Cognitive Vitality Reports® are reports written by neuroscientists at the Alzheimer's Drug Discovery Foundation (ADDF). These scientific reports include analysis of drugs, drugs-in-development, drug targets, supplements, nutraceuticals, food/drink, non-pharmacologic interventions, and risk factors. Neuroscientists evaluate the potential benefit (or harm) for brain health, as well as for age-related health concerns that can affect brain health (e.g., cardiovascular diseases, cancers, diabetes/metabolic syndrome). In addition, these reports include evaluation of safety data, from clinical trials if available, and from preclinical models.

RT001

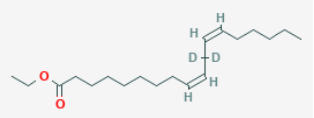
Evidence Summary

RT001 fortifies membranes against oxidative damage. Its potential benefit depends on the contribution of oxidative damage to a given disease, and early intervention is best. It shows good safety, thus far.

Neuroprotective Benefit: RT001 may protect against oxidative stress-related neuron loss, but the degree of benefit is likely dependent on the contribution of oxidative stress to disease progression.

Aging and related health concerns: RT001 may protect cells against age-related oxidative stress damage. Early intervention is likely needed for benefit.

Safety: RT001 has a strong safety profile based on small clinical studies and case reports. Higher doses may impact the gastrointestinal system, and dietary modifications around lipid intake may be necessary.

Availability: In clinical trials	**Dose**: In clinical trials: Loading dose of 2.88 g TID for 1 month then 2.88 g BID	**Chemical formula:** $C_{20}H_{36}O_2$ **MW**: 310.5 g/mol
Half-life: 30.3 ± 18.3 (mean terminal half-life in FA trial)	**BBB**: Penetrant	
Clinical trials: A Phase 1/2 trial in Friedreich's ataxia (n=18) was completed.	**Observational studies**: Case studies have been reported in PSP, ALS, INAD, and Tay-Sachs.	 Source: PubChem

What is it?

RT001 is an orally bioavailable synthetic deuterated polyunsaturated fatty acid (PUFA). It is a di-deuterated linoleic acid ethyl ester, which is an isotopologue of the essential omega-6 PUFA, linoleic acid. It is being developed by Retrotope Inc.

Deuterium (^2H) is a naturally occurring isotope of hydrogen, sometimes called 'heavy hydrogen'. Traditional hydrogen (^1H) contains only one proton, while deuterium contains one proton plus one neutron, resulting in a higher atomic mass [1]. A small percentage of hydrogens in the human body contain deuterium, and thus is not classified by the immune system as foreign. Due to its heavier atomic mass, the replacement of a hydrogen atom with a deuterium influences the rate of chemical reactions in a manner known as the kinetic isotope effect. The rate of the chemical reaction is slowed by the presence of the deuterium. Deuteration is commonly used to influence the pharmacokinetics/therapy of drugs. RT001's mechanism of action is based on this effect.

PUFAs are major components of membranes, especially synaptic and mitochondrial membranes [2]. Reactive oxygen species (ROS) generated by oxidative stress attacks PUFAs by oxidizing them. The oxidization alters the structure of the PUFAs and can disrupt membrane structure making membranes more fluid and less stable [1]. It can also disrupt the ability of essential membrane-localized proteins to embed into the membrane, and lead to cellular dysfunction. Additionally, these oxidized lipids can lead to the generation of reactive aldehydes, which go on to form adducts on proteins which disrupts their function. These reactive species include isoprostanes, 4-HNE, and 4-HHE [2]. The rate limiting step in the peroxidation of PUFAs is the abstraction of hydrogen at bis-allylic sites. Exchanging a hydrogen for a deuterium can reduce the peroxidation rate constant approximately ten-fold [3].

Many of these PUFAs are considered essential, meaning that they cannot be synthesized by the body and need to be obtained from dietary sources. Linoleic acid is an essential omega-6 PUFA, which serves as a precursor for arachidonic acid and for eicosanoids, which play important roles in inflammation. As a di-deuterated form of linoleic acid, consumption of RT001 results in the incorporation of deuterated linoleic acid, as well as its PUFA byproducts, into membranes in place of traditional non-deuterated linoleic acid. These deuterated PUFA are resistant to lipid peroxidation, and their inclusion into the membrane helps to fortify the membrane against oxidative stress damage. Preclinical studies suggest that approximately 20% of the linoleic acid in the membrane needs to be deuterated in order to effectively inhibit lipid peroxidation [4].

The PUFA membrane composition differs in different tissues of the body, with the CNS having the most distinctive profile [5]. For example, the omega-3 PUFA docosahexaenoic acid (DHA) (>25%) is far more abundant than arachidonic acid or linoleic acid in the brain [5; 6]. Preclinical studies have been conducted testing different deuterated PUFAs, and multiple types appear to protect against lipid peroxidation [7]. One study found that deuterated DHA had a stronger than expected effect, suggesting that deuterated DHA or other PUFAs may preferentially benefit particular indications depending on the tissues affected [7]. Indeed, Retrotope is also developing a stabilized DHA for ocular indications, as DHA is the dominant PUFA in the retina (RT011).

RT001 has been tested and is being developed for indications where lipid peroxidation is considered to be a primary driver of disease progression. At this point, it has primarily been tested in conditions involving neurodegeneration. These include Friedreich's ataxia and infantile neuroaxonal dystrophy, for which it has fast-track status, as well as amyotrophic lateral sclerosis and progressive supranuclear palsy.

Neuroprotective Benefit: RT001 may protect against oxidative stress-related neuron loss, but the degree of benefit is likely dependent on the contribution of oxidative stress to disease progression.

Types of evidence:

- 4 case studies/series
- Numerous laboratory studies

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function:

Several studies have shown that lipid oxidation is an early event in the course of dementia, such that levels of lipid oxidation markers, including F2-isoprostanes, are increased in the brain and cerebrospinal fluid (CSF) of patients with early mild cognitive impairment (MCI), suggesting that this form of oxidative damage may play a role in the pathogenesis of cognitive decline [8; 9; 10]. It has not yet been established whether interventions designed to fortify membranes against oxidative stress damage, such as RT001, can prevent or slow cognitive decline in a clinically meaningful manner.

Human research to suggest benefits to patients with dementia:

Progressive Supranuclear Palsy (PSP): POTENTIAL BENEFIT IN A SUBSET

PSP is a neurological disease involving motor and cognitive impairments. Disease course is associated with the accumulation of tau in the cerebral cortex. Rates of lipid peroxidation are also increased, and may play a role.

In patient-derived mesenchymal stem cells (MSCs), rates of lipid peroxidation were found to be elevated in PSP patients relative to controls (161.8 ± 8.2% of control; $p < 0.001$) [11]. Additional detrimental changes to mitochondrial structure and function were also observed in MSCs from PSP patients including, increased levels of mitochondrial reactive oxygen species (ROS), reduced levels of the endogenous antioxidant glutathione, reduced levels of mitochondrial DNA, as well as altered membrane potential, mitochondrial size and shape. Treatment of the MSCs with RT001 in cell culture, ameliorated these mitochondrial defects.

A proof-of-principle study including three patients with PSP was conducted in which the participants were treated with orally administered RT001 2.88 g BID for at least 12 months [11]. The mean plasma levels of deuterated linoleic acid were 19%, while red blood cell membrane levels were 21% of total linoleic acid levels. These levels are consistent with the level (approximately 20%) of D-PUFA incorporation determined necessary for therapeutic effects in preclinical studies [4]. Compared to natural history averages, the slope of disease progression on the PSP Rating Scale (PSP-RS) and Unified Parkinson's Disease Rating Scale (UPDRS) was altered in a manner consistent with a slowing of progression [11]. During the first 12 months, the mean slope on the PSP-RS was +0.04 ± 0.11 points/month relative to +0.91 points/month, while the mean slope on the UPDRS was −0.79 ±−0.005 points/month, relative to +0.95 points/month [12]. By 27 months, the slope on the PSP-RS was 0.16± 0.23, and 0.28 ± 0.44 on the UPDRS, suggestive of a durable response [11]. This was consistent with

symptomatic improvements reported by the patients and caregivers. The patient-level data revealed that this effect was driven by significant disease stabilization in two of the patients, while one patient continued to decline, but at a slightly slower rate than natural history controls. It should be noted that the patient with the lackluster response experienced an unrelated serious adverse event (transient ischemic attack) during the course of the study, and increased the dose of RT001 to 2.88 g TID due to lack of response.

This suggests that RT001 may promote disease stabilization in a subset of patients, and that yet to be determined patient-specific factors may play a role in the degree of treatment responsiveness. A Phase 2 placebo-controlled RCT testing 8.64 g/d (three 960 mg capsules TID) for 1 month followed by 5.76 g/d (three capsules BID) for an additional 11 months is currently underway in this population (NCT04937530).

Alzheimer's disease (AD): UNCLEAR BENEFIT
RT001 is available to patients with certain neurodegenerative diseases as part of an expanded access program. While the details are lacking, an analysis of preclinical and clinical evidence to date indicated that RT001 had been used by a patient with ApoE4+ AD as part of this program and that the clinical response was deemed to be positive based on initial results, but further testing is ongoing [13]. Additionally, it was noted, though no data was provided, that clinical benefit was not seen in non-ApoE4-associated AD.

Mechanisms of action for neuroprotection identified from laboratory and clinical research:

The primary mechanism by which RT001 is expected to protect neurons is via the inhibition of lipid peroxidation, and associated oxidative damage [2]. It may also reduce the pro-oxidant activity of endogenous membrane-associated antioxidants, such as tocopherol (vitamin E) [3]. Additionally, preclinical studies suggest that it may specifically protect against ferroptosis, which is a form of programmed cell death stemming from the loss of glutathione peroxidase 4 and accumulation of ROS [14].

RT001 has been tested in a variety of preclinical models and in patients as part of proof-of-principle clinical studies or as case studies as part of an expanded access program. The results are summarized in Midei et al., 2021[13].

Alzheimer's disease: POTENTIAL BENEFIT EARLY DEPENDING ON ETIOLOGY (Preclinical)
The impact of deuterated PUFAs in models of AD is dependent on the relevance of oxidative stress on pathophysiology in a given model. As such, the benefits seen in a sporadic model of AD, in which mice lack the enzyme aldehyde dehydrogenase 2 (Aldh2), which is involved in the detoxification of lipid peroxidation-induced reactive aldehydes and carbonyls, were greater than in the APP/PS1 model driven by the overproduction of Aβ [6; 15]. While the levels of lipid peroxidation and associated reactive aldehyde products are elevated in both models, lipid peroxidation-induced damage is the predominant driver of neuronal damage and cognitive impairment in the Aldh2-/- model, whereas lipid peroxidation is only one of several mechanisms by which high levels of Aβ promote neurodegeneration.
In the Aldh2-/- model, mice fed a diet supplemented with D-PUFAs for 18 weeks showed an approximately 55% reduction in F2-isoprostanes, and a 20-25% reduction in prostaglandin F2α, in the hippocampus and cortex [6]. F2-isoprostanes primarily produced via non-enzymatic oxidation pathways, while prostaglandin F2α largely stem from enzymatic oxidation. The incorporation of D-PUFA under this paradigm was around 35%, which is above the previously identified threshold of 10-20% that is necessary to therapeutically inhibit lipid peroxidation. This was sufficient to restore performance on cognitive tests (novel object recognition, Y-maze) to the levels seen in wildtype mice.
In the APP/PS1 model, mice fed a diet enriched with D-PUFA (1% 11,11-D2-Ethyl linoleate:11,11,14,14-D4-ethyl linoleate) for five months starting at four to seven months of age did not show significant reductions in Aβ plaque load or significant improvement on cognitive tasks [15]. This is despite the drug working as intended, based on the significant lowering of F4-neuroprostanes in the cerebral cortex, and F2-isoprostances in the liver. There were significant reductions in hippocampal Aβ40 and Aβ38, and a trend toward reduced Aβ42, supporting the notion that oxidative stress is both upstream and downstream of Aβ. It is possible that the intervention was too late, as significant Aβ accumulation had already occurred by the time the treatment was initiated, suggesting that D-PUFA therapy may need to be administered very early in the disease course to offer any clinical benefit.
These findings support the therapeutic concept of RT001, that D-PUFAs can effectively inhibit lipid peroxidation-mediated pathology. Therefore, the potential efficacy of D-PUFAs depends on the relative contribution of lipid peroxidation to a given condition, and suggests that, in most cases, it will need to be part of a combination therapy approach.

Parkinson's disease (PD): POTENTIAL BENEFIT EARLY (Preclinical)
Oxidative stress is implicated as one of the main drivers of neurodegeneration in PD. In a rat model of PD involving the overexpression of human mutant alpha synuclein (A53T) in the substantia nigra, dietary supplementation of D-PUFAs (0.8% D-linoleic and 0.3% H-linolenic) for 12 weeks prevented the loss of

dopaminergic (TH+) neurons [2]. This was accompanied by the preservation of motor function, synaptic proteins, mitochondrial dynamics, and axon transport. The benefits were attributed to the mitigation of α-synuclein-driven oxidative stress, as evidenced by a decrease in levels of the oxidative stress markers, 8-IsoP and PGF2α in the brains of these animals. Notably, unlike other common models of PD, such as those using mitochondrial toxins or mutations in mitochondria-associated proteins, oxidative stress is not necessarily considered the primary driver of pathology in α-synuclein models, thus efficacy here bodes well for the potential translatability of these findings to other models and patients. However, the timing of the intervention (i.e. coincident with the onset of the damage-inducing stressor) may have played a role in the efficacy seen in this model, thus more studies are needed to determine whether the therapeutic efficacy is influenced by the stage of disease.

Huntington's disease (HD): POTENTIAL BENEFIT ON COGNITION (Preclinical)
Markers of lipid peroxidation are elevated in patients with HD, and this phenotype is recapitulated in various animal models of HD [16]. In the Q140 knock-in mouse model, mice fed a diet containing 1.2% D-PUFAs (11,11-D2 linoleic acid ethyl ester) for five months starting at one month of age, showed improved performance on cognitive tests (novel object recognition) [16]. The effect on cognition was associated with a reduction in brain levels of lipid peroxidation, as evidenced by a 75% reduction in F2-isoprostanes in the striatum. Declines in motor function were not affected by the treatment, suggesting that lipid peroxidation is a primary driver of cognitive, but not motor deficits, in this model. It remains to be established whether oxidative stress damage plays a similar role in patients.

Amyotrophic lateral sclerosis (ALS): UNCLEAR BENEFIT
RT001 has been available to patients with ALS as a part of an expanded access program. According to a summary from 2021, 23 patients had been treated with RT001 through this program, and benefits were seen on a validated ALS rating scale relative to natural history controls, though no further details on the response rate or magnitude were provided [13]. A report from ALSUntangled found information regarding one of these cases [17]. Prior to taking RT001 the patient had rapidly declined on the ALSFRS-R (30 to 15), with a forced vital capacity at 60% of predicted. After four months on RT001, the patient showed an improvement in speech and increase of forced vital capacity to 73% of predicted. Since these modest effects could be related to the natural disease course, it is unclear whether the improvements are attributable to RT001. A Phase 2 placebo-controlled RCT testing RT001 in approximately 40 patients with ALS is currently underway (NCT04762589).

Late-Onset Tay Sachs: POTENTIAL MINOR BENEFIT

Tay Sachs is a rare inherited neurodegenerative disorder in which the absence of the enzyme hexosaminidase A results in the toxic accumulation of gangliosides, and thus is a disorder of lipid metabolism. The altered lipid profile may create an environment which fosters lipid peroxidation, and downstream neuronal damage. Although D-PUFAs failed to show benefit in a preclinical model [13], a potentially positive response was seen in a case study where a patient was treated with RT001 (2.7 g BID) [18]. Within one month the plasma levels of deuterated linoleic acid were 36% while red blood cell levels were 28%, indicative of a potentially therapeutic dose. Within one month the patient showed improvement on six activities of daily living, and on nine by three months. Similarly, benefits on the 25-foot walk test (baseline 12.7sec, 30d 12.1 sec, 120d 11.2 sec) and 6-minute walk test (baseline 112M, 30d n/a, 120d 129 M) were also seen.

Infantile neuroaxonal dystrophy (INAD): POTENTIAL MINOR BENEFIT IN A SUBSET

INAD is a rare genetic disorder stemming from mutations in PLA2G6 which leads to progressive declines in cognitive and motor function [19]. PLA2G6 is an A2 phospholipase involved in the breakdown of phospholipids, an integral part of cell membranes [20]. Altered PLA2G6 activity is also implicated in other neurodegenerative disorders, including AD. Loss of PLA2G6 increases the vulnerability of mitochondrial membranes to lipid peroxidation and results in mitochondrial dysfunction. Treatment of fibroblasts derived from patients with PLA2G6 mutations with D-PUFAs (D4-linoleic acid) was able to restore lipid peroxidation to control levels, and reverse some mitochondrial abnormalities [20]. D-PUFA treatment was similarly able to reduce lipid peroxidation and restore mitochondrial membrane potential in a fly model (iPLA2-VIA−/−) [20]. However, treatment had no significant effect on lifespan and only partially impacted motor deficits, suggesting that D-PUFA-based therapy would need to be combined with others targeting additional drivers of disease progression.

This is supported by a case study of two patients with INAD. Although the target of >20% incorporation of deuterated linoleic acid was sustained, there was limited return of developmental milestones and slowing of disease progression [19]. One patient showed improvement on the ability to hold her head upright and grasping of small objects at six months, and improved bulbar function/swallowing, and facial tracking at 12 months. These have been maintained for up to 34 months, but no additional improvements were seen with continued treatment. The second patient showed improvements in alertness, participation, vocalization, and fine motor control at one month, step initiation, reaching and engagement at three months. However, the parents reported motor skill decline at six months and discontinued treatment. Disease severity at time of treatment initiation may play a role in the potential for benefit.

A Phase 2/3 open label clinical trial is still active testing RT001 in INAD (NCT03570931), however, the study failed to meet its primary endpoint based on the modified Ashworth Spasticity Scale. There was a statistically significant 82.5% reduction in morbidity risk, and an 88.8% reduction in mortality risk relative to natural history controls (Press Release). Despite not meeting the primary endpoint, Retrotope plans to bring forward this data to the FDA to determine next steps.

APOE4 interactions: Anecdotal evidence lacking details suggests that RT001 preferentially benefit individuals with ApoE4 [13]. This may be related to the altered lipid profile and higher levels of lipid peroxidation found in ApoE4 carriers [21].

Aging and related health concerns: RT001 may protect cells against age-related oxidative stress damage. Early intervention is likely needed for benefit.

Types of evidence:

- 1 comparator-controlled clinical trial in Friedreich's ataxia
- Numerous laboratory studies

Lifespan: EXTENDED LIFSPAN IN WORMS (Preclinical)
In yeast, the presence of heavy isotopes was found to decrease with age, and supplementation with heavy isotopes, such as deuterium, was able to reduce levels of oxidative stress and extend lifespan in yeast [22]. Due to their ability to influence the rate of reactions, the presence of these isotopes is thought to primarily affect metabolism, and can influence cell growth. Very high doses of deuterium can be toxic, suggesting that there is an optimal level of heavy isotope incorporation in the body to promote healthy aging. More work is needed to understand the biological role of heavy isotopes/deuterium.

In a *C. elegans* model of oxidative stress vulnerability, in which the worms lack omega-3 fatty acid desaturase fat-1, an enzyme required for the synthesis of omega-3 fatty acids that is normally found in worms, but lacking in humans, deuterated PUFAs [D-TG(54:9)] protected against oxidative stress [23]. Treatment with D-PUFA reduced the accumulation of lipid peroxidation and ROS. While treatment with an antioxidant (BHT) had no impact on lifespan, treatment with D-PUFA extended lifespan of worms compared to those supplemented with non-deuterated PUFAs (trilinolenin) (~35 days vs 30 days). Under conditions of paraquat-induced oxidative stress, treatment with D-PUFA also extended lifespan (30 days) relative to those supplemented with non-deuterated PUFAs and exposed to paraquat (~25 days), or

those exposed to paraquat without PUFA supplementation (~23 days). This suggests that the incorporation of D-PUFAs may help protect membranes against mechanisms of oxidative stress which can accelerate mortality. It remains to be established whether a comparable level of protection could be achieved from this approach in humans.

Friedreich's ataxia: POTENTIAL MINOR BENEFIT
Friedreich's ataxia is a genetic disorder involving progressive ataxia, scoliosis, sensory loss, and hypertrophic cardiomyopathy [24]. It involves elevated levels of oxidative stress and mitochondrial dysfunction due to iron dysregulation. Due to its protective effects against lipid peroxidation and ferroptosis in preclinical models, RT001 was hypothesized to have clinical utility in Friedrich's ataxia. RT001 (1.8 or 9.0 g/day) for 28 days was tested against a matching dose of nondeuterated ethyl linoleate in a Phase 1/2 double-blind, comparator-controlled trial in 18 patients with Friedreich's ataxia (NCT02445794) [24]. The percentage of D2-linoleic acid incorporated into red blood cell membranes was lower than the anticipated therapeutic range with the 1.8 g dose (6.52%), but was within range for the 9 g dose (25.8%). RT001 treated patients showed a significant improvement in peak work (0.16 watts/kg) representing a 25.7% improvement relative to the comparator group (P=0.008). Treatment with RT001 was also associated with non-statistically significant improvements on peak oxygen consumption (VO_2max) with a 17.5% change relative to the comparator (0.14 vs -0.02 L/min, P = 0.116), and the FARS-Neurol assessment (-4.75 vs -2.80 FARS points, P = 0.348). There was no clear effect on the timed 25-foot walk test.
Although these effects are modest, the sign of any potential benefit from this study is encouraging given the short duration and suboptimal dosing. RT001 has been granted a fast-track designation for development in Friedreich's ataxia. A Phase 2/3 double-blind, placebo-controlled RCT testing RT001 (2.88 g TID for one month then 2.88 g BID for 11 months) in 65 patients with Friedreich's ataxia (NCT04102501) was completed in late 2021, but results have not yet been released. The primary outcome was change in maximum consumption of oxygen (mlO2/kg/Min) using cardiopulmonary exercise testing.

Atherosclerosis: POTENTIAL BENEFIT EARLY (Preclinical)
Reactive carbonyl species contribute to the pathogenesis of atherosclerosis, as uptake of oxidized LDL particles modified by reactive aldehydes promotes the conversion of macrophages to foam cells [25]. Markers of lipid peroxidation, including F2-isoprostanes and 8-iso-prostaglandin F2α (8-iso-PGF2α) were found to be increased in patients with coronary heart disease by nearly two-fold, suggesting a role for oxidative stress [26].

D-PUFA supplementation (1.2% ethyl 11,11-D2-linoleate and ethyl-11,11,14,14-D4-linolenate in a 1:1 weight ratio) in the context of a Western diet (0.15% cholesterol) in the APOE*3-Leiden.CETP mouse model of hyperlipidemia reduced atherosclerotic lesion area formation throughout the aortic root (−26%) [25]. Treatment reduced body weight gain (up to -54%), especially body fat mass gain (up to -87%) without altered lean mass or food intake. Under hypercholesteremic conditions, D-PUFA supplementation reduced total cholesterol plasma levels (ranging from −20% to −37%; −4.6 mM), which was driven by a reduction in non-HDL-cholesterol (−28%; −4.0 mM). Hepatic cholesterol content (−21%), and sterol markers of intestinal cholesterol absorption were also reduced. These changes were accompanied, and presumed to be driven by reductions in F2-isoprostanes and prostaglandin F2α in the liver (-72% and -44%, respectively) and plasma (-87% and -40%, respectively). The impact and relative effect on each of these markers suggests that D-PUFAs primarily target non-enzymatic lipid peroxidation, but that enzymatic lipid peroxidation may also be affected, to a lesser extent [26].

Lung injury: POTENTIAL MINOR BENEFIT AS PRETREATMENT (Preclinical)
In a mouse model of acute lung injury (intranasal LPS), a diet supplemented with D-PUFA (0.25% bis-allylic hexadeuterated arachidonic acid), significantly reduced interalveolar septal thickness, indicative of decreased lung damage [5]. Dietary consumption of PUFAs can modify pulmonary surfactant composition, and can influence levels of lipid-derived inflammatory products. The effect was impacted by sex effects, which are known to contribute to sex differences in diseases with pulmonary pathology. This preclinical study was undertaken to determine whether RT001 might be a candidate for mitigating Covid-19 related lung inflammation. Considering it is likely that pre-treatment would most likely be needed to significantly impact the lung surfactant and inflammatory profile, the clinical utility of this approach during an active infection is unclear.

Safety: RT001 has a strong safety profile based on small clinical studies and case reports. Higher doses may impact the gastrointestinal system, and dietary modifications around lipid intake may be necessary.

Types of evidence:

- 1 reviews of clinical use of RT001
- 1 clinical trial
- 4 case studies observational studies
- Numerous laboratory studies

RT001 has shown an excellent safety profile in the clinical and preclinical studies conducted thus far. An analysis including 59 patients with 10 different neurodegenerative conditions found that chronic dosing of RT001 up to 36 months was not associated with any use limiting drug-related adverse events [27].

In patients with Friedreich's ataxia treated with up to 9 mg/day for 28 days, RT001 was safe and well-tolerated [24]. One patient with a low body mass experienced steatorrhea, or fatty stool, which may be a sign of fat malabsorption while taking the highest dose, and discontinued the study. The only treatment emergent adverse event which occurred in more than one subject was diarrhea. Based on this study, 9 mg/day was considered the likely maximum tolerated dose, and future studies split the dose to be administered two or three times per day.

In patients administered RT001 as part of the expanded use program, RT001 was generally well-tolerated with only mild to moderate adverse events. The only serious adverse event, a transient ischemic attack, occurred in one patient with PSP [11]. This event is not thought to be RT001-related, but rather an adverse reaction to either amantadine or metformin, which the patient was also taking at the time of the event. In the majority of participants, no toxicities or treatment-related adverse events have been observed [13]. Deuterated lipids have been used to track the transfer of dietary lipids through breast milk, and were not associated with any adverse effects [28].

Preclinical studies testing D-PUFAs also indicate the absence of any overt toxicities [5; 16; 25]. The strong safety profile may be related to doses used in these studies. One study in drosophila found that very high doses of D-PUFA (D2-linoleic acid) reduced the lifespan of mutant flies with increased vulnerability to oxidative stress [20]. PUFAs act as the substrates for a variety of enzymes, and these downstream products exert a plethora of important signaling and other functions in the cell. Since the deuteration of PUFAs slows the rate of these enzymatic reactions, if the levels of D-PUFA are too high, there could be a negative impact on essential cellular processes. The 20% level identified in preclinical studies appears to be the therapeutically optimal level, as it effectively slows lipid peroxidation cascades without significantly impacting essential enzymatic functions [4]. Additionally, the deuterium substitution did not alter the structure of the PUFAs, such that they are able to normally distribute into the membrane lipid bilayer, and allow for the normal distribution of membrane-associated proteins [1]. While safe at low levels, extremely high levels of deuterium can be toxic to organisms, in a species-specific manner. In mammals, deuterium levels reaching greater than 20% of total body water is considered toxic [22]. However, the change in body water deuterium level following therapeutic dosing

of RT001 in clinical studies is estimated to be negligible, and within the range of natural fluctuations (from 132 to 149 ppm), and orders of magnitude lower than a toxic level (150,000 ppm) [27].

Drug interactions: Interactions with RT001 have not been established, but they may be expected to be similar to those for linoleic acid supplements (WebMD). The use of other PUFA-containing drugs/supplements is contraindicated in the clinical trials testing RT001, similarly the protocols indicate that dietary restrictions are also necessary regarding the consumption of linoleic acid/PUFA from dietary sources (i.e. following the monounsaturated fat-rich Mediterranean diet) [27]. These are likely to increase the relative pool of deuterated PUFAs (linoleic acid) relative to the total pool. It is unclear whether use of RT001 with other PUFA-containing therapies would increase the risk for adverse effects, or just reduce the potential efficacy of RT001.

Sources and dosing:

RT001 is being developed by Retrotope Inc. For neurological disorders, it is primarily dosed at 2.88 g TID as a loading dose for one month, followed by a maintenance dose of 2.88 g BID in the form of oral tablets. Therapeutic dosing is expected to require the replacement of 20% of the total linoleic acid with the deuterated form, as measured in the plasma or on red blood cells.

Research underway:

According to Clinicaltrials.gov, there are currently three active clinical trials for RT001. There is a Phase 2 RCT testing RT001 in PSP (NCT04937530). There is a Phase 2 RCT testing RT001 in ALS (NCT04762589). There is a Phase 2/3 open-label study testing RT001 in Infantile Neuroaxonal Dystrophy (NCT03570931).

Search terms:
Pubmed, Google: RT001, D-PUFA

- Alzheimer's disease, neurodegeneration, aging, cardiovascular, clinical trials, safety

Websites visited for RT001:

- Clinicaltrials.gov
- Examine.com (Conjugated linoleic acid)
- WebMD.com (Conjugated linoleic acid)
- PubChem

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