead to an inflammatory condition called cerebral ALD (cALD), which occurs in approximately 35% of boys with X-ALD. cALD is a progressive, demyelinating condition that is usually fatal within several years of diagnosis. This cerebral inflammation is associated with oxidative stress and together these contribute to subsequent demyelination and neuronal cell death.[1–3] ALD is caused by mutations in the *ATP-binding cassette sub-family D member 1* (*abcd1*) gene on the X chromosome that encodes the adrenoleukodystrophy protein (ALDP), an adenosine triphosphate (ATP)-binding-cassette (ABC) transporter located on the peroxisomal membrane. This protein, expressed in the adrenal glands, brain, kidney, liver, lungs, placenta and testis, is responsible for the transport of VLCFAs from the cytosol into the peroxisome for degradation by β-oxidation.[4,5] The genetic mutation in ALD renders this transporter defective, leading to the accumulation of cytosolic VLCFAs.[6,7] Although the systemic effects of elevated VLCFAs are not fully understood, fatty acid imbalance and mitochondrial impairment are known to contribute significantly to the pathophysiology of X-ALD.[2,8–10] The genotype–phenotype

relationship for X-ALD is complicated; the same mutation in the *abcd1* gene is associated with different clinical presentations including primary adrenocortical insufficiency, adrenomyeloneuropathy (AMN) and cALD. Primary adrenocortical insufficiency or Addison's Disease can present prior to the onset of neurological symptoms in X-ALD, occurring when the adrenal glands cannot produce cortisol and aldosterone.[11] AMN is the most common phenotype of X-ALD. In this subtype, patients generally have spinal cord dysfunction that is further categorized by the presence or absence of cerebral involvement.[5] AMN symptoms include ataxia, gait disturbances and spastic paraparesis. cALD is the more severe phenotype of X-ALD. It typically starts with cognitive and vasomotor function impairment, before rapidly progressing to neurologic deficits like spastic quadriparesis, vision loss and, eventually, death.[5,11] Although the peak age for onset of symptoms in children is 7 years, cALD can also occur in adults, initially presenting as adrenal symptoms, then progressing to AMN and eventually developing into cALD.[12] Being an X-linked disease, males are more frequently and severely affected than females. Thus, adrenal insufficiency and cerebral disease are rare among female carriers; however, over 80% of women affected will eventually develop progressive spinal cord disease.[11]

Although diagnosis of X-ALD can be difficult, early identification of the disease has become key to managing disease progression. For instance, in a patient with cALD, the onset of the disease could be asymptomatic, but rapidly progress to the development of brain lesions and white matter loss resulting in the onset of neurological symptoms. The inclusion of X-ALD in the United States Recommended Uniform Screening Panel (RUSP) in 2016 has expanded identification of newborns with a genetic indication for X-ALD. Screening involves a high-throughput biochemical assay that detects the elevation of C26:0-lysophosphatidylcholine (C26:0-LysoPC) in dried blood spot samples.[13,14] This is further confirmed by *abcd1* gene sequencing. Expanding worldwide availability of newborn screening for X-ALD will significantly aid in early diagnosis, monitoring and initiation of appropriate treatment.[15] The incidence of X-ALD has been reported to be approximately 1 in 20000 worldwide,[16–18] but data from newborn screening may be changing that estimate. In the United States, the incidence of X-ALD as determined by newborn screening differs from the worldwide incidence and varies substantially by state, for example: 1:15000 in New York versus 1:4845 in Minnesota.[14] Based on the incidence observed in Minnesota, nearly 500 boys with X-ALD will be born yearly in the US. The reason for this variability in incidence is not known. Upon positive detection, physicians are notified, and families are directed to genetic counselling. The children undergo monitoring including periodic contrast-enhanced MRI studies to detect brain lesions along with measurement of adrenocorticotropic hormone (ACTH) and cortisol for adrenal insufficiency.[14,19,20] Although newborn screening has allowed early detection of the X-ALD mutation, it does not indicate which phenotype will present. This makes management problematic due to varied types of therapy needed, potential adverse effects and high costs of clinical care.

There are few therapeutic options available for X-ALD. Current management strategies include glucocorticoid replacement therapy and allogeneic hematopoietic stem cell transplantation (HSCT), the latter requiring timely initiation for effectiveness. Glucocorticoid replacement therapy, typically using hydrocortisone, is prescribed for X-ALD patients that develop primary adrenocortical insufficiency.[21] HSCT is only offered to boys who show radiological signs of cerebral disease and may help arrest or slow down neuroinflammation in cALD, provided that the procedure is performed before neurological symptoms occur.[3,22] Depending on the onset and presentation of X-ALD, both treatments can be effective; but in the case of HSCT, utilization may be limited based on the specific circumstances of each patient such as their age, degree of disease advancement and financial means. Moreover, none of the existing therapeutic options are curative, but rather provide supportive care. Recently, gene therapy is being investigated in X-ALD as a potential strategy by correcting the defects in the *abcd1* gene in the patient's own bone marrow cells.[1,17]

2 | REPURPOSING DRUGS FOR X-ALD

There is a lack of effective therapies that can prevent or cure the clinical aspects of X-ALD. One approach to addressing this unmet need is drug repurposing (also called drug repositioning), which involves the testing of approved or investigational drugs (under development for other indications) in X-ALD.[23] The general process of drug repurposing is as follows: (1) identification of a candidate compound and its target; (2) acquisition of sufficient supplies of the compound for preclinical and clinical testing; (3) conduct of required pre-clinical and clinical studies of the compound in the new disease; and (4) application for licensure and approval.[14,24] Compared to the conventional method of drug discovery, repurposing is potentially a relatively lower risk, quicker approach to development.[25] Existing approved medications have established safety profiles through preclinical and clinical studies, thus eliminating/reducing the need for repeating extensive/expensive animal and human safety and toxicology assessments.[24] This may not be the case for repurposed drugs being developed for new patient populations such as those with rare disorders. Differences in the physiology of the target population can lead to a different safety profile and dosing requirements.

Optimizing the dosage regimen during pre-clinical and clinical development can eliminate or reduce the frequency of adverse effects and increase the likelihood of successful clinical trials of the repurposed drug. However, the lack of adequate clinical pharmacology information about the drug in the relevant patient population and the small number of patients with the rare disease limits the ability to more efficiently carry out dose optimization research. Approved medications have well-defined therapeutic benefits and safety data within the stipulated dosing ranges.[26] However, this information may not be applicable to the patient population with a rare disease as they may require different dosages, formulations and/or routes of administration. Addressing these challenges can extend the cost and the time required for development. The absence of a relevant animal model that recapitulates a rare disease complicates repurposing as it reduces the information available to optimally design clinical trials. Another

challenge often encountered is a lack of validated biomarkers. Moreover, large clinical studies in rare diseases are not feasible given that the number of available research participants is small. In addition, the legal difficulties in regulation of intellectual property rights of the original drug, management of the drug's life cycle for a new indication, and the likely low return on investment dissuades many pharmaceutical companies from pursuing drug repurposing, particularly for rare diseases.[27,28] These limitations, notwithstanding the immense need for new therapies for rare diseases, obligates investigators to utilize the most efficient and effective approaches to drug development.

Some of the challenges of drug repurposing for treatment of rare diseases can be overcome by incorporating translational and clinical pharmacology investigations into animal and early human studies. A major component of this approach relies on pharmacokinetic/pharmacodynamic (PK/PD) analyses, which describes the relationship of drug exposure with biological or clinical responses. Characterization of PK/PD in relevant animal models prior to the beginning of clinical development permits a more comprehensive assessment of safety and efficacy. The acquired data can be used to construct dose or concentration–response models that permit simulations to guide optimal trial design. Given the limited size of the target population in rare diseases, the PK/PD component can be built into the first part of Phase II/III trials. These results can then be incorporated in the later stages of such trials. By utilizing advanced translational and clinical pharmacology approaches, drug repurposing may yield greater success in developing new therapies for rare disorders. Regarding X-ALD, drug repurposing has the potential to identify new therapeutic options that either normalize VLCFA accumulation or target the associated symptoms. Key preclinical drug repurposing studies in X-ALD cell and mouse models and clinical studies conducted in patients are summarized in Table 1 and briefly explained below.

3 | POTENTIAL THERAPIES FOR X-LINKED ADRENOLEUKODYSTROPHY

3.1 | Antilipaemics

3.1.1 | Bezafibrate

Bezafibrate is a peroxisome proliferator-activated receptor (PPAR)-alpha (PPAR-α) agonist in a class of medications known as the fibrates, clinically indicated for lowering serum triglycerides, decreasing low-density lipoprotein (LDL) cholesterol, and increasing high-density lipoprotein (HDL) cholesterol in primary hyperlipidaemia. It is available in Canada and Europe, but not in the United States. The fibrates work by activating the PPAR-α pathway that regulates lipid metabolism in

TABLE 1 Overview of potential repurposed drugs for X-ALD. Summary of drug products studied in X-ALD that are reviewed in this article

Drug candidate	Drug class	Indication	Potential effects in X-ALD
Bezafibrate[29–31]	Fibrates	Hyperlipidaemia	Decrease VLCFA concentrations
Metformin[32–34]	Biguanides	Type 2 diabetes mellitus	Decrease VLCFA concentrations Improve mitochondrial function
Pioglitazone[35]	Thiazolidinones	Type 2 diabetes mellitus	Improve mitochondrial function
Leriglitazone[36]	Thiazolidinones	Currently no approved indication; under development for X-ALD	Improved mitochondrial function Promote anti-inflammatory response Reduced axonal degeneration Support myelinogenesis
Mesenchymal stem cells (MSC)[37–43]	Stem cells	Cancer Immune-mediated diseases	Promote anti-inflammatory response Promote myelin sheath repair
α-Lipoic acid (ALA) Vitamin C Vitamin E[8,10,44–46]	Antioxidants	Diabetic neuropathy (ALA) Dietary supplement (vitamin C, E) Scurvy (vitamin C)	Arrest axonal degeneration Prevent oxidative stress Reduce reactive oxygen species
N-acetylcysteine (NAC)[10,39,46–54]	Mucolytics/antioxidant	Acetaminophen overdose antidote Cystic fibrosis Dietary supplement	Promote anti-inflammatory response Reversal of oxidative damage
Lorenzo's oil (LO)[55–61]	Monounsaturated fatty acids	Dietary supplement	Decrease VLCFA concentrations
Nervonic acid (NA)[11,62–70]	Monounsaturated fatty acids	Dietary supplement	Decrease VLCFA concentrations Support myelinogenesis
Valproic acid[71–75]	Anticonvulsants	Bipolar disorder Epilepsy	Decrease oxidative damage Decrease VLCFA concentrations
Suberoylanilide Hydroxamic acid (SAHA)[76–82]	Histone deacetylase inhibitors	Cutaneous T-cell lymphoma	Decrease VLCFA concentrations Promote anti-inflammatory response
Vitamin D[83–87]	Vitamins	Dietary supplement	Promote myelin sheath repair

favour of promoting uptake, utilization and catabolism of fatty acids. Common side effects include gastrointestinal upset and nausea that typically occur within the first few days of therapy. Bezafibrate has been studied for anti-inflammatory and monocyte-suppressing effects, which may be beneficial in X-ALD.[29] Engelen et al. tested bezafibrate in human skin X-ALD fibroblasts and showed reduction in VLCFA levels, presumably by direct inhibition of fatty acid elongation via ELOVL1, a VLCFA-specific elongase.[30] The same group conducted a proof-of-concept clinical trial of bezafibrate in 10 male AMN patients using a sustained-release formulation (Bezalip Retard) and standard dosing for hyperlipidaemia (400 mg daily for 12 weeks followed by 800 mg daily for 12 weeks) but did not find a reduction in plasma or lymphocyte VLCFA levels at the doses tested.[31] It was not known if bezafibrate dosing for hyperlipidaemia is clinically appropriate for X-ALD. This is an example where the conduct of PK studies in relevant animal model or early patient studies could provide information to determine dosage regimens for future clinical trials.

3.1.2 | Antidiabetics

Some antidiabetic agents can mitigate or correct dysfunctional pathways such as the AMP-activated protein kinase (AMPK) and the peroxisome proliferator-activated receptor-gamma coactivator/peroxisome proliferator-activated receptor γ (PGC-1α/PPAR-γ) pathways. On this basis, metformin and pioglitazone have been studied for X-ALD.

Metformin

Metformin, a biguanide, inhibits the production of glucose in the liver and increases insulin sensitivity in the body. It is one of the most widely prescribed medications in the United States and is approved in Europe for type 2 diabetes mellitus. Patients on metformin commonly experience side effects such as diarrhoea and nausea, but these usually subside over time. Long-term use of metformin can lead to vitamin B12 deficiency, necessitating supplementation. A 2015 study by Singh et al. documented the loss of a metabolic gene, AMP-activated protein kinase α1 (AMPKα1) in cALD patient-derived fibroblasts and lymphocytes, which suggested that metformin, an AMPK activator, could be useful in this disease.[32] Singh et al. investigated the effect of metformin in AMN patient-derived fibroblasts and lymphocytes as well as *abcd1*- mice.[33,34] They observed that metformin decreased VLCFA levels, increased mitochondrial function and increased AMPK levels.[33] However, it was noted that metformin at high doses could inhibit mitochondrial function, indicating a narrow therapeutic window regarding X-ALD.

Pioglitazone

Pioglitazone is a PPAR-γ agonist approved for use in type 2 diabetes mellitus by lowering blood glucose levels. As a part of the class of medications called thiazolidinones, pioglitazone can also lower blood pressure, improve lipid metabolism and reduce insulin resistance. In the United States, it can be used in combination with other antidiabetic agents such as metformin but has been withdrawn from France and Germany due to the risk of bladder cancer. Common side effects of pioglitazone include oedema and weight gain. It is also contraindicated in patients with New York Heart Association (NYHA) class III/IV heart failure. Notably, onset of blood glucose changes occurs approximately 2 weeks after initiation of pioglitazone with the full therapeutic effect requiring 2–3 months. Morató et al. observed a reduction in mitochondrial DNA and expression of mitochondrial proteins cytochrome c, NDUFB8 and VDAC along with downregulation of the mitochondrial biogenesis pathway driven by PGC-1α/PPAR-γ in a mouse model of X-ALD.[35] Based on these findings, they tested oral administration of pioglitazone in *abcd1*- mice and saw restoration of mitochondrial function and expression of key proteins, which reversed major defects in energy metabolism.[35] However, no significant reduction in VLCFA levels was observed, nor were cardiovascular adverse effects assessed.

Leriglitazone

Another PPAR-γ agonist that crosses the blood brain barrier (BBB) and is a derivative of pioglitazone is leriglitazone, which is being developed for X-ALD.[36] The most common side effects of leriglitazone are oedema and weight gain but otherwise it is well-tolerated overall. In *abcd1*- mice, Rodríguez-Pascau et al. saw that leriglitazone treatment resulted in the reduction of pro-inflammatory cytokines in the spinal cord after 7 days of oral twice daily treatment at 50 mg/kg/day.[36] Similarly, reduced axonal degeneration was observed at the highest dose of 110 mg/kg/day in *abcd1-/abcd2*- mice treated for 6 months.[36] Further investigation demonstrated decreased tumour necrosis factor alpha (TNF-α) expression, an inflammatory cytokine in monocyte-derived macrophages from AMN and increased motor function and mitochondrial survival in neuronal cultures.[36] Leriglitazone also increased myelination and protected oligodendrocytes from cuprizone, a copper-chelating agent that causes demyelination.[36] Leriglitazone was observed to be well-tolerated when increasing doses were administered to healthy volunteers for 8 days and even exerted some anti-inflammatory effects.[36] However, it should be noted that while various doses of leriglitazone were utilized in this study, different positive outcomes were linked to more than one dose. Careful PK/PD analysis of their results will provide valuable information on dose selection for future human studies with leriglitazone. There are two ongoing clinical trials with leriglitazone: (1) a phase II/III trial enrolling males aged 18–65 years of age with AMN to assess the efficacy of leriglitazone using motor function tests over 96 weeks (NCT03231878), and (2) an exploratory trial looking at male paediatric patients with cALD to test the effect of leriglitazone on disease progression (2019-000654-59). It is not clear whether dose–response relationships for leriglitazone were considered for determining doses for these studies.

Although leriglitazone, pioglitazone and metformin appear to have beneficial effects in X-ALD mice, these studies have not established a dose–response relationship for optimal X-ALD treatment. Continued investigation of these drugs and other similar antidiabetic agents are warranted based on existing literature that indicate they could regulate impaired pathways affected by X-ALD.

3.2 | Stem cell transplantation

3.2.1 | Mesenchymal stem cells

Mesenchymal stem cells (MSCs) are multipotent cells easily isolated from adult tissues of animals and humans that can undergo multilineage differentiation into adipocytes, chondrocytes, hepatocytes and osteocytes.[37] MSCs have immunomodulatory, immunosuppressive and tissue-repairing properties and have been studied for clinical uses in cancers and immune-mediated diseases like graft versus host disease (GvHD) and inflammatory bowel disease.[37–39] As a type of repurposing, MSCs are now being studied in neurodegenerative diseases by enhancing neural protection and regeneration. If successful, they could be an alternative to hematopoietic stem cells (HSCs) that only differentiate into cells in the hematopoietic lineage such as erythrocytes and leukocytes. HSCs are therapeutically effective in some conditions such as X-ALD but carry significant toxicity risks.[40,41] MSCs migrate towards damaged tissue where they promote anti-inflammation via inhibition of proliferation and activation of immune cells.[38,39] However, the beneficial effects of MSCs are offset by safety concerns with possible unwanted differentiation and immunosuppression. As an example, MSCs in the setting of malignant conditions have been shown to suppress anti-tumour immune responses and initiate metastasis.[37,39] Furthermore, the process and procedures of transplants incurs risks such as blood clots, excessive bleeding and/or infection.

Multiple sclerosis (MS) is a progressive, neurodegenerative disease that shares pathophysiological aspects with X-ALD such as demyelination, development of brain lesions and neurological decline. In a small, open-label, proof-of-concept study, Connick et al. reported that 10 patients with secondary MS had improvement in visual acuity and a decrease in neural lesion volume at an MSC dose of 1.6×10^6 cells/kg.[42] However, they also stated that there was no change in the rate of lesion accumulation, indicating a lack of neural structural improvement.[42] In terms of safety, Connick et al. reported that two patients developed rash and another two experienced bacterial infections post-infusion, but there was no report of other adverse events during infusions or in the 10-month follow-up.[42] Gupta et al. studied the benefit of intrathecal MSCs in two boys, aged 5 and 7 years, respectively, with childhood cALD. The patients were given a dose of 5×10^6 cells/kg to halt progression of hearing or vision loss, poor coordination and seizures, but MRIs showed unchanged demyelination in both children.[43] It should be noted that one boy received two more doses than the other. The investigators commented that potentially inadequate MSC doses were used.[43] Also, follow-up with MRI was varied between the two boys (2 months after a third dose for the 5-year-old and 1 month after the only dose for the 7-year-old). The lack of perceived benefit should be considered carefully in the light of pharmacological limitations arising from wide variation in dosing frequency and MRI monitoring, and small sample size.[43] Further research is needed to determine the appropriate MSC dose and, subsequently, adequately powered clinical trials are needed to determine what role, if any, MSCs have in X-ALD.

3.2.2 | Antioxidants

Antioxidants are substances that counteract oxidative stress, a chemical reaction that can result in cellular damage due to production and accumulation of reactive oxygen species (ROS).[44] There are several antioxidants available for clinical use including vitamin C, vitamin E, α-lipoic acid (ALA), and N-acetylcysteine (NAC). They have a variety of indications such as scurvy (vitamin C), dietary supplementation (vitamin C, vitamin E), diabetic neuropathy (ALA) and acetaminophen (also known as paracetamol) poisoning (NAC). Some are approved for use in the US and Europe, while others are not. Several studies have demonstrated the role of oxidative stress in X-ALD wherein excessive VLCFA accumulation leads to increased ROS and mitochondrial dysfunction. Consequently, the effects of antioxidants on X-ALD have been investigated in pre-clinical and clinical studies.[8,10,45] López-Erauskin et al. dosed *abcd1*- mice that exhibited the AMN-like phenotype with a cocktail consisting of ALA, NAC and vitamin E.[46] They observed that this mixture arrested locomotor deficits, normalized ROS levels and reduced axonal damage. This study provided a rationale for the investigation of antioxidants in diseases such as Alzheimer's disease, Parkinson's disease and X-ALD, in which oxidative damage is a key component. However, optimal dosages and, ideally, plasma concentrations, of the individual antioxidants in the cocktail need be determined as higher than standard doses routinely used for dietary supplementation may be needed to exert clinical benefit. Further, the contributions of each drug in the antioxidant cocktail and whether the overall clinical effect is synergistic or additive should be considered.

3.2.3 | N-acetylcysteine

N-acetylcysteine is a well-recognized antioxidant that is FDA-approved as a prescription medication for use as a mucolytic and an antidote for paracetamol overdose. It is also available as an over-the-counter dietary supplement. NAC has multiple mechanisms of action: anti-inflammatory, antioxidant effect, free radical scavenging activity, glutamate modulation and chaperon action have been studied in different disease conditions.[47–51] Depending on the route of administration, NAC either acts by releasing bound cysteine in blood or by providing cysteine through deacetylation. Regardless of the pathway, the increased availability of cysteine contributes to glutathione synthesis.

Evidence indicates that oxidative stress and neuroinflammation play a role in X-ALD pathophysiology and disease progression, suggesting that NAC, an anti-inflammatory agent, may be useful in its management.[10,39] Further, HSCT, the standard of care for cALD, involves total or partial myeloablation using chemotherapy that results in oxidative stress. In addition, infectious complications or graft-vs-host disease can further contribute to increased inflammation. Tolar et al. demonstrated improved cumulative survival 8–11 months following HSCT using NAC infusions in the peri-transplant period in boys with advanced cerebral disease as defined by cognitive score (PIQ) and

imaging-based severity score, Loes (PIQ < 80 and Loes score > 14) compared to 8 boys in a comparison group with similar advanced disease, all of whom died within 1 year following the HSCT despite full donor engraftment (Figure 1).[52] Similarly, Orchard et al. reported statistically significant improvement ($p = 0.02$) in cumulative survival for patients with a baseline Loes score of >10 who received a reduced intensity preparatory regimen in addition to NAC infusions compared to those with similar disease stage at pre-transplant who received the fully ablative preparatory regimen, but no NAC treatment.[53] However, many questions regarding the optimum NAC dosage regimen and the duration of treatment remain to be answered. To determine whether NAC disposition changes following HSCT, and thus potentially warrants an NAC dose change, we recently completed an analysis utilizing rich PK data from 18 paediatric patients with inherited metabolic disorders undergoing HSCT. We observed that the primary PK parameters, namely clearance and volume of distribution, were largely unaffected, indicating that any alteration in the NAC dose relative to the time after transplant is most likely not warranted.[54]

Additionally, López-Erauskin et al. and Casasnovas et al. have studied NAC as part of an antioxidant cocktail, which included vitamin E and ALA in pre-clinical investigations to test whether various combinations of antioxidants could decrease oxidative stress and axonal degeneration in *Abcd1*- mice and in AMN patients.[46,55] In their mice studies, they reported that the cocktail reversed oxidative stress and the lesions to proteins, and halted axonal degeneration and locomotor impairment assessed using various immunological and functional tests.[46]

Taken together the research, which indicates that NAC, alone or in combination with other antioxidants, may improve outcomes in X-ALD, warrants additional clinical studies, especially in the early stages of X-ALD.

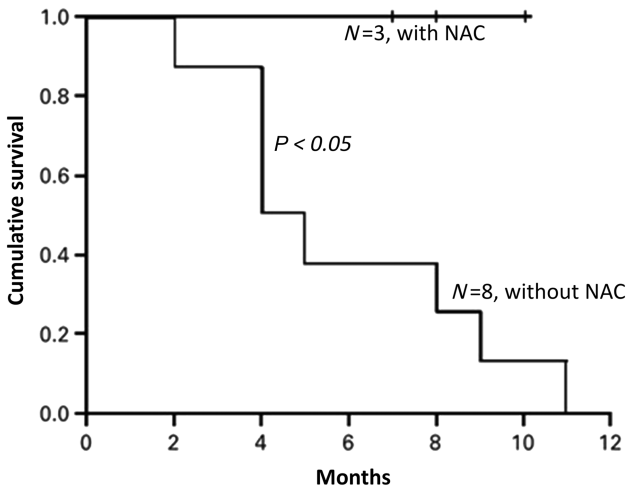

FIGURE 1 Improved survival in X-ALD patients following addition of NAC to the HSCT preparatory regimen. Plot compares cumulative survival rates 8–11 months after HSCT in patients with similar disease severity. This figure is adapted from Tolar et al., *Bone Marrow Transplantation*, PMID: 17290278, DOI: 10.1038/sj.bmt.1705571.[52] *p*-value is calculated using log-rank test for the equality of the survival distributions

3.3 | Dietary fatty acids

3.3.1 | Lorenzo's oil

Lorenzo's oil (LO) is a 4:1 mixture of oleic acid (C18:1 or OA) and erucic acid (C22:1 or EA), which are long-chain, monounsaturated fatty acids found in sphingolipids that are the essential constituents of brain white matter.[56,57] It has been used as a dietary supplement for X-ALD but is not approved for this indication in either the US or Europe.[56] Both OA and EA can reduce saturated VLCFAs in AMN patients, but EA is considered the more potent active ingredient of LO.[56] However, EA is associated with mild transaminase elevations, reversible platelet count reduction and rare instances of development of cardiac steatosis or fibrosis.[58–60] There is a relatively low incidence of these adverse effects.[56,58,60] Although several animal and human studies have been conducted, the pharmacological basis for the dosage regimen is not well established as the PK/PD of EA and other monounsaturated VLCFAs remains poorly understood. As an example of the application of clinical pharmacology approaches, our group used modelling and simulation to characterize the relationship between EA and C26:0 in 104 boys with asymptomatic X-ALD who received a dose of 2–3 mg/kg/day over a mean duration of 4.8 ± 2.76 years.[60] Analysis of plasma C26:0 and EA concentrations showed a significant inverse relationship, as shown in Figure 2.[60–62] This exposure–response relationship may be beneficial in guiding the design of future clinical trials using C26:0 as a biomarker. One other observation arising from this study was that as the boys grew older, the volume of the weight-based LO doses increased, with many patients finding the amount and taste disagreeable, that resulted in poor compliance.[60] Future research should be undertaken to better define LO pharmacology, drug–drug interactions, an improved drug formulation, and to assess safety and efficacy.

3.3.2 | Nervonic acid

Nervonic acid (C24:1 or NA) is also a long-chain, monounsaturated fatty acid that is the immediate elongation product of OA.[63–65] It is primarily derived from olive oil in the human diet. NA is the most abundant VLCFA in sphingomyelins, an important component of myelin.[64] Several studies have suggested that NA is involved in the development and maintenance of brain function as it is one of the fatty acids that rapidly increases during myelinogenesis in infants.[66,67] Furthermore, NA has been found to be deficient in the brains of X-ALD patients.[11,68] Like EA and OA, NA is not approved for treatment of any disease, but is used as a dietary supplement and is generally well-tolerated.[64,68] Monounsaturated VLCFAs such as NA are ELOVL1 substrates that competitively inhibit C24:0 elongation, thereby preventing the generation and accumulation of C26:0.[69] NA supplementation has been proposed for X-ALD as a safer alternative to LO in reducing saturated VLCFAs, supporting normal synthesis of myelin and improving cognitive function.[65–68,70,71] Hence, we conducted a preliminary study to compare the effect of increasing concentrations

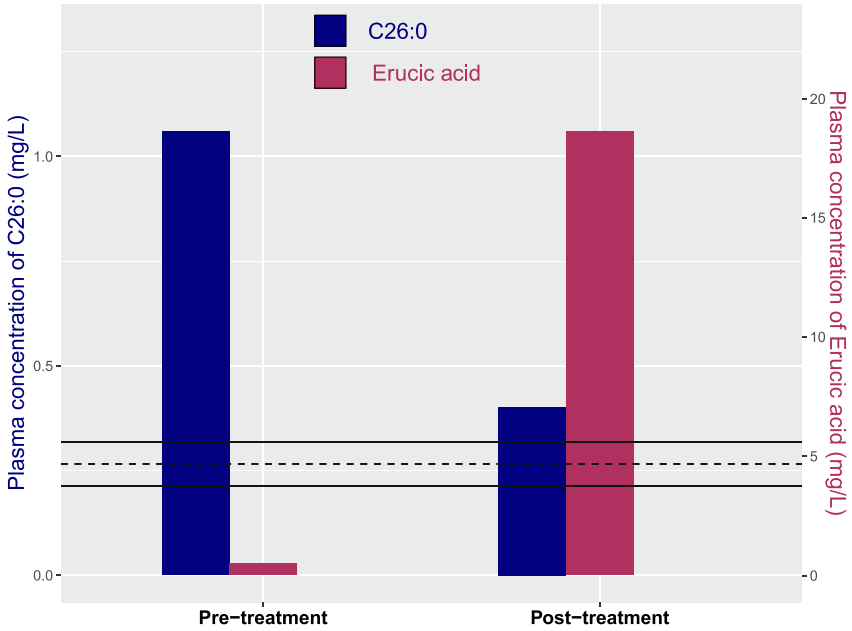

FIGURE 2 Effect of erucic acid (active ingredient of Lorenzo's oil) on C26:0. The figure is generated based on the data provided in table 1 by Ahmed et al., *British Journal of Clinical Pharmacology*, PMID: 26836218, DOI: 10.1111/bcp.12897.[60] Note that there are two Y axes corresponding to the plasma concentration of C26:0 (navy) and erucic acid (maroon) respectively. The coloured bars indicate the median of data ($n = 97$). The dashed line indicates the mean normal C26:0 levels and the solid lines are the deviations from the mean[61,62]

(5–50 μM) of EA and NA on C26:0 in X-ALD patient-derived fibroblasts. We observed that, like EA, NA can significantly decrease accumulation of saturated VLCFA in free and complex lipids in a concentration-dependent manner (manuscript under revision). Further, NA protected these cells from oxidant stimuli, presumably by increasing ATP production. These results indicate that in vivo drug development studies in both animals and humans are warranted to determine if adequate tissue distribution of NA occurs and thus will be useful in the management of X-ALD.

3.3.3 | Antiseizure agents

Valproic acid

Valproic acid (VPA) is a widely used antiseizure medication with histone deacetylase inhibitor properties that could potentially be utilized to reduce VLCFA levels.[72] In a study using patient-derived fibroblasts, *abcd1*- mice, and patients with X-ALD, VPA induced the expression of a functionally overlapping ATP-binding cassette sub-family D member 2 (*abcd2*), a peroxisomal transporter similar to *Abcd1* that encodes a transporter protein involved in moving fatty acids into peroxisomes for degradation.[72] In patients, over a 6-month period, VPA dosing resulted in a reversal of oxidative damage and decreased levels of monounsaturated VLCFAs: C26:1 by 40% without a change in C26:0.[72] The overexpression alone was able to prevent oxidative lesions in proteins in *abcd1*- mice and peripheral blood mononuclear cells.[72] Some X-ALD patients can exhibit seizures and altered psychiatric behavior.[73,74] There are two case reports describing use of VPA in cALD patients to treat these symptoms.[75,76] Salsano et al. reported that VPA 1000 mg twice daily over 6 months resulted in improvement in behaviour (reduced irritability, restlessness, distractibility and poor impulse control) and neurological function (less gait instability, gaze evoked nystagmus, arm tremors) in a 24-year-old male with cALD.[76] Brownstone et al. treated a 50-year-old male with cALD and tonic–clonic seizures with VPA at a dose of 2400 mg/day with favorable response.[75] The authors noted that since recurring seizures in adult-onset cerebral X-ALD are usually followed by neurological decline, prevention of these episodes with an antiseizure medications such as VPA should be considered.

These in vitro and in vivo studies involving mice and humans indicate that VPA may be useful in mitigating oxidative damage and managing neuropsychiatric symptoms often associated with X-ALD. Further preclinical and clinical investigations are needed to establish optimal dosing regimens focused on frequency and timing for this patient population.

3.3.4 | Anticancer agents

Suberoylanilide hydroxamic acid (SAHA)

Suberoylanilide hydroxamic acid (SAHA) (also known as vorinostat), is a histone deacetylase inhibitor used to promote cancer cell apoptosis. It is indicated for progressive or recurrent cutaneous T-cell lymphoma.[77,78] There are concerns of thrombocytopaenia and neutropaenia at higher doses but otherwise SAHA is generally well-tolerated.[77] SAHA has been considered in neurodegenerative diseases such as multiple sclerosis (MS), Huntington's Disease and X-ALD due to its neuroprotective properties, which include minimizing axonal damage, reducing demyelination and inducing neuronal survival factors.[79] Singh et al. reported that SAHA at varying doses (up to 5 μM) over a 72-hour treatment duration normalized the levels of saturated and monounsaturated VLCFAs in human-derived fibroblasts from patients with AMN and downregulated the expression of pro-inflammatory cytokines in astrocytes where *abcd1/2* expression was

silenced.[80] SAHA also upregulated *abcd2* expression in a dose- and time-dependent manner, leading to reduction in VLCFA levels.[80] These investigators also found that SAHA inhibits VLCFA-induced proapoptotic signalling mechanisms and proinflammatory responses in *abcd1*- cells.[81] Given the positive in vitro results, the investigators conducted a proof-of-principle study in *abcd1*- mice given 50 mg/kg/day of SAHA for 62 days. They reported a reduction of VLCFA and maintenance of mitochondrial homeostasis through *abcd2* upregulation and ELOVL1 inhibition.[81] More recently, Baarine et al. treated *abcd1*- human astrocytes cells and rat oligodendrocytes with SAHA at 5 μM/daily for 72 hours which resulted in increased mitochondrial antioxidant activity and ATP levels.[82] This observation indicates that SAHA could target several pathophysiological pathways of X-ALD. Zierfuss et al. studied SAHA pharmacology using monocyte-derived macrophages from seven adults with AMN.[83] They incubated the samples with SAHA for 5 days at doses of 1, 2.5 and 5 μM. They found that the drug lowered secretion of proinflammatory cytokines by correcting interleukin 12B (IL12B) expression, reduced VLCFA levels, stimulating peroxisomal β-oxidation, and upregulated *abcd2* expression.[83] Based on these findings, Zierfuss et al. treated three boys with cALD who were not eligible for HSCT. The patients were given 100–400 mg/day of SAHA for up to 80 days.[83] The disease progressed in all three boys with expansion of brain lesions, but the investigators noted that C26:0 decreased shortly after initiation of therapy and normalized the albumin and immunoglobulin CSF to serum ratios.[83] It should be mentioned that all patients experienced thrombocytopaenia, causing two patients to permanently withdraw from the study. This highlights the need for use of modelling approaches to determine a target dose that has fewer risks and greater efficacy.[83] These in vitro and in vivo studies provide evidence that SAHA may have therapeutic potential for X-ALD by reducing VLCFA levels and correcting mitochondrial dysfunction. The in vitro exposure–response relationship reported by Zierfuss et al. provides valuable information that can be leveraged when designing studies in X-ALD patients. In the future, clinical studies in humans using doses that optimize exposure would be necessary to assess safety and efficacy.

3.3.5 | Vitamin D

Vitamin D (also known as calciferol) is a fat-soluble vitamin that is naturally available in food such as milk and salmon, and endogenously produced from sunlight exposure.[84] It is commonly indicated as dietary supplementation for vitamin D deficiency (VDD).[85] The role of vitamin D involves maintaining calcium and phosphate homeostasis as well as normal bone growth and mineralization.[84,85] Moreover, vitamin D has been suggested to possess immunomodulatory effects on macrophages and T-lymphocytes in neurodegenerative diseases since it has been observed that patients with high levels of vitamin D had a lower risk of developing MS.[84,86,87] In one study, Haren et al. measured vitamin D levels in 16 boys (eight boys with cALD and eight boys without) and reported that the former group who later developed cerebral demyelination had statistically significant lower levels of vitamin D.[88] Given the potential of vitamin D in X-ALD, Haren et al. have undertaken a pilot study involving 21 participants with X-ALD whose primary aim is to establish a safe dose of vitamin D supplementation using two high-dose regimens (1000 or 2000 international units [IU] daily for 6 months, followed by increases to 2000, 3000 or 4000 IU daily thereafter if vitamin D levels have not achieved a target threshold over a 12-month period). The investigators define the target threshold as vitamin D levels within the range of 40–80 ng/mL (NCT02595489). As a secondary aim, the investigators are measuring markers of oxidative stress and inflammation in the blood and brain. The information derived from this trial will inform the design of future, larger clinical trials to determine whether vitamin D supplementation can reduce the risk of developing the cerebral demyelinating form of ALD.

4 | THE ROLE OF TRANSLATIONAL AND CLINICAL PHARMACOLOGY

Repurposing of approved drugs for rare diseases can shorten the drug development timeline by reducing or eliminating certain pre-clinical studies and improving the probability of successful clinical trials. However, with some exceptions, most of the studies summarized in this review do not provide sufficient translational and clinical pharmacology information needed to guide design of Phase II/III type safety and efficacy trials of re-purposed drugs for cALD. There is a general lack of information such as disease natural history, optimum dose, responsive biomarkers, duration of treatment and optimal time to initiate therapy to arrest or reverse pathophysiological manifestations. The absence of such information hinders drug development. The use of advanced translational and clinical pharmacology tools and consideration of all available information about a drug can help address this problem.[89]

Dosing requirements for optimal benefit in a rare disease can differ from the FDA-approved dosing recommendations given the variation in drug PK and PD in a rare disease population. For example, when the highest dose for an approved indication is tested, it may only show no or marginal efficacy in X-ALD owing to a multitude of factors including differences in the patient population such as age or weight; differences in plasma protein binding, biotransformation, hepatic or renal insufficiency, fluid overload; differences in the intended clinical benefit; or what constitutes an acceptable *vs* unacceptable adverse event from the perspective of the patient. Another example that can affect study outcomes can be the lack of formulations suitable for the rare disease population, which are most often young children. This may result in poor adherence and/or bioavailability. Failure to deliver an adequate amount of drug to the site of action due to altered absorption, distribution or elimination, or the formation of toxic metabolites, can easily lead to the perception of treatment failure. The unfortunate result is that the development of a potentially promising drug is abandoned.

Another important component of drug development often lacking in rare diseases is the identification and validation of relevant

biomarkers. An ideal biomarker is one that is responsive to the treatment, lies in the pathway being modulated by the drug under investigation and is a representation of how patients function or feel. Development of such a biomarker needs a clear understanding of the pathophysiologic mechanisms in both pre-clinical and clinical settings; something that is, at best, poorly understood in most rare diseases, including X-ALD. For example, elevated VLCFA levels have been measured in these patients and have been used as a reasonable first target in developing new therapies. However, reducing elevated VLCFAs may not be enough to modify disease progression, especially if other disease manifestations have occurred. Notably, X-ALD mouse models used in several of the summarized studies show the VLCFA accumulation but do not capture the cerebral aspects of the disease. The lack of the inflammatory components in the animal model can limit the identification and development of repurposed drugs that may be effective for AMN but not cALD. Several repurposed drugs normalize VLCFA β-oxidation and VLCFA levels in X-ALD but have not been found to be predictive of response in the clinical trials. Recently, neurofilament light chain (NfL) was demonstrated to be a biomarker of spinal cord degeneration in X-ALD and has shown promise as an early predictor of cerebral disease development in individuals with AMN as NfL reflects the inflammatory activity.[90,91] Future studies should confirm these findings in a larger cohort. Availability of such validated biomarkers will be extremely helpful in the identification of drugs that can arrest or delay disease progression.

Several other factors complicate the development of drugs to treat rare diseases such as X-ALD. These include lack of genotype–phenotype correlation, heterogeneity in disease presentation and progression, small number of potential study participants, and the challenges of conducting studies in children.[92,93] These factors make designing studies more difficult in terms of inclusion and exclusion criteria, outcome measures, determination of sample size and recruitment. For instance, the small patient populations available to participate in rare disease clinical studies increases the risk of type II errors. Additionally, many rare diseases including X-ALD disproportionately affect children. Paediatric studies are challenging because of developmental factors (cognition, emotion, physical), familial consent and ethical considerations.

As shown in Figure 3, utilizing modelling and simulation tools to improve our understanding of target site engagements, target protein modulation, downstream signalling cascades, clinical response and adverse events can address some of the current

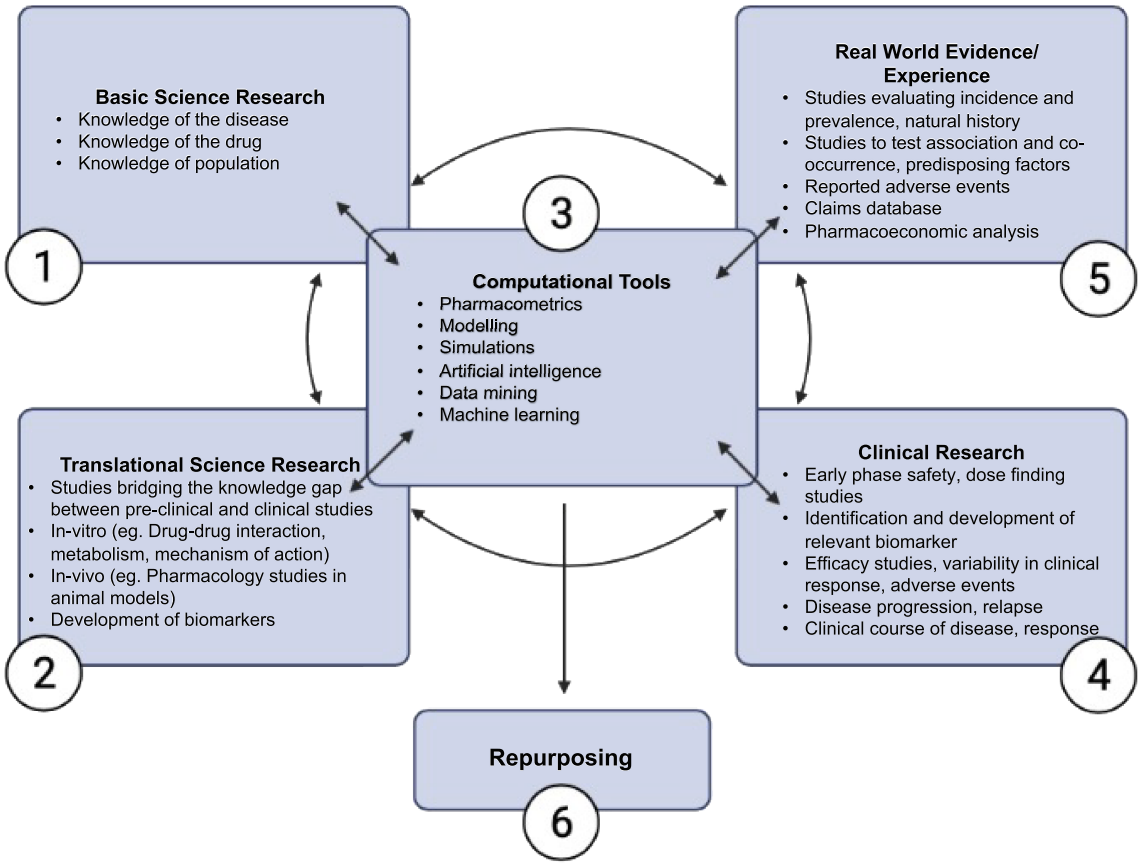

FIGURE 3 Approaches to translational and clinical pharmacology. Clinical pharmacology and computational tools as strategic enabler of repurposing. With multiple data sources ranging from basic science to real-world evidence, repurposing relies on quantitative integration of this information by multiple advanced computational tools to innovatively support forward and reverse translation (shown as bi-directional arrows) with a focus on building confidence in assessed target, biomarker, patient population and safe and effective dosage

challenges in repurposing drugs for rare diseases. Currently, there are no examples of model-informed drug repurposing for the treatment of X-ALD, although the potential for its use is clear. For example, a variety of modelling techniques have been utilized in the successful search for repurposed COVID-19 therapies. Extrapolating that experience to X-ALD can speed up the development of already available drugs for treatment of this disorder. Similarly, artificial intelligence (AI) combined with the ever-growing understanding of the underlying pathophysiology of X-ALD could be used to extract valuable information from radiology images such as MRIs and omics-based data obtained from X-ALD patients to find opportunities to generate new hypotheses that can be experimentally tested. Mechanistic approaches such as quantitative system pharmacology (QSP) can help with mechanism elucidations, rational dose selection, etc. Taken together, AI and QSP hold the promise of identifying potential X-ALD therapies, including drugs that can modify disease progression.

5 | CONCLUDING REMARKS

An incomplete understanding of the PK/PD properties of candidate drugs for repurposing prevents the full utilization of potential therapies in rare diseases such as X-ALD. The result is missed opportunities to develop effective therapies for many conditions that have few or no proven treatments. Scientifically guided drug repurposing holds the promise of increasing the number of safe and effective treatments for rare diseases. Incorporating into the drug development process translational and clinical pharmacology approaches including pharmacometrics modelling, simulation and data mining using big data available as real-world evidence can facilitate drug repurposing for rare diseases and increase the likelihood of successfully repurposing drugs for X-ALD and other rare disorders.

COMPETING INTERESTS

The authors report that they have no conflicts of interest to declare.

CONTRIBUTORS

R.V.K. and J.C.C. planned the review. J.H.T. and S.A.S. wrote the article. J.C.C., R.V.K. and P.J.O. provided key input. All the authors have read and approved the final version of the manuscript.

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REFERENCES

1. Berger J, Forss-Petter S, Eichler FS. Pathophysiology of X-linked adrenoleukodystrophy. *Biochimie*. 2014;98:135-142.
2. Kemp S, Huffnagel IC, Linthorst GE, Wanders RJ, Engelen M. Adrenoleukodystrophy—neuroendocrine pathogenesis and redefinition of natural history. *Nat Rev Endocrinol*. 2016;12(10):606-615.
3. Turk BR, Theda C, Fatemi A, Moser AB. X-linked adrenoleukodystrophy: pathology, pathophysiology, diagnostic testing, newborn screening and therapies. *Int J Dev Neurosci*. 2020;80(1):52-72.
4. Moser HW, Mahmood A, Raymond GV. X-linked adrenoleukodystrophy. *Nat Clin Pract Neurol*. 2007;3(3):140-151.
5. Palakuzhiyil SV, Christopher R, Chandra SR. Deciphering the modifiers for phenotypic variability of X-linked adrenoleukodystrophy. *World J Biol Chem*. 2020;11(3):99-111.
6. McGuinness MC, Lu JF, Zhang HP, et al. Role of ALDP (ABCD1) and mitochondria in X-linked adrenoleukodystrophy. *Mol Cell Biol*. 2003;23(2):744-753.
7. Kemp S, Wanders R. Biochemical aspects of X-linked adrenoleukodystrophy. *Brain Pathol*. 2010;20(4):831-837.
8. López-Erauskin J, Galino J, Bianchi P, et al. Oxidative stress modulates mitochondrial failure and cyclophilin D function in X-linked adrenoleukodystrophy. *Brain*. 2012;135(Pt 12):3584-3598.
9. Baarine M, Ragot K, Athias A, et al. Incidence of Abcd1 level on the induction of cell death and organelle dysfunctions triggered by very long chain fatty acids and TNF-α on oligodendrocytes and astrocytes. *Neurotoxicology*. 2012;33(2):212-228.
10. Fourcade S, Ferrer I, Pujol A. Oxidative stress, mitochondrial and proteostasis malfunction in adrenoleukodystrophy: a paradigm for axonal degeneration. *Free Radic Biol Med*. 2015;88(Pt A):18-29.
11. Engelen M, Kemp S, de Visser M, et al. X-linked adrenoleukodystrophy (X-ALD): clinical presentation and guidelines for diagnosis, follow-up and management. *Orphanet J Rare Dis*. 2012;7(1):51.
12. Zhu J, Eichler F, Biffi A, Duncan CN, Williams DA, Majzoub JA. The Changing Face of Adrenoleukodystrophy. Endocr Rev. 2020;41:(4):577-593. https://doi.org/10.1210/endrev/bnaa013
13. Moser AB, Jones RO, Hubbard WC, et al. Newborn screening for X-linked adrenoleukodystrophy. *Int J Neonatal Screen*. 2016;2(4):15.
14. Wiens K, Berry SA, Choi H, et al. A report on state-wide implementation of newborn screening for X-linked adrenoleukodystrophy. *Am J Med Genet a*. 2019;179(7):1205-1213.
15. Miller WP, Rothman SM, Nascene D, et al. Outcomes after allogeneic hematopoietic cell transplantation for childhood cerebral adrenoleukodystrophy: the largest single-institution cohort report. *Blood*. 2011;118(7):1971-1978.
16. Bezman L, Moser AB, Raymond GV, et al. Adrenoleukodystrophy: incidence, new mutation rate, and results of extended family screening. *Ann Neurol*. 2001;49(4):512-517.
17. Wiesinger C, Eichler FS, Berger J. The genetic landscape of X-linked adrenoleukodystrophy: inheritance, mutations, modifier genes, and diagnosis. *Appl Clin Genet*. 2015;8:109-121.
18. Aubourg P. X-linked adrenoleukodystrophy. *Ann Endocrinol (Paris)*. 2007;68(6):403-411.
19. Regelmann MO, Kamboj MK, Miller BS, et al. Adrenoleukodystrophy: guidance for adrenal surveillance in males identified by newborn screen. *J Clin Endocrinol Metab*. 2018;103(11):4324-4331.
20. Mallack EJ, Turk BR, Yan H, et al. MRI surveillance of boys with X-linked adrenoleukodystrophy identified by newborn screening: meta-analysis and consensus guidelines. *J Inherit Metab Dis*. 2021;44(3):728-739.
21. Laureti S, Casucci G, Santeusanio F, Angeletti G, Aubourg P, Brunetti P. X-linked adrenoleukodystrophy is a frequent cause of idiopathic Addison's disease in young adult male patients. *J Clin Endocrinol Metab*. 1996;81(2):470-474.
22. Kemp S, Berger J, Aubourg P. X-linked adrenoleukodystrophy: clinical, metabolic, genetic and pathophysiological aspects. *Biochim Biophys Acta*. 2012;1822(9):1465-1474.

23. Pushpakom S, Iorio F, Eyers PA, et al. Drug repurposing: progress, challenges and recommendations. *Nat Rev Drug Discov*. 2019;18(1):41-58.
24. Xue H, Li J, Xie H, Wang Y. Review of drug repositioning approaches and resources. *Int J Biol Sci*. 2018;14(10):1232-1244.
25. Low ZY, Farouk IA, Lal SK. Drug repositioning: new approaches and future prospects for life-debilitating diseases and the COVID-19 pandemic outbreak. *Viruses*. 2020;12(9):1058.
26. Oprea TI, Bauman JE, Bologa CG, et al. Drug repurposing from an academic perspective. *Drug Discov Today Ther Strateg*. 2011;8(3–4):61-69.
27. Kato S, Moulder SL, Ueno NT, et al. Challenges and perspective of drug repurposing strategies in early phase clinical trials. *Onco Targets Ther*. 2015;2(6):576-580.
28. Roessler HI, Knoers NVAM, van Haelst MM, van Haaften G. Drug repurposing for rare diseases. *Trends Pharmacol Sci*. 2021;42(4):255-267.
29. Krysiak R, Okopien B. Effect of bezafibrate on monocyte cytokine release and systemic inflammation in patients with impaired fasting glucose. *J Clin Pharmacol*. 2011;51(10):1459-1467.
30. Engelen M, Schackmann MJA, Ofman R, et al. Bezafibrate lowers very long-chain fatty acids in X-linked adrenoleukodystrophy fibroblasts by inhibiting fatty acid elongation. *J Inherit Metab Dis*. 2012;35(6):1137-1145.
31. Engelen M, Tran L, Ofman R, et al. Bezafibrate for X-linked adrenoleukodystrophy. *PLoS One*. 2012;7(7):e41013.
32. Singh J, Suhail H, Giri S. Loss of AMP-activated protein kinase induces mitochondrial dysfunction and proinflammatory response in unstimulated Abcd1-knockout mice mixed glial cells. *Mediators Inflamm*. 2015;2015:176983.
33. Singh J, Olle B, Suhail H, Felicella MM, Giri S. Metformin-induced mitochondrial function and ABCD2 up-regulation in X-linked adrenoleukodystrophy involves AMP-activated protein kinase. *J Neurochem*. 2016;138(1):86-100.
34. Viollet B, Guigas B, Garcia NS, Leclerc J, Foretz M, Andreelli F. Cellular and molecular mechanisms of metformin: an overview. *Clin Sci (Lond)*. 2012;122(6):253-270.
35. Morató L, Galino J, Ruiz M, et al. Pioglitazone halts axonal degeneration in a mouse model of X-linked adrenoleukodystrophy. *Brain*. 2013;136(Pt 8):2432-2443.
36. Rodríguez-Pascau L, Vilalta A, Cerrada M, et al. The brain penetrant PPARγ agonist leriglitazone restores multiple altered pathways in models of X-linked adrenoleukodystrophy. *Sci Transl Med*. 2021;13(596):eabc0555.
37. Ullah I, Subbarao RB, Rho GJ. Human mesenchymal stem cells—current trends and future prospective. *Biosci Rep*. 2015;35(2):e00191.
38. Saeedi P, Halabian R, Imani Fooladi AA. A revealing review of mesenchymal stem cells therapy, clinical perspectives and modification strategies. *Stem Cell Investig*. 2019;6:34.
39. Volarevic V, Markovic BS, Gazdic M, et al. Ethical and safety issues of stem cell-based therapy. *Int J Med Sci*. 2018;15(1):36-45.
40. Joyce N, Annett G, Wirthlin L, Olson S, Bauer G, Nolta JA. Mesenchymal stem cells for the treatment of neurodegenerative disease. *Regen Med*. 2010;5(6):933-946.
41. Kühl JS, Kupper J, Baqué H, et al. Potential risks to stable long-term outcome of allogeneic hematopoietic stem cell transplantation for children with cerebral X-linked adrenoleukodystrophy. *JAMA Netw Open*. 2018;1(3):e180769.
42. Connick P, Kolappan M, Crawley C, et al. Autologous mesenchymal stem cells for the treatment of secondary progressive multiple sclerosis: an open-label phase 2a proof-of-concept study. *Lancet Neurol*. 2012;11(2):150-156.
43. Gupta A, Orchard PJ, Miller WP, et al. Failure of intrathecal allogeneic mesenchymal stem cells to halt progressive demyelination in two boys with cerebral adrenoleukodystrophy. *Stem Cells Transl Med*. 2020;9(5):554-558.
44. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. *World Allergy Organ J*. 2012;5(1):9-19.
45. Vargas CR, Wajner M, Sirtori LR, et al. Evidence that oxidative stress is increased in patients with X-linked adrenoleukodystrophy. *Biochim Biophys Acta*. 2004;1688(1):26-32.
46. López-Erauskin J, Fourcade S, Galino J, et al. Antioxidants halt axonal degeneration in a mouse model of X-adrenoleukodystrophy. *Ann Neurol*. 2011;70(1):84-92.
47. Holmay MJ, Terpstra M, Coles LD, et al. N-Acetylcysteine boosts brain and blood glutathione in Gaucher and Parkinson diseases. *Clin Neuropharmacol*. 2013;36(4):103-106.
48. Coles LD, Tuite PJ, Öz G, et al. Repeated-dose oral N-acetylcysteine in Parkinson's disease: pharmacokinetics and effect on brain glutathione and oxidative stress. *J Clin Pharmacol*. 2018;58(2):158-167.
49. Sahasrabudhe SA, Silamongkol T, Park YW, et al. Identifying biological signatures of N-acetylcysteine for non-suicidal self-injury in adolescents and young adults. *J Psychiatr Brain Sci*. 2021;6:e210007.
50. Kartha RV, Terluk M, Kumar T, Zayed H, Doss GP, Cloyd J. Synergistic chaperone activity of N-acetylcysteine and its metabolite L-cysteine in Gaucher disease. *Mol Genet Metab*. 2020;129(2):S84.
51. Kartha RV, Zhou J, Basso L, Schröder H, Orchard PJ, Cloyd J. Mechanisms of antioxidant induction with high-dose N-acetylcysteine in childhood cerebral adrenoleukodystrophy. *CNS Drugs*. 2015;29(12):1041-1047.
52. Tolar J, Orchard PJ, Bjoraker KJ, Ziegler RS, Shapiro EG, Charnas L. N-acetyl-L-cysteine improves outcome of advanced cerebral adrenoleukodystrophy. *Bone Marrow Transplant*. 2007;39(4):211-215.
53. Orchard P, Boelens JJ, Raymond G. Multi-institutional assessments of transplantation for metabolic disorders. *Biol Blood Marrow Transplant*. 2013;19(1 Suppl):S58-S63.
54. Sahasrabudhe SA, Kartha RV, Ng M, et al. Population pharmacokinetic analysis of N-acetylcysteine in pediatric patients with inherited metabolic disorders undergoing hematopoietic stem cell transplant. *J Clin Pharmacol*. 2021. https://doi.org/10.1002/jcph.1943
55. Casasnovas C, Ruiz M, Schlüter A, et al. Biomarker identification, safety, and efficacy of high-dose antioxidants for adrenomyeloneuropathy: a Phase II pilot study. *Neurotherapeutics*. 2019;16(4):1167-1182.
56. Moser HW, Moser AB, Hollandsworth K, Brereton NH, Raymond GV. "Lorenzo's oil" therapy for X-linked adrenoleukodystrophy: rationale and current assessment of efficacy. *J Mol Neurosci*. 2007;33(1):105-113.
57. Sassa T, Wakashima T, Ohno Y, Kihara A. Lorenzo's oil inhibits ELOVL1 and lowers the level of sphingomyelin with a saturated very long-chain fatty acid. *J Lipid Res*. 2014;55(3):524-530.
58. Moser HW, Raymond GV, Lu SE, et al. Follow-up of 89 asymptomatic patients with adrenoleukodystrophy treated with Lorenzo's oil. *Arch Neurol*. 2005;62(7):1073-1080.
59. Kramer JK, Sauer FD, Wolynetz MS, Farnworth ER, Johnston KM. Effects of dietary saturated fat on erucic acid induced myocardial lipidosis in rats. *Lipids*. 1992;27(8):619-623.
60. Ahmed MA, Kartha RV, Brundage RC, et al. A model-based approach to assess the exposure–response relationship of Lorenzo's oil in adrenoleukodystrophy. *Br J Clin Pharmacol*. 2016;81(6):1058-1066.
61. Valianpour F, Selhorst JJM, van Lint LEM, van Gennip AH, Wanders RJA, Kemp S. Analysis of very long-chain fatty acids using electrospray ionization mass spectrometry. *Mol Genet Metab*. 2003;79(3):189-196.
62. Moser HW, Moser AB, Steinberg SJ & Kemp S Diagnosis of ALD. 2019. https://adrenoleukodystrophy.info/clinical-diagnosis/diagnosis-of-ald. Updated March 8, 2019. Accessed October 1, 2021.

63. Moser HW, Borel J. Dietary management of X-linked adrenoleukodystrophy. *Annu Rev Nutr*. 1995;15(1):379-397.
64. Fan Y, Meng HM, Hu GR, Li FL. Biosynthesis of nervonic acid and perspectives for its production by microalgae and other microorganisms. *Appl Microbiol Biotechnol*. 2018;102(7):3027-3035.
65. Rizzo WB, Watkins PA, Phillips MW, Cranin D, Campbell B, Avigan J. Adrenoleukodystrophy: oleic acid lowers fibroblast saturated C22-26 fatty acids. *Neurology*. 1986;36(3):357-361.
66. Strandvik B, Ntoumani E, Lundqvist-Persson C, Sabel KG. Long-chain saturated and monounsaturated fatty acids associate with development of premature infants up to 18 months of age. *Prostaglandins Leukot Essent Fatty Acids*. 2016;107:43-49.
67. Martínez M, Mougan I. Fatty acid composition of human brain phospholipids during normal development. *J Neurochem*. 1998;71(6):2528-2533.
68. Sargent JR, Coupland K, Wilson R. Nervonic acid and demyelinating disease. *Med Hypotheses*. 1994;42(4):237-242.
69. Ofman R, Dijkstra IME, van Roermund CWT, et al. The role of ELOVL1 in very long-chain fatty acid homeostasis and X-linked adrenoleukodystrophy. *EMBO Mol Med*. 2010;2(3):90-97.
70. Taylor DC, Francis T, Guo Y, et al. Molecular cloning and characterization of a KCS gene from Cardamine graeca and its heterologous expression in Brassica oilseeds to engineer high nervonic acid oils for potential medical and industrial use. *Plant Biotechnol J*. 2009;7(9):925-938.
71. Suliman NA, Mat Taib CN, Mohd Moklas MA, Adenan MI, Hidayat Baharuldin MT, Basir R. Establishing natural nootropics: recent molecular enhancement influenced by natural nootropic. *Evid Based Complement Alternat Med*. 2016;2016:4391375.
72. Fourcade S, Ruiz M, Guilera C, et al. Valproic acid induces antioxidant effects in X-linked adrenoleukodystrophy. *Hum Mol Genet*. 2010;19(10):2005-2014.
73. Rosebush PI, Garside S, Levinson AJ, Mazurek MF. The neuropsychiatry of adult-onset adrenoleukodystrophy. *J Neuropsychiatry Clin Neurosci*. 1999;11(3):315-327.
74. Zgorzalewicz-Stachowiak M, Stradomska TJ, Bartkowiak Z, Galas-Zgorzalewicz B. Cerebral childhood and adolescent X-linked adrenoleukodystrophy. Clinical presentation, neurophysiological, neuroimaging and biochemical investigations. *Folia Neuropathol*. 2006;44(4):319-326.
75. Brownstone E, Voigtländer T, Baumhackl U, Finsterer J. Epilepsy in adult X-linked adrenoleucodystrophy due to the deletion c.1415-1416delAG in exon 5 of the ABCD1-gene. *Gene*. 2013;513(1):71-74.
76. Salsano E, Gambini O, Giovagnoli AR, Farina L, Uziel G, Pareyson D. Effectiveness of valproate for the treatment of manic-like behavior in X-linked adrenoleukodystrophy. *Neurol Sci*. 2012;33(5):1197-1199.
77. Marks PA. Discovery and development of SAHA as an anticancer agent. *Oncogene*. 2007;26(9):1351-1356.
78. al-Yacoub N, Fecker LF, Möbs M, et al. Apoptosis induction by SAHA in cutaneous T-cell lymphoma cells is related to downregulation of c-FLIP and enhanced TRAIL signaling. *J Invest Dermatol*. 2012;132(9):2263-2274.
79. Shukla S, Tekwani BL. Histone deacetylases inhibitors in neurodegenerative diseases, neuroprotection and neuronal differentiation. *Front Pharmacol*. 2020;11:537.
80. Singh J, Khan M, Singh I. HDAC inhibitor SAHA normalizes the levels of VLCFAs in human skin fibroblasts from X-ALD patients and downregulates the expression of proinflammatory cytokines in Abcd1/2-silenced mouse astrocytes. *J Lipid Res*. 2011;52(11):2056-2069.
81. Singh J, Khan M, Pujol A, Baarine M, Singh I. Histone deacetylase inhibitor upregulates peroxisomal fatty acid oxidation and inhibits apoptotic cell death in abcd1-deficient glial cells. *PLoS One*. 2013;8(7):e70712.
82. Baarine M, Beeson C, Singh A, Singh I. ABCD1 deletion-induced mitochondrial dysfunction is corrected by SAHA: implication for adrenoleukodystrophy. *J Neurochem*. 2015;133(3):380-396.
83. Zierfuss B, Weinhofer I, Kühl JS, et al. Vorinostat in the acute neuroinflammatory form of X-linked adrenoleukodystrophy. *Ann Clin Transl Neurol*. 2020;7(5):639-652.
84. Nair R, Maseeh A. Vitamin D: the "sunshine" vitamin. *J Pharmacol Pharmacother*. 2012;3(2):118-126.
85. Charoenngam N, Shirvani A, Holick MF. Vitamin D for skeletal and non-skeletal health: what we should know. *J Clin Orthop Trauma*. 2019;10(6):1082-1093.
86. Adrenoleukodystrophy and Vitamin D. 2019. https://adrenoleukodystrophynews.com/adrenoleukodystrophy-and-vitamin-d/. Accessed October 1, 2021.
87. Goldsmith JR. Vitamin D as an immunomodulator: risks with deficiencies and benefits of supplementation. *Healthcare (Basel)*. 2015;3(2):219-232.
88. Van Haren K, Mowry E, Raymond G, Moser A, Steinman L. Vitamin D status as a risk factor for cerebral demyelination in X-linked adrenoleukdystrophy. *Neurology*. 2013;80(7 Supplement):S60.005.
89. Bashaw ED, Huang SM, Coté TR, et al. Clinical pharmacology as a cornerstone of orphan drug development. *Nat Rev Drug Discov*. 2011;10(11):795-796.
90. van Ballegoij WJC, Stadt SIW, Huffnagel IC, et al. Plasma NfL and GFAP as biomarkers of spinal cord degeneration in adrenoleukodystrophy. *Ann Clin Transl Neurol*. 2020;7(11):2127-2136.
91. Weinhofer I, Rommer P, Zierfuss B, et al. Neurofilament light chain as a potential biomarker for monitoring neurodegeneration in X-linked adrenoleukodystrophy. *Nat Commun*. 2021;12(1):1816.
92. Augustine EF, Adams HR, Mink JW. Clinical trials in rare disease: challenges and opportunities. *J Child Neurol*. 2013;28(9):1142-1150.
93. Fetro C, Scherman D. Drug repurposing in rare diseases: myths and reality. *Therapie*. 2020;75(2):157-160.

How to cite this article: Tieu JH, Sahasrabudhe SA, Orchard PJ, Cloyd JC, Kartha RV. Translational and clinical pharmacology considerations in drug repurposing for X-linked adrenoleukodystrophy—A rare peroxisomal disorder. *Br J Clin Pharmacol*. 2022;88(6):2552-2563. doi:10.1111/bcp.15090