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.

Cyclophilin Inhibitors

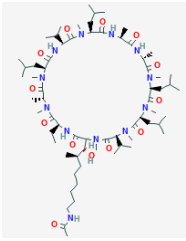
Evidence Summary

Cyclophilin inhibitors exhibit anti-viral and anti-fibrotic activity, and may reduce cell loss in response to oxidative stress. They are generally safe as monotherapy, but future clinical development is unclear.

Neuroprotective Benefit: Elevations in cyclophilin D are observed with brain aging and dementia such that inhibition may be neuroprotective. However, the development of mitochondrial-targeted cyclophilin D inhibitors for the CNS is technically challenging.

Aging and related health concerns: Cyclophilin inhibitors have anti-fibrotic effects and liver accumulating inhibitors may have the most utility in preventing liver cancer in those with fatty liver disease and/or hepatitis.

Safety: Cyclophilin inhibitors have generally been safe as monotherapy, but have the potential to increase levels of blood lipids and elevate blood pressure. They increase the risk for serious side effects related to interferon therapy when used in combination.

Availability: In clinical trials	**Dose**: Not established Clinically tested in oral formulations	**Rencofilstat** **Chemical formula:** $C_{67}H_{122}N_{12}O_{13}$ **MW**: 1303.8 g/mol  Source: PubChem
Half-life: Rencofilstat: 33.7 ± 11.1 hours Alisporivir: 60-90 hours (in plasma) NIM811: <48 hours SCY-635: 24-43 hours (in monkeys)	**BBB**: Varies, generally low/modest penetrance with preferential accumulation in the liver	
Clinical trials: Alisporivir was tested in Phase 1, 2, 3 (n=1,081) trials for hepatitis C. NIM811 was tested in a Phase 2a trial for hepatitis C (n=75). SCY-635 was tested in Phase 1 trials (n=37; n=20) for hepatitis C. Rencofilstat was tested in Phase 1 trials in healthy volunteers, and in a Phase 2a (n=49) trial for F2/F3 NASH.	**Observational studies**: Elevations in cyclophilin D have been observed in the brain with aging, diabetes, and dementia. Elevations in cyclophilins are associated with worse prognosis in liver cancer.	

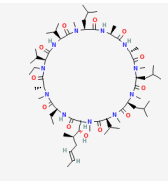
What is it?

Cyclophilins belong to the family of peptidyl-prolyl-cis-trans isomerases, which catalyze the cis-trans isomerization of peptide bonds at proline residues [1]. This isomerization event influences protein folding, which provides cyclophilins the capacity to modulate the structure and function of a wide array of proteins. There are 17 known cyclophilins in humans, which are localized to different cellular compartments, such as the cytosol, nucleus, endoplasmic reticulum, and mitochondria. Due to high homology, cyclophilin inhibitors generally inhibit multiple cyclophilin isoforms, and are typically considered pan-cyclophilin inhibitors [2]. The first described cyclophilin inhibitor is cyclosporine, which is primarily used as an immunosuppressant agent in the context of organ transplantation. In addition to acting as a cyclophilin inhibitor, cyclosporine also acts as a calcineurin inhibitor, which confers its immunosuppressive properties [2]. Therefore, this report will generally not discuss cyclosporine, but instead will focus on the more recently developed class of non-immunosuppressive cyclophilin inhibitors. These drugs tend to accumulate in the liver and were generally tested for liver-related indications. The first group, alisporivir, NIM811, and SCY-635 were developed as anti-viral agents, and tested for hepatitis C. Their clinical development for this indication was subsequently halted due to the

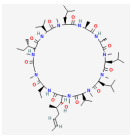
increased risk for serious side effects when combined with interferon therapy. In addition to hepatitis, cyclophilin inhibitors have been shown to have anti-viral activity against a variety of other viruses in preclinical studies, primarily *in vitro* assays. Cyclophilin inhibitors have also shown anti-fibrotic effects in preclinical models of liver disease, and one, rencofilstat, has been clinically tested for this indication. The future clinical development of this class of drugs is currently unclear.

Four cyclophilin inhibitors have been tested in clinical trials, to date.

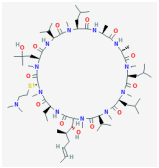
Rencofilstat, originally called CRV431, is a cyclophilin inhibitor developed by Hepion Pharmaceuticals, formerly called Contravir Pharmaceuticals. It inhibits cyclophilins with an IC_{50} range of 2.5–7.3 nM [3]. It has been tested in Phase 1 trials in healthy volunteers as well as a Phase 2a study in patients with fibrotic liver disease (F2/F3 NASH) [1]. An ongoing Phase 2b trial in the same population is winding down early due to limited resources by Hepion (Press release). The future clinical status of rencofilstat is unclear. Hepion is in discussions for a proposed merger with Pharma Two B Ltd (Press release).



Alisporivir is a cyclophilin inhibitor that was in clinical development by DebioPharm for hepatitis C. It is an analog of cyclosporine [2]. It was tested in Phase 1, 2, and 3 trials for this indication, but development was halted due to the increased risk for serious adverse events when used in combination with interferon therapy [4]. It was also clinically tested in patients with covid-19. (Formula $C_{63}H_{113}N_{11}O_{12}$; Molecular weight 1216.6 g/mol; Source: PubChem)



NIM811 is a cyclophilin inhibitor that was in clinical development by Novartis for hepatitis C, and was tested in a Phase 2a study [5]. It is an analog of cyclosporine isolated from the fungus *Tolypocladiumniveurn* [2]. (Formula $C_{62}H_{111}N_{11}O_{12}$; Molecular weight 1202.6 g/mol; Source: PubChem)



SCY-635 is a cyclophilin inhibitor that was in clinical development by Scynexis for hepatitis C, and was tested in Phase 1 studies [6]. It inhibits cyclophilins in the low nanomolar range, and unlike most of the other tested cyclophilin inhibitors, it is not a substrate for the major cytochrome P540 enzymes, suggesting it has less potential for drug interactions [2]. (Formula $C_{66}H_{120}N_{12}O_{13}S$; Molecular weight 1321.8 g/mol; Source PubChem).

Neuroprotective Benefit: Elevations in cyclophilin D are observed with brain aging and dementia such that inhibition may be neuroprotective. However, the development of mitochondrial-targeted cyclophilin D inhibitors for the CNS is technically challenging.

Types of evidence:

- 2 postmortem brain tissue studies examining cyclophilin D levels
- 2 biomarker studies assessing cyclophilin A levels in dementia
- 1 Mendelian randomization study examining link between cyclophilin A and dementia
- Numerous laboratory studies

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

Cyclophilin inhibitors have not been tested for cognition, but there is evidence to suggest that cyclophilin activity may be altered in the context of aging, and that these changes are exacerbated in the context of dementia and diabetes, suggesting that they may contribute to cognitive decline. Cyclophilin D is located within the mitochondrial matrix, and plays a role in regulating the sensitivity of the mitochondrial permeability transition pore (mPTP) to changes in calcium levels [7]. Prolonged activation of the mPTP can result in the inhibition of mitochondrial energy production (oxidative phosphorylation), mitochondrial swelling and rupture, and ultimately cell death. Consequently, by influencing the sensitivity of the mPTP, cyclophilin D can regulate cellular energy status as well as the vulnerability to cell death in the context of cellular stress. Levels of cyclophilin D are typically kept much lower in the brain relative to other organs, such as the heart and liver in which cyclophilin D levels are around three times higher than the brain [7].
Studies using postmortem brain tissue suggest that brain levels of cyclophilin D increase with age, which may impact energy production and neuronal survival. Cyclophilin D levels in temporal cortical mitochondria were found to be 1.5-fold higher in the brain tissue from elderly individuals (age: 81.4 ± 3.06, n = 9) relative to young adults (age: 33.75 ± 2.69, n=4) [8]. Cyclophilin D levels were further increased in the temporal cortex of patients with Alzheimer's disease (AD) (age: 85.8 ± 1.23, n =12), such that they were around three-fold higher than in young adults. The increase was restricted to disease affected brain regions, as levels were 2 to 2.5-fold higher in AD patients relative to age-matched controls in the temporal cortex and hippocampus, but levels were similar in a non-affected brain region, the cerebellum. A separate study (n=14) found that cyclophilin D levels were over three-fold higher in brain tissue from the inferior temporal cortex in postmortem tissue from patients with diabetes relative to

non-diabetics [9]. The elevation in cyclophilin D was inversely correlated with cognitive performance, such that participants with high levels of cyclophilin D tended to have lower scores on the Mini-Mental State Examination (MMSE). Increased interaction of cyclophilin D with ATP synthase may contribute to mitochondrial dysfunction, reduced energy production, and neuronal loss in the brains of diabetics and dementia patients, thereby impairing cognitive function.
To date, however, no drugs have been developed that reliably or selectively target CNS cyclophilin D.

Human research to suggest benefits to patients with dementia:

Cyclophilin inhibitors have not yet been tested in dementia patients. The immunosuppressive cyclophilin inhibitor, cyclosporine A, has been found to impact cognitive function and dementia risk in some studies, but these associations are thought to stem from its activity as a calcineurin inhibitor. Cognition is typically not impacted with short-term use of cyclosporine A [10]. While cognitive decline has been observed with long-term use in some transplant patients [11], other studies indicate little to no effects on cognition [12]. A study including 125,564 adults aged 65 and older found that in propensity-score matched cohorts, treatment with the immunosuppressant calcineurin inhibitors tacrolimus, sirolimus, or cyclosporine, was associated with a lower prevalence of dementia diagnosis, though the effect was weakest with cyclosporine [13]. The modest effects on cognition in either direction for cyclosporine A likely stem from its poor penetration into the CNS.
Effects on cognition have not been observed in clinical trials testing non-immunosuppressant cyclophilin inhibitors for viral and hepatic indications, thus far, though the clinically tested inhibitors primarily accumulate in the liver, with marginal impact on the CNS [14].
The impact of CNS penetrant cyclophilin inhibitors on cognition in those with normal cognition or dementia has not been established.

Biomarker studies suggest that alterations in cyclophilin levels may be associated with dementia. As described above, cyclophilin D levels have been found to be increased in affected brain regions of AD patients [8]. Cyclophilin A has been implicated in the inflammatory status of vascular endothelial cells [15], such that it may impact vessel permeability and barrier integrity. Plasma cyclophilin A levels were found to be significantly higher in patients with vascular dementia (n=27) (median: 36.57 ng/mL, Q25 17.51 to Q75 73.32) relative to patients with AD (n=26) (median: 18.95 ng/mL, Q25 10.46 to Q75 35.05), such that cyclophilin A levels could be used to differentiate the two groups in this study [16]. Levels of healthy controls (n=27) were in-between the two groups (median: 30.78 ng/mL, Q25 21.00 to Q75 49.15). However, the biological relevance of this finding is unclear, as BBB disruption tends to be a

feature of both AD and vascular dementia, and cyclophilin A levels were not associated with cognitive scores. Another study (n=118) found that decreased blood levels of cyclophilin A were associated with the loss of gray matter volume and worse cognitive function in AD patients [17]. A Mendelian randomization and polygenic risk score analysis suggests that the changes in cyclophilin A may be a consequence of disease processes rather than a causal factor, as it did not find an association between cyclophilin A and risk of AD [18].

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

Cyclophilin A: BBB breakdown

Preclinical studies have identified a mechanistic pathway by which elevation of cyclophilin A promotes the breakdown of the BBB, particularly in the context of ApoE4. In the 5XFAD mouse model, mice containing the ApoE4 allele showed evidence of accelerated BBB breakdown, including reduced cerebral blood flow and greater fibrinogen deposits in the cortex and hippocampus, relative to AD mice with the ApoE3 allele [19]. The cyclophilin A-matrix metalloproteinase (MMP)-9 pathway was overactivated in vascular pericytes in the ApoE4 mice, resulting in the degradation of tight junction proteins, and breakdown of the BBB. Treatment of these ApoE4/5XFAD mice with the cyclophilin inhibitor alisporivir (Debio-025) (10 mg/kg i.p.) for 30 days starting at the time when the BBB starts breaking down in this model (10-12 months of age) slowed the destruction of tight junctions, mitigating the pace of BBB damage. The cyclophilin inhibitor did not impact the disease trajectory of the mice related to amyloid pathology, as the treatment restored BBB integrity and cognitive function to the level observed in the ApoE3/5XFAD mice, but the mice were still impaired relative to age-matched control mice. The cyclophilin inhibitor (alisporivir) had no effect on the disease course in the ApoE3/5XFAD mice. Additionally, it is unclear whether the cyclophilin inhibitor would have a clinically meaningful effect if administered at a point later in the disease, when significant BBB breakdown has already occurred. The relevance of this pathway in human AD patients is unclear. CSF studies suggest that levels of cyclophilin A and MMP9 are elevated in AD patients, while some studies in blood, such as described above, indicate a decrease or no change [17; 18]. It is possible that this pathway is most relevant for a subset of AD patients, such as ApoE4 carriers, or ApoE4 homozygotes, in which case these associations may only emerge upon stratified analysis.

Cyclophilin D: Mitochondrial dysfunction

Cyclophilin D is located in the mitochondrial matrix and plays a variety of roles in the maintenance of mitochondrial homeostasis [7]. One of the most critical roles involves the regulation of the mPTP, by

controlling the level of calcium required to open the channel. Brief opening of the channel can help protect against oxidative stress, while prolonged opening of the channel results in mitochondrial dysfunction and cell death. Sustained opening of this non-selective channel leads to osmotic and ionic changes that result in mitochondrial swelling and the collapse of the gradients that allow for energy production. Eventually, this can lead to mitochondrial rupture and cell death. Higher levels of cyclophilin D promote easier channel opening, which can be detrimental in an environment with high levels of oxidative stress. Studies in postmortem brain tissue have found an upregulation of cyclophilin D in conditions associated with heightened oxidative stress, including aging, diabetes, and AD [8; 9].

Preclinical studies in AD models suggest that inhibiting cyclophilin D could be neuroprotective. Cyclophilin D has been shown to interact with Aβ oligomers and form complexes within cortical mitochondria [8]. This interaction was found to promote oxidative stress and accelerate neuronal loss. AD model (mAPP) mice deficient in cyclophilin D were partially protected against Aβ-induced oxidative stress and mitochondrial dysfunction. These mice also had better preservation of synaptic plasticity and cognitive function on spatial memory tasks, relative to mAPP mice expressing cyclophilin D. Aged (18-month-old) tau deficient (tau-/-) mice were found to be protected from age-related cognitive impairment and mitochondrial dysfunction [20]. Notably, they had lower levels of cyclophilin D than age-matched wildtype mice, resulting in higher calcium buffering capacity and a lower degree of mPTP opening. Overexpression of cyclophilin D in the hippocampus reversed this effect, leading to impaired mitochondrial bioenergetics and worse performance on memory and social interaction tests.

The major challenge is in specifically targeting cyclophilin D levels in the brain. As a mitochondrial localized protein, cyclophilin D is more resistant to regulation relative to cytosolic cyclophilins, which may serve as a sink for cyclophilin inhibitors [7]. Additionally, the clinically tested cyclophilin inhibitors to date preferentially localize within the liver. A study testing the cyclophilin inhibitor NIM811 (50 or 100 mg/kg i.p.) starting 12-14 days post induction of MOG_{35-55}-induced experimental allergic encephalitis (EAE) found that although there were protective effects, they were not mediated through the inhibition of cyclophilin D, as NIM811 did not appreciably inhibit cyclophilin D in the CNS with *in vivo* administration [21].
One preclinical study developed a 'nano-brake' to target cyclophilin D in the brains of 5XFAD mice [22]. The nano-brake includes the encapsulation of magnesium ion, which is a natural antagonist of calcium, along with an siRNA to cyclophilin D, and an MMP9 activatable cell-penetrating peptide anchored on the surface of the nano-brake for enhanced delivery across the BBB. Likely due to leakiness of the barrier, uptake of the nano-brake particles into the brain following tail vein injection was higher in 5XFAD mice

relative to wildtype mice. Cyclophilin D levels were reduced in the brains of the treated mice, which was accompanied by an improvement in energy production and performance on cognitive tests. The glutathione peroxidase mimetic, ebselen, was identified as a cyclophilin D inhibitor in a screen, and demonstrates proof-of-principle benefit in AD models [23]. Intraperitoneal administration (2.5 mg/kg) of ebselen achieved 80% inhibition of cyclophilin D activity in the mouse brain, which was accompanied by an improvement in mitochondrial energy production and reduction in oxidative stress in AD mouse models. In the 5XFAD model, treatment for eight weeks starting at six months of age, protected against spatial learning deficits on the Morris water maze. The binding interaction between ebselen and cyclophilin D may serve as a scaffold to develop more potent and selective brain penetrant cyclophilin D inhibitors.
Cyclophilin D plays multifaceted roles in mitochondrial function, thus while inhibiting it may protect against some types of mitochondrial dysfunction, it could lead to the impairment of other aspects, thus the degree of inhibition may need to be carefully balanced [7].

Tau aggregation: Cyclophilins have chaperone-like activity, and were found to inhibit tau aggregation in cellular and *in vitro* assays [24]. Cyclophilins B, C, D, and E were found to reduce both soluble and insoluble tau, while only cyclophilin C was able to protect against tau seeding. It is unclear whether this chaperone activity would be disrupted by the use of cyclophilin inhibitors.

APOE4 interactions: Preclinical studies suggest that cyclophilin A-mediated breakdown of the BBB may be exacerbated in ApoE4 carriers, but this has not yet been confirmed in human patients.

Aging and related health concerns: Cyclophilin inhibitors have anti-fibrotic effects and liver accumulating inhibitors may have the most utility in preventing liver cancer in those with fatty liver disease and/or hepatitis.

Types of evidence:

- 1 review on Phase 1 and 2 clinical trials testing alisporivir
- 1 Phase 3 RCT testing alisporivir in hepatitis C
- 1 Phase 2a RCT for rencofilstat in NASH
- 1 Phase 2a trial for NIM811 in hepatitis C
- 1 Phase 1 trial for SCY-635 in hepatitis C
- Numerous laboratory studies

NAFLD/NASH: POTENTIAL BENEFIT

Non-alcoholic fatty liver disease (NAFLD), now referred to as metabolic dysfunction-associated steatotic liver disease (MASLD) is a metabolic disorder involving the buildup of fat in the liver [25]. Over time it can progress to nonalcoholic steatohepatitis (NASH), now referred to as metabolic dysfunction-associated steatohepatitis (MASH), which is characterized by hepatic inflammation and fibrosis, in addition to the accumulation of fat (steatosis). Numerous drugs are in clinical development for the treatment of NAFLD/NASH, however, most target the accumulation of fat or inflammatory aspects of the disease, and there has been less progress on drugs that can stop or reverse liver fibrosis [26]. Cyclophilin inhibitors have been proposed as therapeutic candidates to mitigate the fibrotic process. Several have shown benefit in preclinical studies, and one, rencofilstat, is being clinically tested for this indication [26].

Cyclophilin B plays a role in collagen production, as it catalyzes a rate-limiting step in type 1 collagen folding, therefore, inhibition of cyclophilin B may help mitigate fibrosis by slowing collagen synthesis [27].

The cyclophilin inhibitor ***rencofilstat*** was tested in a single-blind, placebo-controlled Phase 2a trial in 49 patients with F2/F3 NASH, defined as levels of the liver enzyme aspartate aminotransferase (AST) > 20 IU/L, levels of the collagen fibrogenesis marker Pro-C3 > 15.5 ng/ml or enhanced liver fibrosis (ELF) score > 9.8, and a vibration-controlled transient elastography (VCTE) kPa value > 8.5 kPa (NCT04480710) [1]. Rencofilstat was tested at oral doses of 75 mg and 225 mg per day for 28 days. The primary endpoint was safety, and the study was not powered to address its exploratory efficacy endpoints. There were non-significant trends toward reduced levels of the collagen biomarkers C1M, C6M, and Pro-C8, as well as the downregulation of several collagen genes (COL181, COL6A5, COL7A1, COL8A2) in those treated with rencofilstat. Serum levels of alanine aminotransferase (ALT) were reduced in all groups, but to a greater degree in those treated with rencofilstat (−18.36 ± 25.75%, and −16.31 ± 25.50% for the 75 mg and 225 mg doses, respectively), relative to the corresponding placebo groups (−0.65 ± 13.44%, and −10.24 ± 12.32% respectively). It should be noted that the groups had significantly different levels of ALT at baseline, which may reflect differences in the degree of liver inflammation. Pro-C3 has emerged as a predictor of liver fibrosis progression, with higher levels indicative of more advanced fibrosis. Prior studies suggest that patients with Pro-C3 levels >15.0 ng/ml may be the appropriate clinical population for rencofilstat, and more likely to respond. Consistent with this, participants with Pro-C3 levels ≥ 15.0 ng/ml at baseline were more likely to experience reductions in collagen markers in response to rencofilstat. Based on these results, a Phase 2b trial testing

rencofilstat in patients with NASH and advanced liver fibrosis (F2 or F3) was initiated (NCT05402371). However, due to constraints on resources by the parent company, the trial is winding down early, such that it will not be possible to obtain meaningful efficacy data (Press release).

Rencofilstat, formerly called CRV431, protected against liver fibrosis in rodent models, including a six-week carbon tetrachloride (CCl_4) model and the STAM NASH model, involving the induction of insulin dysfunction via injection with streptozotocin followed by a high-fat diet [3]. In the CCl_4 model, treatment with rencofilstat reduced collagen fiber content by 43%, based on Sirius red staining. In the NASH model, treatment reduced hepatic fibrosis levels by 37%–46%. Notably, benefits were observed during treatment periods starting during early (3–14 weeks), intermediate (8-14 weeks), or late (week 20-30) stages of disease in this model. Significant reductions were also observed in NASH activity scores, which is a composite of liver steatosis, inflammation, and ballooning.
The cyclophilin inhibitor ***NV556*** also showed anti-fibrotic effects in the STAM and methionine-choline-deficient (MCD) models of NASH in male mice [28]. In the MCD model, treatment with NV556 (100 mg/kg for 7 weeks) reduced levels of the liver enzymes ALT and AST, but had no effect on liver weight, cholesterol, triglycerides, or fatty acids. Similarly, in the STAM model, NV5556 decreased liver collagen content, but had no effect on liver weight or triglyceride levels. The anti-fibrotic effect may be mediated by interfering with collagen cross-linking.

Hepatocellular carcinoma: POTENTIAL BENEFIT (Preclinical)
Hepatocellular carcinoma (HCC) is becoming one of the leading causes of cancer mortality in the US. NAFLD/NASH is a significant risk factor for the development of HCC [29]. The mechanisms underlying this relationship are unclear, but are thought to be related to changes in hepatic metabolism, inflammatory signaling, and fibrotic processes, which may promote oncogenic transformation and foster a pro-tumorigenic environment. Hepatitis C (HCV) is another major risk factor for HCC through the induction of pro-oncolytic pathways that drive cell proliferation, inflammation, and fibrosis, as well as through the modification of the epigenome [30].
As a result of their potential to target these two leading risk factors for HCC, cyclophilin inhibitors may be particularly well-suited for the prevention of HCC in populations with NAFLD/NASH and/or HCV. Moreover, preclinical studies suggest that the cyclophilin inhibitors may have additional, direct anti-tumor effects.

The overexpression of cyclophilins is associated with poor prognosis in HCC [31]. The cyclophilin inhibitor NV651 exhibited anti-proliferative effects in the majority of the 31 liver cancer cell lines tested

[31]. The combination of ***NV651*** with the chemotherapeutic cisplatin had a synergistic effect, likely stemming from the combined effect of cisplatin-induced DNA damage, and NV651-mediated inhibition of DNA repair in the cancer cells [31]. NV651 also had an anti-proliferative effect in an HCC xenograft model in nude mice, as evidenced by a reduction in tumor growth [32].

The cyclophilin inhibitor ***NV556*** protected against the development of NASH-driven HCC, and significantly decreased the size and number of the nodules in the mice that developed tumors [33]. Similarly, rencofilstat significantly decreased overall tumor burden (size and number) by 52% in a mouse model of NASH-driven HCC [3]. While the anti-fibrotic effect appears to be driven primarily through the inhibition of cyclophilin B, the anti-tumor effect may be at least partially mediated through the inhibition of cyclophilin D [34]. Cyclophilin D deficient mice largely (by 80%) protected against the development of NASH-driven HCC, with gene expression analysis demonstrated a downregulation of numerous HCC-related genes in these animals [34].
Rencofilstat also showed protection against HCV-induced HCC in humanized mice through effects related to its anti-HCV activity as well as effects independent of its anti-HCV activity [35]. Treatment initiated at the time of infection prevented the development of HCC by inhibiting the replication of HCV [35]. However, mice starting treatment at later stages, up to 16 weeks after HCV infection and HCC had been established, also experienced a reduction in tumor growth through both the slowing of new tumors and modest shrinking of pre-existing tumors. This suggests that rencofilstat may have clinical utility within a therapeutic window that is applicable to patients.

Hepatitis C: POTENTIAL BENEFIT BUT BOTH EFFICACY AND SAFETY RISKS INCREASE WITH COMBINATION THERAPY
Cyclophilin inhibitors were originally tested for their anti-viral properties, and several were clinically tested in patients with hepatitis C (HCV). Cyclophilins are involved in regulating the interaction of HCV with RNA polymerase, such that cyclophilin inhibitors disrupt this interaction [36]. This form of antiviral activity, which targets host proteins rather than viral proteins is considered to be more robust since resistance does not emerge as readily. Cyclophilin inhibitors have also been shown to regulate innate interferon responses, and thus exert a synergistic anti-viral effect when used in combination with interferon therapy. Although the tested inhibitors showed evidence of anti-HCV activity, clinical development was halted after the emergence of potential safety signals when used in combination with pegylated interferon-α2a therapy [4].

Alisporivir (Debio-025) was tested in numerous clinical trials for antiviral activity, including for HCV and HIV, as a monotherapy or combination therapy [37]. The largest study was the randomized, double-blind, placebo-controlled, Phase 3 ESSENTIAL II trial (NCT01318694) including 1,081 treatment-naïve patients with chronic HCV genotype 1 infection [4]. Participants received alisporivir at a dose of 600 mg per day plus with peginterferon-α2a (180 µg/week) and ribavirin (1000 or 1200 mg/day) using response guided therapy based on viral load, alisporivir at a dose 400 mg twice per day in combination with peginterferon-α2a and ribavirin for 24 or 48 weeks, or peginterferon-α2a and ribavirin alone for 48 weeks. The trial was put on a partial clinical hold by the FDA in April 2012 due to cases of pancreatitis in patients taking alisporivir in combination with peginterferon-α2a and ribavirin, such that all participants were switched to peginterferon-α2a and ribavirin alone. At the time of the hold, 87% of patients had received ≥12 weeks of triple therapy, but only 20% had received treatment for ≥24 weeks, and only three participants received the full 48 weeks of therapy. Despite the hold, antiviral responses were stronger with the triple combination, as more patients achieved the primary endpoint of SVR12, or the proportion of patients with HCV RNA levels under the limit of detection at 12 weeks, in the triple combination with alisporivir (69%) relative to those taking peginterferon-α2a and ribavirin alone (53%). Additionally, the antiviral response rates (SVR) were consistently better for those treated with alisporivir, irrespective of HCV 1 genotype subtype or IL28B polymorphism status. The inclusion of alisporivir was also associated with a lower relapse rate.

NIM811 was tested in a randomized, placebo-controlled Phase 2a trial (CNIM811B2102) in patients with genotype 1 HCV [5]. The study included 72 patients in the monotherapy arm, who received NIM811 at oral doses of 10 to 600 mg once or twice per day for 14 days, as well as 21 patients who received NIM811 in combination with pegylated interferon alpha (180 µg as two doses) over a 14-day interval. Meaningful anti-viral activity was absent with NIM811 monotherapy, but the combination led to a greater decline in HCV RNA (2.85 log) relative to pegylated interferon therapy alone (0.56 log). Additionally, NIM811 treatment led to reductions in the liver enzyme ALT as a monotherapy at doses over 75 mg as well as in combination therapy, but not with pegylated interferon therapy alone.

SCY-635 was tested in a randomized, double-blind, placebo-controlled dose-escalation Phase 1b trial in chronic genotype 1 HCV [6]. No anti-viral effects were observed in part 1 of the study, which included 37 adult patients treated with SCY-635 at a dose of 30 mg, 100 mg, or 300 mg once per day for 15 days. The second part of the study included 20 HCV patients treated with SCY-635 at doses of 100, 200, or 300 mg three times per day (t.i.d.) for 15 days. Anti-viral activity was observed with the 900 mg/day dose, with individual maximum changes in viral load ranging from 0.84 to 5.47 log10 IU/m. SCY-635 treatment

promoted the induction of type I and III interferons. The greatest reductions in viral load were achieved by those with the IL28B genotype CC (rs12979860).

Covid-19: UNCLEAR

Cyclophilins play a role in the lifecycle of coronaviruses [38]. The N protein of SARS-CoV binds strongly to cyclophilin A, and this interaction may promote cell invasion. The cyclophilin inhibitor alisporivir was shown to block the life cycle of SARS-CoV-2 in cell culture models, though alisporivir plus ribavirin did not prevent SARS-CoV infection in a mouse model [38; 39]. Accumulation of alisporivir in the lung occurs to a greater extent than the plasma (37-fold) in a rat model, suggest that even though the *in vitro* EC_{50} of alisporivir towards SARS-CoV-2 is about ten times higher than for HCV, therapeutic dosing of alisporivir may still be possible in covid-19 patients [38].

Alisporivir was tested in a proof-of-concept randomized, open-label Phase 2 trial (NCT04608214) in 26 patients hospitalized with covid-19 at a dose of 600 mg twice per day for 14 days orally, or via a nasogastric tube. Results have not been made publicly available.

Ischemic injury: POTENTIAL BENEFIT (Preclinical)

Cyclophilin inhibitors have been shown to protect against oxidative damage-driven cell death in models of ischemia by preventing prolonged activation of the mPTP via the inhibition of cyclophilin D. Treatment has typically occurred close in proximity or prior to the ischemic event, thus it is unclear how well these results would translate to benefit in a clinical population.

Liver: The novel cyclophilin inhibitor C105 SR diastereomer (***C105SR***) was shown to mitigate hypoxia/reoxygenation-induced cell death in cultured hepatocytes (AML-12 cell line) through the inhibition of cyclophilin D and the opening of the mPTP [40]. Treatment with C105SR via an osmotic pump also reduced liver cell death during a hepatic ischemic-reperfusion injury.

Muscle: Treatment with the cyclophilin inhibitor ***NIM811*** (10 mg/kg) attenuated muscle degeneration and inflammatory responses, while preserving motor function, in a mouse model of hindlimb ischemia when administered 10 minutes prior to reperfusion and 30 minutes afterwards, in a sex-dependent manner [41].

Kidney: Pre-treatment with the cyclophilin inhibitor ***GS-642362***, from Gilead Sciences, (10 or 30 mg/kg) one hour prior to surgery, protected against renal tubular cell damage and death in models of acute kidney injury [42]. In the bilateral renal ischemia/reperfusion injury model, GS-642362 dose-dependently preserved renal function, with improvements of 54 and 85% in plasma creatinine levels at the low and high doses, respectively. This was accompanied by a reduction in neutrophil and macrophage infiltration to the kidney. In the unilateral ureteric obstruction model, which is a milder

injury model, GS-642362 reduced the deposition of interstitial collagen IV and the accumulation of interstitial α-SMA+ myofibroblasts.

Safety: ***Rated C for potential and C for evidence.*** Cyclophilin inhibitors have been generally safe as monotherapy, with the potential to increase levels of blood lipids and elevate blood pressure. They increase the risk for serious side effects related to interferon therapy when used in combination.

Types of evidence:

- 1 review on Phase 1 and 2 clinical trials testing alisporivir
- 1 Phase 3 RCT testing alisporivir in hepatitis C
- 1 Phase 2a RCT for rencofilstat in NASH
- 1 Phase 1 trial and overview of other Phase 1 studies for rencofilstat
- 1 Phase 2a trial for NIM811 in hepatitis C
- 1 Phase 1 trial for SCY-635 in hepatitis C
- Numerous laboratory studies

Rencofilstat: Rencofilstat was generally found to be safe and well-tolerated in Phase 1 studies in healthy volunteers as well as in a Phase 2a trial in patients with F2/F3 NASH [1; 43]. In Phase 1 studies, doses between 75 mg to 225 mg, the doses tested in the Phase 2a study, were found to achieve drug concentrations in the liver that exceed IC_{50} values. No safety signals emerged in Phase 1 studies. In the Phase 2a study, the most common treatment-emergent adverse events were constipation, diarrhea, back pain, dizziness, and headache, and most were mild to moderate severity [1]. There were no treatment emergent serious adverse events. Gastrointestinal events were more common at the 225 mg dose. There was one instance each of decreased body temperature, hypercholesterolemia, and elevated blood pressure at the 225 mg dose, otherwise there were no other alterations in laboratory tests, vital signs, or ECG parameters. Additionally, rencofilstat has a food effect, such that a high-fat meal may increase the extent of exposure [43]. Delayed gastric emptying in response to a high-fat meal may increase the absorption and bioavailability of rencofilstat.

Alisporivir: The most common adverse events observed in Phase 1 studies testing oral doses of alisporivir up to 1,200 mg twice per day (b.i.d.) in patients co-infected with HIV and HCV were abdominal pain, feeling hot, vomiting, fatigue, and fever [37]. Cases of transient hyperbilirubinemia led to treatment discontinuation in some patients. Alisporivir was tested as a monotherapy, in combination

with pegylated interferon, or in combination with pegylated interferon and ribavirin in patients with HCV in Phase 2 trials [37]. Transient hyperbilirubinemia was observed with alisporivir across studies stemming from the alisporivir-mediated block of the uptake of bilirubin by transporters OATP1B1 and OATP1B3, and efflux by transporter multidrug resistance-associated protein 2, resulting in the elevation of bilirubin. Consequently, the increase in bilirubin with alisporivir is not a reflection of liver injury. The overall safety profile of alisporivir is strongest when it is not combined with interferon. One case of pancreatitis occurred in the CDEB025A2210 (FUNDAMENTAL) study testing alisporivir with pegylated interferon-α2a (IFNα) and ribavirin. The most frequent adverse events were neutropenia, anemia, hyperbilirubinemia, and thrombocytopenia, with serious adverse events occurring at a higher frequency with alisporivir relative to pegylated IFNα and ribavirin alone.
The combination of alisporivir with pegylated IFNα and ribavirin was found to exacerbate safety signals associated with interferon therapy, such as pancreatitis, likely due to the augmentation of interferon responses with cyclophilin inhibitors [4]. Seven cases of pancreatitis occurred in a Phase 3 trial, though rates were generally similar between those taking alisporivir with pegylated IFNα and ribavirin relative to pegylated IFNα and ribavirin alone (5 cases [0.6%] vs. 2 cases [0.8%]) [4]. Two cases occurred in the context of elevated levels of triglycerides. Rates of anemia, thrombocytopenia, hyperbilirubinemia and hypertension were higher in those receiving alisporivir, including severe cases of hyperbilirubinemia (1%), hypertriglyceridemia (0.4%) and hypertension (0.3%), only observed with alisporivir. Three cases of hypertensive crisis occurred in the alisporivir (400 mg b.i.d.) plus pegylated IFNα and ribavirin group. Rates of adverse events considered severe by the investigator were higher in those treated with alisporivir, with rates of 22% in those taking 400 mg (b.i.d.) alisporivir, 16%-17% in those taking 600 mg/day alisporivir, and 8% in those taking pegylated IFNα and ribavirin alone. Hypertension occurred in 19% of participants taking the alisporivir 400 mg (b.i.d.) dose, relative to only 2% in those taking pegylated IFNα and ribavirin alone. Those with a history of hypertension were more likely to experience elevations in blood pressure in response to alisporivir.

NIM811: In a Phase 2a trial, NIM811 was tested at doses ranging from 10 to 600 mg once or twice a day in patients with genotype 1 HCV for 14 days as monotherapy or in combination with pegylated interferon alpha [5]. All doses were generally well-tolerated. Mild elevations in bilirubin and declines in platelets were observed at the 400 mg and 600 mg doses, but the changes were not considered clinically meaningful. One patient in the combination arm experienced neutropenia, likely related to interferon therapy. There were minor elevations (~0.6 mmol increase) in triglycerides with NIM811 as a monotherapy or in combination. Cases of nausea were more common with NIM811. Thrombopenia was

observed starting at the 200 mg b.i.d. dose, which was exacerbated when NIM811 was combined with pegylated interferon.
SCY-635: In a Phase 1 trial, SCY-635 was generally safe and well-tolerated in single daily doses ranging from 30 to 300 mg per day for 15 days in patients with genotype 1 HCV (n=37). In part two of this study (n=20), there were no dose-limiting toxicities in HCV patients receiving SCY-635 up to 900 mg per day for 15 days [6]. The most common treatment-emergent adverse events were elevated serum creatinine phosphokinase (all grade 1), headache, hypokalemia (all had history of diuretic use), asymptomatic elevated liver function tests (grade 1 or 2 increases in ALT/AST) and nausea. One participant with grade 1 elevation in triglycerides at baseline experienced a grade 4 elevation in triglycerides following treatment, which resolved through diet modification without treatment interruption.

Drug interactions: Cyclophilin inhibitors are likely to have both drug-specific and class-related drug interactions. Due to an impact on interferon production and inflammatory mediators, cyclophilin inhibitors can increase the risk for side effects when combined with interferon therapies. Other interactions may depend on the metabolism of the drug. For example, alisporivir is a substrate/inhibitor of cytochrome P450 34A (CYP3A4) and P-glycoprotein, and thus can interact with other substrates of these enzymes, such as ketoconazole, rifampin, and azithromycin [44]. Rencofilstat is also a substrate for CYP34A. In a drug-interaction study, ketoconazole increased the exposure of rencofilstat by approximately four-fold, while midazolam increased the exposure of rencofilstat around two-fold. Reconfilstat did not affect the pharmacokinetics of midazolam [43].

Sources and dosing:

Non-immunosuppressive cyclophilin inhibitors have not yet been approved for any indication.
Rencofilstat (CRV431) is in clinical development by Hepion Pharmaceuticals. It is currently in clinical development for liver diseases, at doses ranging from 75 mg to 225 mg/day in the form of oral capsules. To date, no clinically beneficial dose has been established for any indication. Due to limited resources, the company is winding down its current Phase 2b study early, and the future clinical development of rencofilstat is unclear. Its development could potentially be revived following a proposed merger (Press release).
The clinical development of cyclophilin inhibitors that were tested for hepatitis C/anti-viral indications, such as alisporivir, NIM811, and SCY-635 appears to have been terminated. Additional cyclophilin inhibitors have been described in the literature and tested in preclinical studies. The prospective clinical development of these inhibitors is currently unclear.

Research underway:

Rencofilstat is currently being tested, at doses of 75 mg, 150 mg, and 225 mg, in a randomized, placebo-controlled, double-blind Phase 2b trial in adults with nonalcoholic steatohepatitis (NASH) and advanced liver fibrosis (F2 or F3) (NCT05402371). The trial has an estimated completion date in 2025. In 2024, the trial's sponsor, Hepion Pharmaceuticals announced that it would be winding down the trial early, and would likely only be able to obtain meaningful safety data (Press release).

Search terms:

Pubmed, Google: Cyclophilin inhibitor; reconfilistat, alisporivir, NIM811

- Alzheimer's disease, cognition, liver disease, antiviral, clinical trial, safety

Websites visited for Cyclophilin inhibitors:

- Clinicaltrials.gov (Reconfilstat, Alisporivir, NIM811, SCY-635)
- PubChem (Reconfilstat, Alisporivir, NIM811, SCY-635)
- DrugBank.ca (Reconfilstat, Alisporivir, NIM811, SCY-635)

References:

1. Rajan S, Wischmeyer E, Karschin C *et al.* (2001) THIK-1 and THIK-2, a Novel Subfamily of Tandem Pore Domain 1. Shan B, Pan H, Najafov A *et al.* (2018) Necroptosis in development and diseases. *Genes & development* **32**, 327-340https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5900707/.

2. Degterev A, Ofengeim D, Yuan J (2019) Targeting RIPK1 for the treatment of human diseases. *Proceedings of the National Academy of Sciences* **116**, 9714-9722https://www.pnas.org/content/pnas/116/20/9714.full.pdf.

3. Oliveira SR, Amaral JD, Rodrigues CMP (2018) Mechanism and disease implications of necroptosis and neuronal inflammation. *Cell death & disease* **9**, 903-903https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6125291/.

4. Caccamo A, Branca C, Piras IS *et al.* (2017) Necroptosis activation in Alzheimer's disease. *Nature Neuroscience* **20**, 1236https://doi.org/10.1038/nn.4608.

5. Ofengeim D, Mazzitelli S, Ito Y *et al.* (2017) RIPK1 mediates a disease-associated microglial response in Alzheimer's disease. *Proceedings of the National Academy of Sciences of the United States of America* **114**, E8788-E8797https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5642727/.

6. Xu D, Jin T, Zhu H *et al.* (2018) TBK1 Suppresses RIPK1-Driven Apoptosis and Inflammation during Development and in Aging. *Cell* **174**, 1477-1491.e1419http://www.sciencedirect.com/science/article/pii/S0092867418309693.

7. Belkhelfa M, Beder N, Mouhoub D *et al.* (2018) The involvement of neuroinflammation and necroptosis in the hippocampus during vascular dementia. *Journal of Neuroimmunology* **320**, 48-57http://www.sciencedirect.com/science/article/pii/S0165572818300316.

8. Jayaraman A, Htike TT, James R *et al.* (2021) TNF-mediated neuroinflammation is linked to neuronal necroptosis in Alzheimer's disease hippocampus. *Acta Neuropathol Commun* **9**, 159https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8501605/.

9. Dermentzaki G, Politi KA, Lu L *et al.* (2019) Deletion of Ripk3 Prevents Motor Neuron Death In Vitro but not In Vivo. *eNeuro* **6**, ENEURO.0308-0318.2018https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6391588/.

10. Vissers M, Heuberger J, Groeneveld GJ *et al.* (2022) Safety, pharmacokinetics and target engagement of novel RIPK1 inhibitor SAR443060 (DNL747) for neurodegenerative disorders: Randomized, placebo-controlled, double-blind phase I/Ib studies in healthy subjects and patients. *Clin Transl Sci* **15**, 2010-2023https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9372423/.

11. Yang S-H, Lee DK, Shin J *et al.* (2017) Nec-1 alleviates cognitive impairment with reduction of A β and tau abnormalities in APP/PS1 mice. *EMBO molecular medicine* **9**, 61-77https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5210088/.

12. Takabe W, Urano Y, Vo D-KH *et al.* (2016) Esterification of 24S-OHC induces formation of atypical lipid droplet-like structures, leading to neuronal cell death. *Journal of lipid research* **57**, 2005-2014https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5087868/.

13. Hernández DE, Salvadores NA, Moya-Alvarado G *et al.* (2018) Axonal degeneration induced by glutamate excitotoxicity is mediated by necroptosis. *Journal of Cell Science* **131**, jcs214684http://jcs.biologists.org/content/joces/131/22/jcs214684.full.pdf.

14. Cheng Z, Shang Y, Gao S *et al.* (2017) Overexpression of U1 snRNA induces decrease of U1 spliceosome function associated with Alzheimer's disease. *Journal of Neurogenetics* **31**, 337-343https://doi.org/10.1080/01677063.2017.1395425.

15. Arrázola MS, Saquel C, Catalán RJ *et al.* (2019) Axonal Degeneration Is Mediated by Necroptosis Activation. *The Journal of Neuroscience* **39**, 3832-3844http://www.jneurosci.org/content/jneuro/39/20/3832.full.pdf.

16. Sanofi (2021) A multicenter, randomized, placebo-controlled, double-blind, Phase 1b study to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of SAR443060 (DNL747) on subjects with amyotrophic lateral sclerosis (TDR16536 [DNLI-D-0003]) https://www.sanofi.com/assets/dotcom/content-app/clinical-studies/pharma/Letter-R/TDR16536-results-summary.pdf.

17. Wei J, Li M, Ye Z *et al.* (2023) Elevated peripheral levels of receptor-interacting protein kinase 1 (RIPK1) and IL-8 as biomarkers of human amyotrophic lateral sclerosis. *Signal Transduct Target Ther* **8**, 451https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10716192/.

18. Ito Y, Ofengeim D, Najafov A *et al.* (2016) RIPK1 mediates axonal degeneration by promoting inflammation and necroptosis in ALS. *Science (New York, NY)* **353**, 603-608https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5444917/.

19. Re DB, Le Verche V, Yu C *et al.* (2014) Necroptosis drives motor neuron death in models of both sporadic and familial ALS. *Neuron* **81**, 1001-1008https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3951532/.

20. Ofengeim D, Ito Y, Najafov A *et al.* (2015) Activation of necroptosis in multiple sclerosis. *Cell reports* **10**, 1836-1849https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4494996/.

21. Wang T, Perera ND, Chiam MDF *et al.* (2020) Necroptosis is dispensable for motor neuron degeneration in a mouse model of ALS. *Cell Death Differ* **27**, 1728-1739https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7206015/.

22. Zhang Y, Su SS, Zhao S *et al.* (2017) RIP1 autophosphorylation is promoted by mitochondrial ROS and is essential for RIP3 recruitment into necrosome. *Nature Communications* **8**, 14329https://doi.org/10.1038/ncomms14329.

23. Iannielli A, Bido S, Folladori L *et al.* (2018) Pharmacological Inhibition of Necroptosis Protects from Dopaminergic Neuronal Cell Death in Parkinson's Disease Models. *Cell reports* **22**, 2066-2079https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5842028/.

24. Wu J-R, Wang J, Zhou S-K *et al.* (2015) Necrostatin-1 protection of dopaminergic neurons. *Neural regeneration research* **10**, 1120-1124https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4541245/.

25. Kartik S, Pal R, Chaudhary MJ *et al.* (2023) Anti-oxidative and anti-neuroinflammatory role of Necrostatin-1s and docosahexaenoic acid in RIP-1-mediated neurotoxicity in MPTP-induced Parkinson's disease model. *Fundam Clin Pharmacol* **37**, 794-806https://pubmed.ncbi.nlm.nih.gov/36807936/.

26. Kim DY, Leem YH, Park JS *et al.* (2023) RIPK1 Regulates Microglial Activation in Lipopolysaccharide-Induced Neuroinflammation and MPTP-Induced Parkinson's Disease Mouse Models. *Cells* **12**https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9913664/.

27. Qiao C, Niu G, Zhao W *et al.* (2023) RIPK1-Induced A1 Reactive Astrocytes in Brain in MPTP-Treated Murine Model of Parkinson's Disease. *Brain Sci* **13**https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10216483/.

28. Lin QS, Chen P, Wang WX *et al.* (2020) RIP1/RIP3/MLKL mediates dopaminergic neuron necroptosis in a mouse model of Parkinson disease. *Lab Invest* **100**, 503-511https://pubmed.ncbi.nlm.nih.gov/31506635/.

29. Linkermann A, Bräsen JH, Darding M *et al.* (2013) Two independent pathways of regulated necrosis mediate ischemia–reperfusion injury. *Proceedings of the National Academy of Sciences* **110**, 12024-12029https://www.pnas.org/content/pnas/110/29/12024.full.pdf.

30. Zhang Y, Su Y, Wang Z *et al.* (2023) TAK1 Reduces Surgery-induced Overactivation of RIPK1 to Relieve Neuroinflammation and Cognitive Dysfunction in Aged Rats. *Neurochem Res* **48**, 3073-3083https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10471686/.

31. Yin C, Zhang Q, Zhao J *et al.* (2022) Necrostatin-1 Against Sevoflurane-Induced Cognitive Dysfunction Involves Activation of BDNF/TrkB Pathway and Inhibition of Necroptosis in Aged Rats. *Neurochem Res* **47**, 1060-1072https://pubmed.ncbi.nlm.nih.gov/35040026/.

32. Karunakaran D, Geoffrion M, Wei L *et al.* (2016) Targeting macrophage necroptosis for therapeutic and diagnostic interventions in atherosclerosis. *Science advances* **2**, e1600224-e1600224https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4985228/.

33. Zhang Y-Z, Wang L, Zhang J-J *et al.* (2018) Vascular peroxide 1 promotes ox-LDL-induced programmed necrosis in endothelial cells through a mechanism involving β-catenin signaling. *Atherosclerosis* **274**, 128-138http://www.sciencedirect.com/science/article/pii/S0021915018302168.

34. Karunakaran D, Nguyen MA, Geoffrion M *et al.* (2018) Therapeutic inhibition of RIP1 improves metabolic dysfunction and inhibits atherosclerosis in mouse models of cardiometabolic diseases. *The FASEB Journal* **32**, 38.31-38.31https://www.fasebj.org/doi/abs/10.1096/fasebj.2018.32.1_supplement.38.1.

35. Zhang Y, Li H, Huang Y *et al.* (2021) Stage-Dependent Impact of RIPK1 Inhibition on Atherogenesis: Dual Effects on Inflammation and Foam Cell Dynamics. *Front Cardiovasc Med* **8**, 715337https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8572953/.

36. McCaig WD, Patel PS, Sosunov SA *et al.* (2018) Hyperglycemia potentiates a shift from apoptosis to RIP1-dependent necroptosis. *Cell death discovery* **4**, 55-55https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5945624/.

37. Xu H, Du X, Liu G *et al.* (2019) The pseudokinase MLKL regulates hepatic insulin sensitivity independently of inflammation. *Molecular metabolism* **23**, 14-23https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6480316/.

38. Yu Q, Chen Y, Zhao Y *et al.* (2023) Nephropathy Is Aggravated by Fatty Acids in Diabetic Kidney Disease through Tubular Epithelial Cell Necroptosis and Is Alleviated by an RIPK-1 Inhibitor. *Kidney Dis (Basel)* **9**, 408-423https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10624943/.

39. Tao L, Lin H, Wen J *et al.* (2018) The kinase receptor-interacting protein 1 is required for inflammasome activation induced by endoplasmic reticulum stress. *Cell death & disease* **9**, 641-641https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5974395/.

40. Jhun J, Lee SH, Kim S-Y *et al.* (2019) RIPK1 inhibition attenuates experimental autoimmune arthritis via suppression of osteoclastogenesis. *Journal of translational medicine* **17**, 84-84https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6419814/.

41. Weisel K, Berger S, Thorn K *et al.* (2021) A randomized, placebo-controlled experimental medicine study of RIPK1 inhibitor GSK2982772 in patients with moderate to severe rheumatoid arthritis. *Arthritis Res Ther* **23**, 85https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7962407/.

42. Weisel K, Berger S, Papp K *et al.* (2020) Response to Inhibition of Receptor-Interacting Protein Kinase 1 (RIPK1) in Active Plaque Psoriasis: A Randomized Placebo-Controlled Study. *Clin Pharmacol Ther* **108**, 808-816https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7540322/.

43. Weisel K, Scott N, Berger S *et al.* (2021) A randomised, placebo-controlled study of RIPK1 inhibitor GSK2982772 in patients with active ulcerative colitis. *BMJ Open Gastroenterol* **8**https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8365785/.

44. Mohammed S, Thadathil N, Selvarani R *et al.* (2021) Necroptosis contributes to chronic inflammation and fibrosis in aging liver. *Aging Cell* **20**, e13512https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8672775/.

45. Kondo T, Macdonald S, Engelmann C *et al.* (2021) The role of RIPK1 mediated cell death in acute on chronic liver failure. *Cell Death Dis* **13**, 5https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8683430/.

46. Takemoto K, Hatano E, Iwaisako K *et al.* (2014) Necrostatin-1 protects against reactive oxygen species (ROS)-induced hepatotoxicity in acetaminophen-induced acute liver failure. *FEBS open bio* **4**, 777-787https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4208088/.

47. Vucur M, Ghallab A, Schneider AT *et al.* (2023) Sublethal necroptosis signaling promotes inflammation and liver cancer. *Immunity* **56**, 1578-1595.e1578https://pubmed.ncbi.nlm.nih.gov/37329888/.

48. Gong Y, Fan Z, Luo G *et al.* (2019) The role of necroptosis in cancer biology and therapy. *Molecular cancer* **18**, 100-100https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6532150/.

49. Vogelsang TLR, Kast V, Bagnjuk K *et al.* (2023) RIPK1 and RIPK3 are positive prognosticators for cervical cancer patients and C2 ceramide can inhibit tumor cell proliferation in vitro. *Front Oncol* **13**, 1110939https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10183606/.

50. (2021) US Patent Application for ECLITASERTIB FOR USE IN TREATING CONDITIONS INVOLVING SYSTEMIC HYPERINFLAMMATORY RESPONSE Patent Application (Application #20230233576). https://patents.justia.com/patent/20230233576.

51. Clot PF, Farenc C, Suratt BT *et al.* (2024) Immunomodulatory and clinical effects of receptor-interacting protein kinase 1 (RIPK1) inhibitor eclitasertib (SAR443122) in patients with severe COVID-19: a phase 1b, randomized, double-blinded, placebo-controlled study. *Respiratory research* **25**, 107https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10903152/.

52. Howley B, Fearnhead HO (2008) Caspases as therapeutic targets. *Journal of cellular and molecular medicine* **12**, 1502-1516https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3918066/.

53. Wegner KW, Saleh D, Degterev A (2017) Complex Pathologic Roles of RIPK1 and RIPK3: Moving Beyond Necroptosis. *Trends in pharmacological sciences* **38**, 202-225https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5325808/.

54. Weisel K, Scott NE, Tompson DJ *et al.* (2017) Randomized clinical study of safety, pharmacokinetics, and pharmacodynamics of RIPK1 inhibitor GSK2982772 in healthy volunteers. *Pharmacology research & perspectives* **5**, e00365https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5723699/.

55. Tompson DJ, Davies C, Scott NE *et al.* (2021) Comparison of the Pharmacokinetics of RIPK1 Inhibitor GSK2982772 in Healthy Western and Japanese Subjects. *Eur J Drug Metab Pharmacokinet* **46**, 71-83https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7811991/.

56. Tompson DJ, Whitaker M, Pan R *et al.* (2021) Development of a Prototype, Once-Daily, Modified-Release Formulation for the Short Half-Life RIPK1 Inhibitor GSK2982772. *Pharm Res* **38**, 1235-1245https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8292240/.

57. Hincelin-Mery A, Nicolas X, Cantalloube C *et al.* (2023) Safety, pharmacokinetics, and target engagement of a brain penetrant RIPK1 inhibitor, SAR443820 (DNL788), in healthy adult participants. *Clin Transl Sci* **17**https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10772668/.

58. Lickliter J, Wang S, Zhang W *et al.* (2023) A phase I randomized, double-blinded, placebo-controlled study assessing the safety and pharmacokinetics of RIPK1 inhibitor GFH312 in healthy subjects. *Clin Transl Sci* **16**, 1691-1703https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10499419/.

59. Jones NS, Kshirsagar S, Mohanan V *et al.* (2023) A phase I, randomized, ascending-dose study to assess safety, pharmacokinetics, and activity of GDC-8264, a RIP1 inhibitor, in healthy volunteers. *Clin Transl Sci* **16**, 1997-2009https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10582670/.

60. Grievink HW, Heuberger J, Huang F *et al.* (2020) DNL104, a Centrally Penetrant RIPK1 Inhibitor, Inhibits RIP1 Kinase Phosphorylation in a Randomized Phase I Ascending Dose Study in Healthy Volunteers. *Clin Pharmacol Ther* **107**, 406-414https://pubmed.ncbi.nlm.nih.gov/31437302/.

61. Li Y, Zhang L, Wang Y *et al.* (2022) Generative deep learning enables the discovery of a potent and selective RIPK1 inhibitor. *Nat Commun* **13**, 6891https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9653409/.

62. Tang J, Wu Y, Zhao W *et al.* (2023) Scaffold hopping derived novel benzoxazepinone RIPK1 inhibitors as anti-necroptosis agents. *Bioorg Med Chem* **91**, 117385https://pubmed.ncbi.nlm.nih.gov/37364415/.

63. Ling ZY, Lv QZ, Li J *et al.* (2023) Protective Effect of a Novel RIPK1 Inhibitor, Compound 4-155, in Systemic Inflammatory Response Syndrome and Sepsis. *Inflammation* **46**, 1796-1809https://pubmed.ncbi.nlm.nih.gov/37227549/.