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.

RIPK1 Inhibitors

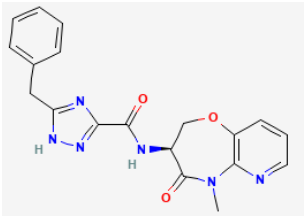
Evidence Summary

They have the potential to reduce cell loss and inflammation, but anti-inflammatory clinical benefit has not yet been observed with tested compounds, though safety has generally been good.

Neuroprotective Benefit: RIPK1 inhibitors may protect against neuronal loss to a variety of cell stressors, reduce inflammation, and promote a neuroprotective phenotype in microglia, however, clinical validation is needed.

Aging and related health concerns: Tested RIPK1 inhibitors have not shown clinical benefit in autoimmune/inflammatory conditions thus far. Preclinical studies suggest they may help reduce inflammation-mediated damage and improve insulin sensitivity.

Safety: RIPK1 inhibitors have been well-tolerated in Phase 1 studies. Adverse events tend to be drug-specific, and related to off-target effects. Headache and gastrointestinal events are the most common. Elevated liver enzymes were observed with some drugs.

Availability: In clinical trials	**Dose**: Not established. Tested drugs have all been oral formulations.	Eclitasertib **Chemical formula:** $C_{19}H_{18}N_6O_3$ **MW**: 378.4 g/mol  Source: PubChem Structural information for SAR443820, GFH312, GDC-8264, LY3871801, or SIR2446 is not currently available.
Half-life: SAR443820: 7.2-8.9 h in a Phase 1 trial GFH312: 6.3- 23.3 h in a Phase 1 trial GDC-8264: 10-13 h in a Phase 1 trial	**BBB**: SAR443820 is penetrant. Eclitasertib is not penetrant. GFH312, GDC-8264, and LY3871801are being developed for peripheral indications, while SIR2446 is being developed for CNS indications, but penetrance data not available.	
Clinical trials: Small phase 1 trials in healthy volunteers have been completed for SAR443060, GSK2982772, DNL104, SAR443820, Eclitasertib, LY3871801, GFH312, GDC-8264, and SIR1-365. Phase 1 trials in covid-19 were conducted for eclitasertib (n=68) and SIR1-365 (n=45). Small Phase 2 trials have been conducted for SAR443060 in ALS (n=15), and AD (n=16); GSK2982772 in rheumatoid arthritis (n=52), psoriasis (n=65), and ulcerative colitis (n=36); and eclitasertib in lupus (n=78). There are ongoing Phase 1 trials in acute graft vs host disease, and Phase 2 trials in ALS, MS, rheumatoid arthritis, and ulcerative colitis.	**Observational studies**: RIPK1 expression elevated in tissue from patients with neurodegenerative and inflammatory diseases	

What is it?

Receptor-interacting serine/threonine-protein kinase 1 (RIPK1) is a multi-domain protein that serves as a mediator for cell death and inflammation. RIPK1 serves as an initiating factor for necroptosis, which is a programmed form of necrosis. Necrotic cell death is associated with the release of damage-associated molecular pattern molecules (DAMPs), which promote inflammation and exacerbate damage to surrounding cells. Necroptosis, then, is a programmed method of inflammatory cell death, whereas apoptosis is a programmed method of non-inflammatory cell death. RIPK1 serves as an integrator of

various cell signaling events which can regulate cell responses and determine cell fate [1]. Its activation is regulated by multiple post-translational modifications, especially ubiquitination and phosphorylation. The activation of RIPK1 can trigger the formation of the necrosome, which initiates the inflammatory necroptotic cell death cascade. The execution of necroptosis requires the activation of the downstream mediators RIPK3 and MLKL, such that cells lacking MLKL may undergo a shift in phenotype, but fail to undergo necroptotic cell death in response to RIPK1 activation. RIPK1 is not essential for all forms of necroptotic cell death, but is responsible for the activation of necroptosis downstream from TNF, which is the best studied necroptosis stimulator [2]. Furthermore, RIPK1 mediated necroptosis is inhibited in the presence of caspase activity, particularly caspase-8, which instead drives a cell toward apoptotic cell death.

RIPK1 kinase inhibitors have been tested in a variety of animal models for inflammatory and neurodegenerative diseases, however, the early compounds based off a molecule called Necrostatin-1 have poor drug properties [3]. Next generation RIPK1 kinase inhibitors have been developed and several have been tested in Phase 1 trials. They are primarily in clinical development for peripheral autoimmune diseases or neurodegenerative diseases.

Active programs:

SAR443820 (DNL788) is a BBB penetrant small molecule, oral RIPK1 inhibitor in clinical development by Sanofi, licensed from Denali Therapeutics, for CNS indications, including amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), and Alzheimer's disease (AD). It has been tested in Phase 1 trials in healthy volunteers. It is being tested in Phase 2 trials in patients with ALS and patients with MS.

Eclitasertib (SAR443122; DNL758) is a peripherally restricted small molecule, oral RIPK1 inhibitor in clinical development by Sanofi, licensed from Denali Therapeutics. It has been tested in Phase 1 trials in healthy volunteers and covid-19 patients, as well as in a Phase 2 trial in patients with cutaneous lupus erythematosus. It is currently being tested in a Phase 2 trial in patients with ulcerative colitis.

GFH312 is a small molecule, oral RIPK1 inhibitor in clinical development for autoimmune diseases by GenFleet Therapeutics. It was tested in a Phase 1 trial in healthy volunteers. A planned trial in patients with peripheral artery disease was withdrawn due to an adjusted clinical development strategy by the sponsor.

GDC-8264 is a small molecule, oral RIPK1 inhibitor in clinical development by Genentech (subsidiary of Roche). It has been tested in a Phase 1 trial in healthy volunteers and is currently being tested in a Phase 1 trial in patients with acute graft-versus-host disease.

LY3871801 (R552) is a small molecule, oral RIPK1 inhibitor in clinical development by Eli Lilly and Rigel Pharmaceuticals. It appears to be in development for peripheral autoimmune/inflammation-related conditions. It has been tested in Phase 1 trials in healthy volunteers, and is currently being tested in patients with rheumatoid arthritis.

SIR2446 is a small molecule, oral RIPK1 inhibitor in clinical development for CNS indications, by Sironax. It is being tested in a Phase 1 trial in healthy volunteers.

(***SIR1-365***) is a small molecule, oral RIPK1 inhibitor that was in clinical development for peripheral inflammatory conditions by Sironax. It was tested in Phase 1 trials in healthy volunteers and in covid-19 patients. SIR-365 is no longer listed on the pipeline on the website for Sironax, and the development status of this drug is unclear.

Discontinued programs:

SAR443060 (DNL747) was in clinical development by Sanofi, licensed from Denali Therapeutics, for neurodegenerative diseases, and was tested in Phase 1 trials in healthy volunteers, and Phase 2 trials in ALS patients and AD patients. Clinical development was terminated following the detection of immune-related safety signals in preclinical toxicology studies.

GSK2982772 was in clinical development by GlaxoSmithKline for peripheral autoimmune conditions, and was tested in clinical trials for rheumatoid arthritis, plaque psoriasis, and ulcerative colitis. It has a short half-life of around 2-3 hours, requiring three times per day (t.i.d.) dosing. Clinical development was discontinued after clinical trials for these indications failed to meet their primary efficacy endpoints.

DNL104 was in clinical development by Denali Therapeutics for CNS conditions, however development was terminated following the detection of off-target liver toxicity signals in a Phase 1 trial.

Neuroprotective Benefit: RIPK1 inhibitors may protect against neuronal loss to a variety of cell stressors, reduce inflammation, and promote a neuroprotective phenotype in microglia, however, clinical validation is needed.

Types of evidence:

- 7 observational studies for RIPK1 expression in postmortem tissue for AD, VD, ALS, MS
- 2 pilot Phase 2 trials for SAR443060 in ALS and AD
- Numerous laboratory studies (for Necrostatin series RIPK1 inhibitors)

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

There is currently no evidence to date to indicate that RIPK1 inhibitors are neuroprotective in humans, however, RIPK1 activation is implicated in neurodegeneration based on studies in human postmortem tissue.

RIPK1 can act as a transcriptional regulator, and RIPK1 regulated genes are highly enriched in the transcriptomic profile of genes altered in Alzheimer's disease (AD) [4], and other neurodegenerative diseases [5]. A transcriptomic microarray analysis found that the mRNA for the endogenous RIPK1 inhibitor Tak1decreases in the brain with age [6]. Brain Tak1 mRNA was decreased 1.33-fold in people over age 60 compared to the those under age 40, and the protein level was also reduced in the aged brain prefrontal cortex. This loss of inhibition suggests that the aged brain is more sensitive to RIPK1 activating stimuli, such as TNFα.

Activation of RIPK1 in microglia is associated with pro-inflammatory cytokine production, while activation of RIPK1 in neurons and oligodendrocytes is associated with cell death. The different responses stem from differential expression of the downstream mediators of the necroptosis cell death signaling cascade, RIPK3 and MLKL. Levels of activated RIPK1, based on S166 phosphorylation, have been found to be increased in the microglia of AD patients, indicative of elevated damage-inducing inflammation [5]. In the AD brain, RIPK1 colocalizes with RIPK3 and MLKL in neurons with high levels of phosphorylated tau and the levels correlate with Braak stage [4]. RIPK1 levels are also inversely correlated with brain weight ($R=-0.333$; $P=1.1 \times 10^{-3}$) and cognitive scores based on the Mini-Mental State Examination (MMSE). This suggests that RIPK1 mediated necroptosis may contribute to neuronal loss in AD.

In a comparative histological study, the hippocampus from an individual with vascular dementia had higher levels of inflammatory cytokines associated with RIPK1 activation, including TNF, as well as evidence of both apoptotic and necrotic cells [7]. In contrast, the hippocampus from the individual without dementia showed some apoptotic cells, but no necrotic cells, suggesting that high inflammation may activate necroptotic cell loss and contribute to the development of cognitive impairment.

The necroptotic mediators, RIPK1, RIPK3, and MLKL were found to be elevated by two to three times in the hippocampi of AD patients (n=30), relative to controls (n=11) in postmortem tissue analysis [8]. Additionally, there was co-localization of the activated forms within neurons in AD patients, as well as an increase in levels of TNFR within these neurons, suggestive of a role for inflammatory TNF signaling in the activation of the necroptotic pathway. A sex difference was observed with 2-to-2.5-fold higher levels of activated necrotic mediators in females relative to males. Additionally, levels of ESCRT-III components, such as CHMP2B, were elevated, which is suggestive of a protective compensatory response, as these components may have a role in countering necroptotic cell death.

Caution is warranted in interpreting the contribution of necroptosis mediated cell loss in neurodegenerative diseases based on postmortem tissue because hypoxia can induce RIPK1, and many genes involved in cell death are upregulated during the early postmortem period [9]. However, the localization of elevated RIPK1 specifically in areas with prominent disease-associated pathology suggest that the increase in RIPK1 is disease relevant.

Human research to suggest benefits to patients with dementia:

The BBB penetrant RIPK1 inhibitor DNL747 (SAR443060) was tested in Phase 1 clinical trials in 16 patients with AD (NCT03757325) and in 15 patients with ALS (NCT03757351) [10]. The AD participants were amyloid confirmed and had a Clinical Dementia Rating (CDR) score of 0.5–1.0. Participants in both trials were treated with 50 mg of SAR443060 or placebo twice per day (b.i.d.) for 28 days. Based on PK-PD modeling from a prior Phase 1 trial, this dose was expected to provide for >80% inhibition of pRIPK1. In AD patients, the level of inhibition in peripheral blood mononuclear cells (PBMCs) was >80% (93.98% at 2 hours to 81.83% at 12 hours) over the 12-hour dosing period, but fell below this level in ALS patients (92.34% at 2 hours to 65.92% at 12 hours). There were no statistically significant effects on the DCTclock, a digital version of the clock drawing test, in the AD study, or on the ALSFRS-R clinical rating scale in the ALS study. But these short studies were not adequately powered to detect a change in cognitive or functional measures. An open-label extension study in ALS patients was terminated

prematurely because of the decision to end clinical development of SAR443060 due to preclinical safety signals. Instead, development for neurodegenerative diseases shifted to the BBB penetrant RIPK1 inhibitor SAR443820 (DNL788), which is being tested in clinical trials for ALS (NCT05237284) and MS (NCT05630547).

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

Elevated levels of RIPK1 in the CNS are associated with microglial mediated inflammation, axon degeneration, and necrotic cell death.

Alzheimer's disease: POTENTIAL BENEFIT (preclinical)
Inhibition of the kinase activity of RIPK1 has been shown to be neuroprotective in several rodent models of AD. Treatment with the RIPK1 kinase inhibitor Necrostatin-1s has been shown to reduce A plaque burden, tau aggregation, and levels of pro-inflammatory cytokines, and improve performance on spatial memory tests in APP/PS1 mice [4; 5; 11], and reduce cell loss in the 5XFAD mouse model [4].
RIPK1 is an attractive target for AD because it serves as a mediator of cellular toxicity that is downstream of a variety of cell stressors and processes that are dysregulated in the context of AD, which is advantageous in a patient population where the underlying disease etiology is heterogeneous. Examples of stressors that induce RIPK1 include disruption of cholesterol homeostasis, excitotoxicity, and dysregulated mRNA processing [12; 13; 14]. Inhibition of RIPK1 kinase activity has been shown to protect against the loss of cell viability in response to these stressors in preclinical models, due it is role in regulating neuronal cell death [2]. RIPK1 also plays a critical role in driving axon degeneration independent of apoptosis and its inhibition has been shown to prevent subsequent mitochondrial fragmentation and loss of axon function following injury in culture and animal models [13; 15].
RIPK1 can also promote neurological damage by promoting a pro-inflammatory environment and driving microglia toward a disease-associated phenotype [5]. Disease-associated microglia are thought to be derived from the population of homeostatic microglia by acquiring a unique transcriptional profile. Since RIPK1 regulates many of the genes associated with the disease-associated microglia profile, its activation in response to chronic cell stress is hypothesized to be one of major drivers of this pathogenic cell population. In mice, RIPK1 activation can lead to an impairment of lysosomal function in microglia, while RIPK1 inhibition can promote effective microglial clearance of Aβ[5].
Therefore, RIPK1 inhibition may protect against microglial dysfunction, the exacerbation of inflammation, and neuronal loss induced by a wide variety of cell stressors.

Amyotrophic Lateral Sclerosis: POTENTIAL BENEFIT (preclinical)
The BBB penetrant RIPK1 inhibitor, SAR443060 (DNL747), was tested in a 28-day Phase 1b trial in 15 ALS patients at a dose of 50 mg b.i.d. (NCT03757351) [10]. Eight patients continued treatment during the open-label extension phase, though two discontinued early due to disease progression, so only six patients continued for nine months, until the projected 12-month study was terminated due to discontinuation of the drug stemming from potential safety concerns. The initial proposed dose was 200 mg b.i.d., and the 50 mg b.i.d. dose did not allow for continuous pRIPK1 inhibition at a level >80% in this population. By the end of the 12-hour dosing period, median levels of pRIPK1 inhibition were 65.92% (Confidence Interval [CI] 79.3 to 45.39%) in PBMCs, the peripheral marker of target engagement. As a result, the level of the study drug may not have been high enough to achieve a therapeutic effect, and the lack of benefit on clinical measures, such as the ALSFRS-R clinical rating scale, or on fluid biomarker measures of neurodegeneration and disease progression, including neurofilament light, YKL-40, sTREM2, or p75 extracellular domain, cannot be reliably interpreted [16]. The BBB penetrant RIPK1 inhibitor SAR443820 (DNL788) is currently being tested in a Phase 2 trial in ALS patients (NCT05237284). The study includes a 24-week controlled treatment period followed by an 80-week open-label extension. It was recently reported that the placebo-controlled period (n=305) failed to meet its primary endpoint of change from baseline on the ALSFRS-R, but further details have not yet been disclosed (Fierce Biotech). It is currently unclear whether the open-label extension study will proceed as scheduled.

Serum levels of RIPK1 were found to be elevated in ALS patients (n=162) in a trial testing the neuronal voltage-gated sodium channel blocker primidone (62.5 mg/day), which has shown protective effects in delaying symptom onset in the $SOD1^{G93A}$ mouse model [17]. Levels of RIPK1 were correlated with the inflammatory mediator IL-8, and were positively correlated with the severity of bulbar symptoms. Serum levels of RIPK1 and IL-8 were reduced at the end of the 24-week study.

The dysregulation of RIPK1 is implicated in the pathogenesis of ALS based on the interaction between RIPK1 with several genes associated with ALS. Loss of function mutations in the ubiquitin binding protein optineurin sensitize cells to TNF induced inflammation and necroptosis, which stems from the activation of RIPK1 in microglia and oligodendrocytes, respectively [18]. The partial loss of TKB1, an endogenous inhibitor of RIPK1, is a major genetic case of combined ALS/FTD [6]. The expression of necroptotic mediators, including RIPK1, has also been found to be elevated in the spinal cord of the SOD^{G93A} mouse model [18]. Cell death could be reduced, and behavioral deficits delayed in these genetic ALS mouse models when RIPK1 kinase activity was inhibited using Necrostatin-1 or transgenic models.

In cell culture, astrocytes from ALS patients were shown to secrete a soluble factor that could promote RIPK1 dependent necroptosis in co-cultured neurons [19]. It is still unclear, however, how much RIPK1 activity contributes to disease progression in ALS patients, and whether it is a primary driver of motor neuron loss. One study examining postmortem tissue from the primary motor cortex of ALS patients found no change in RIPK1 expression [9], while a separate study found it was increased in the spinal cord in areas of demyelination [18]. The discrepancy may be related to a role for pathogenic RIPK1 activation only in certain cell types, and/or a localization specifically to regions of white matter damage, which has also been reported in multiple sclerosis [20]. In the $SOD1^{G93A}$ mouse model, ablation of the obligatory necroptotic effector MLKL had no effect on disease severity or progression. While RIPK1 accumulated in spinal cord, it did not trigger the activation of the necroptotic machinery in the $SOD1^{G93A}$ mice, suggesting it may play a distinct unclear role in this condition [21].

Parkinson's disease: POTENTIAL BENEFIT (preclinical)
Several genes associated with Parkinson's disease are involved in mitochondrial homeostasis, leading to dysregulated mitophagy, mitochondrial fragmentation, and excessive ROS production. Preclinical models suggest that RIPK1 acts as a sensor for ROS and is activated in response to oxidative stress, which then triggers neuronal cell death [22]. The RIPK1 kinase inhibitor Necrostatin-1 has been shown to protect against the loss of dopaminergic cell viability and mitigate neuroinflammation in response to a deficiency in the mitochondria protein Opa1, or the neurotoxins 6-OHDA and MPTP [23; 24; 25; 26; 27; 28]. Necrostatin-1 also mitigated the activation of pro-inflammatory A1 astrocytes in the striatum [27]. While RIPK1 inhibition does not correct the underlying driver of mitochondrial dysfunction, it can mitigate downstream cell loss, and thus may slow disease progression.

Ischemic brain injury: POTENTIAL BENEFIT (preclinical)
RIPK1 dependent necroptosis is the dominant driver of cell loss in the context of ischemic brain injury, such as stroke or intracerebral hemorrhage [29]. RIPK1 kinase inhibitors have been shown to be neuroprotective in a variety of rodent models of ischemic brain injury by blocking necroptotic cell death [2].

Postoperative cognitive dysfunction: POTENTIAL BENEFIT (Preclinical)
In a rat model of postoperative cognitive dysfunction (POCD), the postsurgical production of pro-inflammatory cytokines and incidence of POCD were elevated in old animals (24 months), relative to young animals (2 months old) [30]. Older rats had lower levels of the endogenous RIPK1 inhibitor, TAK1. Inhibition of TAK1 increased the incidence of POCD in young rats, but could be prevented by treatment

with the RIPK1 inhibitor, Necrostatin-1. In a separate study, pretreatment with necrostatin-1 one hour prior to anesthesia prevented sevoflurane-induced cognitive dysfunction in aged rats [31]. This suggests that RIPK1 inhibitors may help mitigate the incidence of POCD in the elderly.

APOE4 interactions: Unknown

Aging and related health concerns: Tested RIPK1 inhibitors have not shown clinical benefit in autoimmune/inflammatory conditions thus far. Preclinical studies suggest they may help reduce inflammation-mediated damage and improve insulin sensitivity.

Types of evidence:

- 6 observational studies for RIPK1 expression in atherosclerotic tissue, tumors, adipose tissue, renal tissue, or liver tissue
- 3 RCTs testing GSK2982772 in autoimmune conditions (rheumatoid arthritis, plaque psoriasis, and ulcerative colitis)
- 1 RCT testing eclitasertib in cutaneous lupus erythematosus
- 1 RCT testing eclitasertib in covid-19
- Several laboratory studies (for Necrostatin series RIPK1 inhibitors)

Atherosclerosis: POTENTIAL MIXED BENEFIT (preclinical)
Necroptosis has been found to be activated in advanced carotid plaque samples from the Biobank of Karolinska Endarterectomy (BiKE) based on expression of phosphorylated MLKL, which is the downstream effector of necroptosis [32]. Expression of necroptosis mediators RIPK1, RIPK3, and MLKL is upregulated in vascular endothelial cells in the presence of atherogenic oxidized LDL (oxLDL) [32; 33]. The induction of these necroptotic factors may stem from the oxLDL mediated upregulation of vascular peroxidase 1 (VPO1) and the activation of □-catenin signaling. In hyperlipidemic patients, the plasma level of necroptotic mediators, including RIPK1, is positively correlated with VPO1 (R=0.8710) [33].

Treatment of atherosclerotic (ApoE-/-) mice with the RIPK1 kinase inhibitor Necrostatin-1 reduced the size of established lesions by 27% and reduced overall lesion burden by 68% [32]. Lesion size was similarly reduced through the use of RIP1 antisense oligonucleotides [34]. Necroptotic macrophages also play a key role in the pathogenesis of atherosclerosis as a source of proinflammatory cytokines and DAMPs which promote plaque inflammation and instability. This inflammatory response can be

significantly attenuated by the knockdown of RIP1 in macrophages [34], suggesting that RIPK1 inhibitors may protect against atherosclerosis-associated adverse events by targeting a key driver of plaque formation and instability.
However, another study found that RIPK1 has stage-dependent effects on atherosclerosis, such that treatment with the selective RIPK1 inhibitor GSK547 had short-term anti-atherosclerotic and long-term pro-atherosclerotic effects in the $ApoE^{SA/SA}$ mouse model of hypercholesterolemia and hypertension [35]. The initial anti-atherosclerotic effects were related to a reduction in vascular monocyte infiltration and inflammation, while the subsequent pro-atherosclerotic effects were associated with the accumulation of lipid filled foam cells by suppressing their cell death, as well as the inhibition of ApoA1 synthesis in the liver. This suggests that there may be a particular therapeutic window during which RIPK1 inhibitors may be best suited for atherosclerosis, and potentially other cardiovascular diseases.
A planned Phase 2 clinical trial testing the RIPK1 inhibitor GFH312 in patients with peripheral artery disease and intermittent claudication (NCT05618691) was withdrawn by the sponsor.

Diabetes: POTENTIAL BENEFIT (preclinical)
A genetic SNP analysis in the METSIM cohort of over 1,800 people identified five SNPs in linkage disequilibrium with RIP1 associated with 75-89% increased risk for diabetes (adjusted odds ratios [OR]: 1.75–1.89; $P<10^{-5}$) [34]. Individuals with the risk allele also had increased expression of RIP1 in adipose tissue.

The activation of cell death pathways is influenced by metabolic condition, and hyperglycemia can promote a shift from apoptosis to RIPK1 dependent necroptosis [36]. The core mediators of necroptosis, RIPK1, RIPK3, and MLKL have been found to be increased in the liver and adipose tissue of obese (ob/ob, high-fat diet) and type 2 diabetic (db/db) mouse models [37]. Inhibition of these factors using transgenic knockout lines or with the RIPK1 kinase inhibitor Necrostain-1 improved insulin sensitivity and glucose tolerance in these mice by regulating insulin signaling. RIP1 antisense oligonucleotides could also improve insulin resistance, and decrease fat mass (by 50-65%) in obese mice [34]. This suggests that RIPK1 inhibitors may improve glucometabolism.
Inhibition of RIPK1 and the induction of necroptosis may also protect against diabetes-related complications. Levels of the active necroptosis mediators, phosphorylated RIPK1/RIPK3/MLKL, were found to be elevated in the renal tubular epithelial cells of patients with diabetic kidney disease [38]. The level of phosphorylated MLKL was positively correlated with disease severity, particularly with respect to the degree of tubular injury. There was also an association between the necroptosis markers and the accumulation of lipid droplets in the cells, suggesting that lipid deposition may play a role in the

activation of necroptosis. Pretreatment with the selective RIPK1 inhibitor, RIPA-56 (300 mg/kg in mouse chow), prevented the activation of the necroptotic pathway, reduced renal lipid droplet accumulation and inflammation, and attenuated renal injury in high-fat diet fed db/db diabetic mice.

Inflammatory autoimmune diseases

RIPK1 is associated with both necrosis-associated inflammation and cell death independent inflammation. RIPK1 kinase activity plays a primary role in the initiation of multiple mechanisms of inflammatory cell death in response to activation of the TNFR1 receptor by TNFα. RIPK1 also plays a role in ER-stress induced activation of the inflammasome through induction of ROS production and mitochondrial dysfunction [39]. Therefore, RIPK1 is an attractive target for diseases associated with high levels of TNFα mediated inflammation. RIPK1 kinase inhibitors have been shown to reduce inflammation and cell loss in animal models of inflammatory autoimmune diseases [1; 20; 40]. However, to date, clinical trials testing RIPK1 inhibitors in rheumatoid arthritis, plaque psoriasis, ulcerative colitis, and cutaneous lupus erythematosus have not shown clinical benefit. Additional clinical trials testing RIPK1 inhibitors in rheumatoid arthritis and ulcerative colitis are ongoing. The RIPK1 inhibitor GDC-8264 is being tested in a Phase 1 trial in patients with acute graft-versus-host disease (NCT05673876).

Rheumatoid arthritis: NO BENEFIT WITH GSK2982772

A Phase 2 randomized, placebo-controlled clinical trial (NCT02858492) testing the RIPK1 inhibitor ***GSK2982772*** failed to show clinical benefit in patients with moderate to severe rheumatoid arthritis (n=52) [41]. GSK2982772 was tested at doses of 60 mg b.i.d. or t.i.d. for 84 days. Overall, there were no significant differences between GSK2982772 and placebo on disease-associated measures, including, Disease Activity Score in 28 Joints–C-reactive protein (DAS28-CRP) scores, ACR20/50/70 response, and rates of low disease activity and remission. As a result, clinical development of GSK2982772 has been discontinued for this indication.

The RIPK1 inhibitor, ***LY3871801*** (R552) is currently being tested in an adaptive randomized, double-blind, placebo-controlled Phase 2a/2b trial in adults with moderate to severe active rheumatoid arthritis (NCT05848258).

Plaque psoriasis: POTENTIAL MINOR BENEFIT

The RIPK1 inhibitor ***GSK2982772*** has been tested in a Phase 2a randomized, double-blind, placebo-controlled, repeat-dose trial in patients with active plaque psoriasis (n=65) (NCT02776033) [42]. Patients were treated orally with GSK2982772 at a dose of 60 mg b.i.d. or t.i.d. for 84 days. The Plaque Lesion Severity Sum (PLSS) improved in the 60 mg b.i.d. dose relative to placebo with a mean difference of -

18% at day 85, however, there was no significant difference relative to placebo in the 60 mg t.i.d. group, which may have been related to an unusually large improvement in the placebo group in this cohort possibly stemming from an imbalance in baseline PLSS scores. Similarly, a decrease in the Psoriasis Area and Severity Index (PASI) was observed in the 60 mg b.i.d. group relative to placebo (difference of -0.31), but not in the 60 mg t.i.d. group. Body Surface Area (BSA), a measure of disease severity, decreased from baseline in both GSK2982772 groups, with a difference from placebo of −1.41 for 60 mg b.i.d. and −1.27 for 60 mg t.i.d. on day 85. On the Physician Global Assessment (PGA), three patients in the 60 mg t.i.d. and two in the placebo group achieved a score of clear or almost clear. Reductions in immune cell infiltration to the skin were observed with GSK2982772. There were 28% and 29% reductions in CD3+ T cells, as well as 43% and 37% reductions in CD11+ myeloid dendritic cells in the dermis, in the 60-mg b.i.d. arm and 60-mg t.i.d. groups, respectively. Clinical development of GSK2982772 has been discontinued for this indication.

Ulcerative colitis: NO BENEFIT WITH GSK2982772
The RIPK1 inhibitor ***GSK2982772*** did not show clinical benefit in a Phase 2a randomized, double-blind, placebo-controlled trial in patients with active ulcerative colitis (n=36) (NCT02903966) [43]. GSK2982772 was tested in a placebo-controlled study at a dose of 60 mg t.i.d. for 42 days, followed by an open-label study for 42 days. Relative to patients in the placebo group, treatment with GSK2982772 was not associated with significant differences in histological disease activity, clinical disease activity or quality-of-life measures. By day 85, three patients (14%) treated with GSK2982772 achieved Mayo endoscopic scores of zero, while one patient (11%) in the placebo/open-label GSK2982772 group achieved a score of zero. There were no differences in levels of the systemic inflammatory marker C-reactive peptide (CRP) across groups. Based on the lack of efficacy, clinical development of GSK2982772 has been discontinued for this indication.
Eclitasertib (SAR443122; DNL758) is currently being tested in a randomized, double-blind, placebo controlled, dose-finding Phase 2 trial (NCT05588843) in patients with moderate to severe active ulcerative colitis.

Lupus: NO BENEFIT WITH ECLITASERTIB
The RIPK1 inhibitor ***eclitasertib*** (SAR443122; DNL758) was tested in a Phase 2 placebo-controlled proof of concept study in patients with cutaneous lupus erythematosus (n=78) for 12 weeks (NCT04781816). Based on the results of the study, the sponsor (Sanofi) decided to end clinical development of SAR443122 for this indication, as the study failed to meet its primary endpoint of percent change in cutaneous erythematosus disease area and severity index activity (CLASI-A) at week 12 (Fierce Biotech).

Liver disease: POTENTIAL BENEFIT (preclinical)
RIPK1 is involved in liver homeostasis, and inhibition of RIPK1 kinase activity has been shown to protect against hepatoxicity in rodents. Markers of necroptosis, including phosphorylated (activated) RIPK1 and RIPK3 have been found to be elevated in the livers of old mice (18-24 months old) relative to young mice (7 months old), which was accompanied by an increase in hepatic M1-type macrophages with age. This age-related hepatic inflammation could be reversed by treatment with the RIPK1 inhibitor necrostatin-1 [44]. Necroptosis markers were found to be elevated in hepatic tissue from patients with acute-on-chronic liver failure (ACLF) (n=417). Plasma RIPK3 levels predicted risks for disease progression and mortality [45]. Pre-treatment with the RIPK1 inhibitors, necrostain-1 and RIP56 attenuated disease severity in rat and mouse models of ACLF, respectively. In a model of acetaminophen induced acute liver failure, pretreatment with the RIPK1 kinase inhibitor Necrostatin-1 decreased the production of pro-inflammatory cytokines and reactive oxygen species (ROS), and protected against hepatocyte cell loss [46]. The use of RIPK1 inhibitors may protect the liver by conferring resistance to cellular stressors. However, a separate study found that the reprogramming of necroptosis responses may be a more effective strategy relative to the inhibition of necroptosis for preventing cancer in patients with liver disease [47]. Hepatocarcinogenesis was found to be associated with an NF-kB driven reprogramming of hepatocytes into a sublethal state characterized by low levels of RIPK3. In this case, activation of necroptosis machinery does not trigger cell death, but rather results in leaky cells that drive a pro-carcinogenic inflammatory state. Therefore, RIPK1 modulators may need to be tailored for different disease indications.

Cancer: POTENTIAL BENEFIT/HARM DEPENDING ON TUMOR TYPE (preclinical)
The activation of necroptosis has been proposed as a possible mechanism to promote tumor cell death via necrosis in the context of resistance to chemotherapeutic agents which primarily promote tumor cell death via apoptosis. However, there is evidence to suggest that RIPK signaling may play different roles in different tumor types based on the tissue type and tumor microenvironment. Necroptotic mediator (RIPK1, RIPK3, or MLKL) expression has been found to be low or absent in a variety of cancer cell lines, such as breast cancer, colorectal cancer, leukemia, ovarian cancer, cervical cancer, and melanoma, which is associated with worse prognosis for some types of cancer [48; 49]. Meanwhile, RIPK1 expression is upregulated in other types of cancer, such as glioblastoma, lung cancer, and pancreatic cancer, where RIPK1 signaling is oncogenic. Development of the RIPK1 inhibitor GSK095 for pancreatic cancer by GlaxoSmithKline was terminated (Fierce Biotech).

Covid-19: NO CLEAR BENEFIT

Eclitasertib (SAR443122; DNL758) was tested in a randomized, placebo-controlled Phase 1b clinical trial (NCT04469621) in patients hospitalized with covid-19 (n=68). The study did not meet its primary endpoint of change in the systemic inflammatory marker CRP at day 7, however due to early discharge, day 7 CRP values were not available for around half of the patients, and the concomitant use of corticosteroids in ~65% of patients may have impacted the ability to detect a meaningful change in this metric [50; 51]. The adjusted change of CRP for those treated with eclitasertib relative to placebo was 0.85 (90% CI 0.49 to 1.45). There was a trend toward a faster decline in CRP, as the median time to a 50% improvement in CRP was 3 days in the eclitasertib group and 5 days in the placebo group. Development of eclitasertib for this indication has been discontinued.

SIR1-365 was tested in a randomized, double-blinded, placebo-controlled Phase 1 trial (NCT04622332) in patients with severe covid-19 (n=45). The study was completed in 2021, but no results have been made available to date.

Safety: RIPK1 inhibitors have been well-tolerated in Phase 1 studies. Adverse events tend to be drug-specific, and related to off-target effects. Headache and gastrointestinal events are the most common. Elevated liver enzymes were observed with some drugs.

Types of evidence:

- 10 Phase 1 clinical trials in healthy volunteers
- 1 Phase 1 trial for eclitasertib in covid-19 patients
- 3 Phase 2 clinical trials for GSK2982772
- 2 Phase 2 trials for SAR443060
- Numerous laboratory studies

Attempts to target apoptotic cell death through the use of caspase inhibitors for inflammatory diseases have been hindered by safety concerns, such as the compensatory induction of necrotic cell death stemming from the induction of RIPK1 mediated necroptosis in the absence of caspase-8 [2; 52]. Targeting necroptosis is expected to be a safer alternative since, unlike apoptosis, it generally plays very little role in homeostatic mechanisms in healthy tissue. The genes associated with necroptosis are only found in higher order organisms, and necroptosis is thought to have evolved as a mechanism to abort

defective embryos [1]. Therefore, necroptosis appears to be a developmental program that gets reactivated under pathological conditions involving excessive levels of cell stress.

Of the major necroptotic factors, RIPK1 is expected to be the best drug target with respect to both safety and efficacy for inflammatory and neurodegenerative diseases. Loss of MLKL shows less protection in animal models, while inhibition of RIPK3 can trigger apoptosis in some cases [1]. Necroptosis is activated in response to some viral pathogens which is driven by TLR activation of RIPK3, thus targeting only RIPK1 preserves this host defense mechanism [2]. Although RIPK1 activation can play different roles in different cell types depending on the presence or absence of interacting signaling partners, its primary role is in promoting necroptotic cell death and inflammation [53]. Furthermore, RIPK1 inhibitors are also thought to be a safer alternative to TNFα inhibitors for CNS indications because TNFR2 is important for neural regeneration and RIPK1 selectively affects TNFα mediated processes downstream from the TNFα-TNFR1 interaction [2].

Inhibition of RIPK1 kinase activity through transgenic models or small molecules, such as the Necrostatin compounds, have been effective in a variety of animal models, and no major safety concerns have been noted in these studies. However, the Necrostatins are not amenable for clinical development due to their low metabolic stability [3].

The good safety profile for RIPK1 inhibitors is due, in part, to their high degree of specificity and selectivity, which is highly unusual for kinase inhibitors, since they tend to target multiple classes of kinases. The selectivity of these allosteric small molecule RIPK1 inhibitors stems from the kinase structure of RIPK1 due to the unusual flexibility of its allosteric back pocket [53].

Several RIPK1 kinase inhibitors have been tested in Phase 1 RCTs. Although the clinical development of some of these compounds has been discontinued due to potential safety signals, these adverse events appear to be related to off-target effects, and the targeting of RIPK1 itself appears to be relatively safe, at least in short-term studies.

SAR443060 (DNL747) is a brain penetrant RIPK1 inhibitor that was tested in a double-blind, placebo controlled RCT in healthy volunteers (n=56) for safety, tolerability, pharmacokinetics, and pharmacodynamics, as well as in patients with ALS and in patients with AD [10].
Healthy volunteers: SAR443060 was tested at doses from 100 to 400 mg in capsules as a spray-dried nanosuspension formulation or a micronized drug substance as oral single doses in the single ascending

dose (SAD) study and for 14 days b.i.d. in the multiple ascending dose (MAD) study, as preclinical modeling predicted a half-life compatible with twice daily dosing [10]. The starting dose was 45-fold below the original 200 mg/kg/day no observed adverse event level (NOAEL). The adverse event profile was similar between drug and placebo. The most common adverse events not related to the medical devices used for assessments were headache (6.1%), diarrhea (6.1%), and nasopharyngitis (6.1%) in the SAD study, and nausea (4.7%) in the MAD study. There were no clinically significant changes in the clinical laboratory results, vital signs, 12-lead ECGs, physical examinations, or suicidal ideations.
The original NOAEL was determined to be 200 mg/kg based on 28-day GLP toxicology studies. In cynomolgus monkeys, adverse events to the immune system, including the lymph nodes, bone marrow, and spleen, as well as cutaneous lesions to the skin stemming from lymphocytic infiltration, were detected at 1,000 mg/kg/day. A subsequent three-month toxicology study in monkeys run in parallel to the phase 1 trial in healthy volunteers detected additional immune-related serious toxicology, including thrombocytopenia, anemia, and bleeding at doses ≥40 mg/kg/day (20 mg/kg b.i.d.). As a result, the NOAEL was reduced to 20 mg/kg. Following a nine-month GLP toxicology study in which anemia was observed in monkeys at a dose of 20 mg/kg/day (10 mg/kg b.i.d.), the NOAEL was further reduced to 6 mg/kg.
ALS and AD: Due to these changes to the NOAEL, the dose for 28-day phase 1 trials in patients with ALS (n=15) and AD (n=16) was lowered from 200 mg b.i.d. to 50 mg b.i.d. [10]. In the AD trial, adverse events of special interest included mild, asymptomatic, and self-limiting anemia in two subjects taking the study drug, as well as events in five subjects in the placebo group. In the ALS trial there was a case of mild erythema considered possibly drug related. Aside from the cases of anemia, there were no other clinically significant changes in laboratory tests, vital signs, 12-lead ECGs, physical and neurological examinations, or suicidal ideations.
During the nine-month open label extension trial in ALS patients (n=8), two patients had serious adverse events considered not drug related (pneumonia aspiration and ALS worsening). Four patients experienced adverse events related to skin disorders, and one patient experienced liver enzyme elevations, which were attributed to riluzole [16].
Clinical development of SAR443060 was discontinued based on the preclinical toxicology findings [10]. The toxicities are expected to be drug related, stemming from off-target effects, rather than target/class related.

GSK2982772 is a RIPK1 inhibitor that binds to the back allosteric pocket of RIPK1 and was tested in several clinical trials, including in healthy volunteers, patients with rheumatoid arthritis, and patients with ulcerative colitis. It was generally well-tolerated across studies, though there were some cases of

asymptomatic elevations in liver enzymes, as well as isolated cases of cardiac abnormalities in individuals with predisposing risk factors. Due to its short half-life, it is typically dosed three times per day.

Healthy volunteers: In a Phase 1 double-blind, placebo controlled RCT in healthy male volunteers aged 18-65 (n=79) for safety, tolerability, pharmacokinetics, and pharmacodynamics (NCT02302404), GSK2982772 was tested in a SAD study up to 120 mg and in a MAD study from 20 mg once daily up to 120 mg twice daily [54]. The C_{max} of the drug was 1 hour and the half-life ($T_{1/2}$) was 2-3 hours, with no evidence of drug accumulation with multiple dosing. The drug showed dose-dependent target engagement based on based on an immunoassay specific for RIPK1 and the inhibition of MIP-1a and MIP-1b. The adverse events were mild, and the drug was generally well-tolerated. The most common adverse events were dermatitis and headache.

GSK2982772 was also tested in randomized, double-blind, placebo-controlled studies in Western subjects (NCT03305419) at doses of 20 mg three times per day (t.i.d.), 240 mg t.i.d., or 360 mg b.i.d. for one day or at doses of 120 mg t.i.d., 240 mg t.i.d. for 14 days, and in Japanese subjects (NCT03590613) at doses of 60, 120, 240 mg t.i.d. for one day [55]. GSK2982772 was generally well tolerated. In the SAD/MAD study, the most common adverse event was contact dermatitis, which was attributed to the ECG and telemetry electrodes. In the SAD study, all adverse events were mild, and the incidence of adverse events was highest in the GSK2982772 360 mg b.i.d. (78%), followed by the 120 mg t.i.d. group (67%). In the MAD study, the incidence of adverse events was highest in the 240 mg t.i.d. (92%). One participant in the GSK2982772 120 mg t.i.d. group experienced a severe adverse event of an asymptomatic elevation of the liver enzymes alanine aminotransferase (ALT) of 7.5 times the upper limit of normal (ULN) and aspartate aminotransferase (AST) of 4.5 times the ULN one day after the last dose, but levels returned to normal within four weeks. Six participants experienced arrhythmia events that led to a study pause, but were later determined to be unrelated to the study drug. In the study in Japanese participants, one subject in the 60 mg t.i.d. group experienced a mild increase in ALT to 84 IU/l (normal range is 5 to 45 IU/l) and a mild increase in AST increase to 100 IU/l (normal range 10 to 40 IU/l). The drug pharmacokinetics were similar in the two populations, and the drug did not significantly affect CYP3A4 activity *in vivo*.

A once daily, modified-release formulation of GSK2982772 was tested in a Phase 1 (NCT03266172) trial in healthy volunteers (n=45). It was tested at a dose of 120 mg, administered in two matrix minitab formulations (MT-8 h and MT-12 h), in comparison to the standard immediate release formulation [56]. The pharmacokinetics of the delayed release formulations were consistent with once daily dosing. Drug-related adverse events included mild dizziness in one participant taking the immediate release formulation, and mild jaw pain and headache in one participant taking the MT-12 h formulation. There

were no clinically important changes for laboratory assessments, or vital signs. One participant experienced asymptomatic bigeminy on his ECG two hours after a single dose of 120-mg MT-12 h, and discontinued the study.

Rheumatoid arthritis: In patients with rheumatoid arthritis receiving GSK2982772 at a dose of 60 mg b.i.d. or t.i.d. for 84 days, the most commonly reported adverse events were arthralgia and headache, though in both cases the incidences were <10%, and below levels in the placebo group [41]. There were two adverse events leading to study withdrawals in the GSK2982772 t.i.d. group. One patient had an event of severe visual disturbance and retinal vein thrombosis at day 66, while another patient experienced a case of moderate bronchitis. Another patient in this group with a history of ventricular arrhythmia experienced sinus arrhythmia. There were no changes in hematology or clinical chemistry measurements, or vital signs.

Psoriasis: Patients with active plaque psoriasis were also treated with GSK2982772 at a dose of 60 mg b.i.d. or t.i.d. for 84 days [42]. In this population, adverse events were balanced across study arms, and the most commonly reported events were nasopharyngitis and headache. A case of moderate intensity herpes zoster in the GSK2982772 b.i.d. cohort led to study withdrawal. There were no clinically relevant changes in laboratory assessments, ECGs and vital signs.

Ulcerative colitis: GSK2982772 was tested in patients with active ulcerative colitis at a dose of 60 mg t.i.d. for 42 days [43]. The most commonly reported adverse events with GSK2982772 were headache (33%), followed by nasopharyngitis (21%), nausea (13%), and abdominal pain (13%). There were no clinically relevant changes in clinical chemistry or hematology values. A participant experienced a QTcF increase between 450 and 480 ms.

SAR443820 (DNL788) is a BBB penetrant RIPK1 inhibitor with an IC_{50} of 3.16 nM in human PBMCs. It was tested in a Phase 1 randomized, double-blind, placebo-controlled trial (NCT05795907) in healthy volunteers (n=84) [57]. In the SAD study, SAR443820 was tested as single oral doses from 10 mg up to 40 mg, and was tested as repeated oral doses ranging from 10 once daily (q.d.) up to 20 mg b.i.d. in the MAD study. In an open-label PK-CSF sub-study, SAR443820 was administered as two single doses (10 mg and 40 mg, each dose per cohort) approximately 30 min after breakfast. SAR443820 was generally well-tolerated, with no drug-related serious adverse events. The most common adverse events were dizziness and headache. There were no clinically meaningful changes in laboratory values, vital signs, or electrocardiogram parameters. In the MAD study, one participant in the SAR443820 15 mg b.i.d. group experienced an increase in the liver enzyme ALT of 2.29 times the ULN on day 9, which increased up to 2.71 ULN on day 15, and was associated with an increase in AST of 1.43 times the ULN, while other liver function parameters were within normal range. In the PK-CSF sub-study, one participant in the

SAR443820 10 mg group experienced an increase in ALT of 1.5 times the ULN on day 2, which increased up to 2.4 times the ULN on day 7. The liver enzyme elevations were asymptomatic in both cases. SAR443820 showed no potential for CYP3A4 induction.
The mean CSF-to-unbound plasma concentration ratio ranged from 0.8 to 1.3. The reduction of pS166-RIPK1 measured in PBMC lysates was used as a measure of target engagement. The maximum inhibition ranged from 82% to 97% after a single dose, and greater than 80% inhibition was maintained up to 24 h following the last morning dose in the 14-day MAD study.

GFH312 is a selective small molecule inhibitor of RIPK1 with an IC_{50} of 40 nM. It was tested in a randomized, double-blinded, placebo-controlled Phase 1 trial (NCT04676711) in healthy volunteers (n=76) [58]. In the SAD study it was tested in single oral doses from 5 mg up to 500 mg, and in the MAD study, it was tested in once-daily repeated oral doses up to 200 mg for 14 days. The drug was not dose proportional across dose cohorts, and the intersubject variability was high, which may have been related to different absorption kinetics for the 5 mg vs the 100 mg tablets. There were no trends related to the frequency or severity of treatment-emergent adverse events with increasing dose. There were no serious adverse events. The most common treatment-emergent adverse event was headache (21.1%). Treatment-emergent adverse events were more common in the fasted state related to the fed state. There were no clinically significant changes in laboratory measures, vital signs, physical findings or ECG recordings. Two participants in the GFH312 group experienced Grade 2 elevations in blood creatine, resulting in study discontinuation. Additionally, there were trends to elevations in serum creatinine in the higher-dose cohorts, in both absolute terms and in relation to baseline. In terms of target engagement, reductions in the level of pRIPK1 level of up to 80% were maintained for over 24 hours for single doses, and for over the course of the 14-day dosing period in the MAD study.

GDC-8264 is selective and reversible small molecule inhibitor of RIP1 kinase activity with a PK-PD model estimated IC_{50} of 0.58 ng/mL. It was tested in a Phase I, randomized, placebo-controlled, ascending-dose trial in healthy volunteers (n=53) [59]. Single doses were tested from 5 to 225 mg, and multiple once daily doses of 50 mg and 100 mg were tested for up to 14 days. GDC-8264 was well-tolerated, as all adverse events were of mild severity, and the percentage of adverse events was similar across drug and placebo arms. The most frequently reported treatment emergent adverse events were contact dermatitis (15.8% of subjects), headache (15.8% of subjects), and nausea (13.2% of subjects). Additional reported treatment emergent adverse events included abdominal pain, diarrhea, dyspepsia, gingivitis, somnolence, and dizziness. One participant in the MAD 100-mg GDC-8264 q.d. cohort experienced earlobe inflammation lasting 14 days. No significant changes were observed in clinical laboratory results,

vital signs, ECGs, or physical examinations. In a food effect study testing a 75 mg dose of GDC-8264 after a high fat meal, no significant food effect was observed on the drug's pharmacokinetic properties. Greater than 90% inhibition of CCL4, the study's marker of target engagement, was observed after single doses, and was maintained throughout the 14-day MAD study, lasting an additional seven days following the last dose.

DNL104 is a BBB penetrant RIPK1 inhibitor with an IC_{50} of 39.71 ± 1.15 nM that was tested in a randomized, placebo-controlled, Phase 1 SAD and MAD study in healthy volunteers (n=68) [60]. DNL104 was tested in single doses from 5 mg to 225 mg, and was found to be well-tolerated. Adverse events were mild and included headache, fatigue, paresthesia, somnolence, pollakiuria, and presyncope. In the MAD study, DNL104 was tested in multiple doses of 50 mg and 100 mg for 14 days. The most frequently reported events were headache, dizziness and somnolence, and nausea, back pain, paresthesia, and feeling hot. Elevations in liver function tests were observed in six participants in the MAD study. One participant in the 50 mg group exhibited an abnormal liver function test of moderate severity after the end of dosing that lasted 35 days. Three participants experienced mild elevations in ALT (1.5-fold ULN) and AST that returned to normal within 50 days of the first dose. The other three participants experienced substantial increases in ALT (15-fold, 2.7-fold, and 3.2-fold ULN, respectively) and AST (6-fold, 1.1-fold, and 2-fold ULN, respectively), emerging after the last dose had been administered. One participant also has an increase in lactate dehydrogenase (1.2-fold ULN) along with an increase in gamma GT three-fold from baseline, but still within normal limits. The ratio between ALT and alkaline phosphatase (R-ratio) indicates hepatocellular drug-induced liver injury when values are >5, and a mix of cholestatic and hepatocellular injury at values >2. An R-ratio >5 was observed in one participant, and an R-ratio >2 was observed in two other participants. These individuals also exhibited elevated eosinophil counts (1.9-fold, 2-fold, and 2-fold of ULN, respectively), and IgE levels (1.8-fold, 2.2-fold, and 4.9-fold of ULN, respectively), which returned to normal within 60 days of the first dose.
Hepatobiliary impairment was also identified in preclinical toxicity studies of DNL104 in rats and dogs consistent with a mild cholestasis. The late onset drug-induced liver injury appears to stem from an immunoallergic reaction to DNL104 and/or its metabolites, as an off-target effect. Based on the liver toxicity findings, clinical development of DNL104 has been discontinued.

Eclitasertib (SAR443122; DNL758) is a peripherally restricted RIPK1 inhibitor that has been tested in clinical trials for covid-19 (n=68) and cutaneous lupus erythematosus (n=78).
Healthy volunteers: In a Phase 1 trial in healthy volunteers, eclitasertib was found to be safe and well tolerated at single doses ranging from 10 to 800 mg, and in multiple doses for 14 days ranging from 50

to 600 mg [50]. Doses up to 500 mg/kg/day were not associated with any safety signals in a preclinical toxicology study in non-human primates.
Covid-19: In the phase 1b trial in patients hospitalized with covid-19, patients were treated with eclitasertib at a dose of 300 mg b.i.d. for up to 14 days [50; 51]. Adverse events were balanced across treatment arms, most of which were grade 2 in the eclitasertib arm, and grade 3 in the placebo arm, and were associated with covid-19. Increases in the liver enzyme ALT were observed in two out of 20 (10.0%) participants in the placebo group and six out of 47 (12.8%) participants in the eclitasertib group. These ALT increases were reversible, and there were no other changes in liver function parameters. There were four deaths due to covid-19 or related complications: two in the placebo group, and two in the eclitasertib group. There were also 15 serious adverse events due to covid-19, which were balanced across the groups. There were no clinically meaningful changes in vital signs parameters.
Lupus: It was reported that eclitasertib was well-tolerated in lupus patients, however, detailed results have not yet been made available.

SIR1-365 was tested in two Phase 1 trials, one in patients with severe covid-19 (n=45) (NCT04622332) and one in healthy young adults (n=20) examining the effect of a single oral dose of 300 mg on the activities of CYP450 isoform 3A (ACTRN12621000400864). Safety results have not been made available for either study.

LY3871801 (R552) has been tested in several Phase 1 trials, including in a trial in healthy volunteers (n=15) (NCT05222399), a trial in healthy Asian and non-Asian participants (n=32) (NCT05960851), a drug interaction study in healthy volunteers (n=39) examining interactions with methotrexate, warfarin, dextromethorphan, midazolam, and repaglinide (NCT05602675), as well as a pharmacokinetic study using [^{14}C]-LY3871801 in healthy male participants (n=8) (NCT06049108). Study results have not yet been disclosed, but the regulatory approval to conduct a Phase 2 trial in patients with rheumatoid arthritis suggests that no major safety concerns were identified in the Phase 1 studies.

Sources and dosing:

To date, all of the clinically tested RIPK1 inhibitors have been small molecule oral formulations. No clinical validated doses have been established, and none have been approved for any indication.

Denali Therapeutics has partnered with Sanofi for the development of ***SAR443820*** (DNL788) for neurological diseases, including ALS, MS, and AD, and for the development of the peripherally restricted

eclitasertib (SAR443122; DNL758) for peripheral autoimmune conditions. SAR443820 has been tested at repeated oral doses up to 20 mg b.i.d. The doses tested in the ongoing ALS and MS trials have not been disclosed, but it is being tested twice per day in ALS patients and once per day in MS patients. Eclitasertib was tested in repeated oral doses up to 600 mg in healthy volunteers, and was tested in covid-19 patients at a dose of 300 mg b.i.d. The dose being tested in patients with rheumatoid arthritis has not been disclosed.

Rigel Pharmaceuticals has partnered with Eli Lilly and Co., for the clinical development of ***LY3871801*** (R552) for autoimmune and inflammatory conditions. It is being tested as an oral formulation, and the doses have not been disclosed.

GFH312 is in clinical development by GenFleet Therapeutics and has been tested in repeated oral doses up to 100 mg/day in healthy volunteers.

GDC-8264 is in clinical development for inflammatory conditions by Genentech and has been tested in repeated oral doses up to 200 mg/day in healthy volunteers. It is currently being tested at doses of 35 mg/day and 75 mg/day in patients with acute graft-versus-host disease.

SIR2446 is in clinical development for neurological diseases by Sironax.

Research underway:

SAR443820 (DNL788) is currently being tested in Phase 2 clinical trials for ALS (NCT05237284), which is expected to be completed in 2027, and MS (NCT05630547), which is expected to be completed in 2025.

Eclitasertib (SAR443122; DNL758) is currently being tested in a Phase 2 clinical trial in adult patients with moderate to severe active ulcerative colitis (NCT05588843), which is expected to be completed in 2026.

LY3871801 (R552) is currently being tested in an adaptive Phase 2a/2b clinical trial in adult patients with active moderate to severe rheumatoid arthritis (NCT05848258), which is expected to be completed in 2026.

GDC-8264 is currently being tested in a Phase 1 trial in patients with acute graft-versus-host disease in patients who have undergone allogeneic hematopoietic stem cell transplantation (NCT05673876), which is expected to be completed in 2025.

SIR2446 is currently being tested in a Phase 1 trial in healthy volunteers (ACTRN12621001621808).

There are medicinal chemistry efforts underway to develop novel RIPK1 inhibitors that have better pharmacokinetic properties (recently published efforts include [61; 62; 63]). There are also several companies developing RIPK1 inhibitors which are still in preclinical development stages, including Anaxis Pharma, and Voronoi Pharma. Voronoi is a Korean biotech developing VRN04 for inflammatory autoimmune conditions, including inflammatory bowel disease.

Search terms:

Pubmed, Google: RIPK1 inhibitors

Alzheimer's, Parkinson's, ALS, neurodegeneration, inflammation, aging, diabetes, cardiovascular, cancer, necroptosis, safety, clinical trials

Websites visited for RIPK inhibitors:

- Clinicaltrials.gov (SAR443820, SAR443122, DNL747, LY3871801, GDC-8264, GFH312, GSK2982772)
- PubChem GSK2982772, Eclitasertib

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