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.

HDAC3 Inhibitors

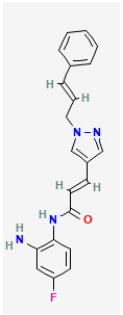
Evidence Summary

HDAC3 regulates gene programs involved in inflammation, metabolism, cognition, and cell survival. Drug-like selective inhibitors have not been developed. Cell specific inhibition may be needed for safety.

Neuroprotective Benefit: Acute HDAC3 inhibition may protect against pathological neuroinflammation following neuronal damage. Alleviating excessive HDAC3 activity in neurons may improve learning and memory in neurodegenerative disease.

Aging and related health concerns: HDAC3 inhibitors likely have the most utility in cancer. Cell type targeted inhibitors could benefit type 2 diabetes and atherosclerosis by modulating lipid metabolism.

Safety: Selective HDAC3 inhibitors suitable for clinical use have not been developed. Animal studies suggest acute HDAC3 inhibition is likely safe, but long-term safety is unknown. The safety and efficacy profile will likely vary across drugs in this class.

Availability: Tool compounds are available for research use	**Dose**: Not established	**RGFP-966** (not suitable for clinical use)
Half-life: N/A	**BBB**: Varies based on drug	**Chemical formula:** $C_{21}H_{19}FN_4O$
Clinical trials: None	**Observational studies**: Elevated HDAC3 is associated with poor prognosis in several cancers.	**MW**: 362.4 g/mol  Source: PubChem

What is it?

Histone deacetylases (HDACs) are a class of enzymes that act as epigenetic regulators, meaning that they regulate the ability of genes to be turned on and off by binding to and modifying chromatin [1]. HDACs remove acetyl groups from lysines on histones, which makes the chromatin more compact, effectively repressing genes by making it harder for the transcription machinery to access them. They work in concert with an opposing class of enzymes, histone acetyl transferases (HATs), thus the balance of activity between HDACs and HATs influences the gene expression profile in a given cell. This dynamic is highly cell type specific, such that changing the activity of HDACs or HATs can have very different effects in different cells. HDACs can also influence gene transcription through non-enzymatic activity, whereby they associate with other proteins as part of repressive complexes that can regulate the binding of the transcription factors that turn on or off a particular gene. HDAC3 is a class 1 HDAC [2]. Unlike other members of this class, HDAC3 can localize to both the cytoplasm and the nucleus. HDAC3 has been shown to participate in multiprotein repressive complexes with NCoR and SMRT [3]. Through these complexes, HDAC3 regulates the expression of genes associated with lipid metabolism, including the liver X receptors (LXRs) and peroxisome proliferator-activated receptors (PPARs), and thus has been implicated in obesity and type 2 diabetes [4]. The activity of HDAC3 can be regulated by post-translational modifications that impact its localization and ability to interact with chromatin or other

proteins [5]. HDAC3 is the most highly expressed HDAC in the brain, and abnormal HDAC3 has been implicated in neurodegenerative disease [2]. Dysregulated HDAC3 is also a hallmark and prognostic factor of several types of cancers [6]. As a result, HDAC3 inhibitors have been proposed as anti-cancer therapeutics with a better therapeutic profile than current broad spectrum HDAC inhibitors. However, due to the high degree of similarity across the HDACs, it has been difficult to develop inhibitors that are highly specific and selective for HDAC3 [7]. Additionally, there have been challenges in developing inhibitors with pharmacokinetic properties suitable for clinical development and use. As a result, selective HDAC3 inhibitors are still in the early preclinical development phase. Most of the research regarding the potential utility of HDAC3 inhibitors in disease models comes from the use of the blood brain barrier (BBB) penetrant drug RGFP-966. This drug is three- and five-times more selective for HDAC3 relative to HDAC1 and HDAC2, respectively, but only modestly selective relative to HDAC6 [7; 8]. Due to the combination of the moderate selectivity profile and compound profile that is unsuitable for clinical development, the results of research studies using RGFP-966 should be interpreted with caution, as they may not provide a reference for the anticipated therapeutic profile of selective HDAC3 inhibitors. It is likely as they are developed, each HDAC3 inhibitor will have a unique profile, and will need to be evaluated individually.

Neuroprotective Benefit: Acute HDAC3 inhibition may protect against pathological neuroinflammation following neuronal damage. Alleviating excessive HDAC3 activity in neurons may improve learning and memory in neurodegenerative disease.

Types of evidence:

- 3 gene association studies for HDAC3 SNPs and schizophrenia
- 1 study assessing HDAC3 expression in human postmortem brain tissue
- Numerous laboratory studies

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

HDAC3 inhibitors have not been tested in humans, so their ability to impact cognition has not been established. However, HDAC3 is implicated in cognitive function and the regulation of neurotransmitters based on gene association studies, namely its association with schizophrenia. A family-based genetic association including 951 Caucasian participants identified several single nucleotide polymorphisms (SNPs) in HDAC genes associated with schizophrenia, including a SNP (rs14251) in HDAC3 [9]. By

affecting transcriptional regulation of HDAC3 in the 5' UTR, this SNP is hypothesized to alter the enzymatic activity of HDAC3. The association of this SNP with schizophrenia was further validated in two case-control studies in a Chinese Han population, including 4,244 and 2,711 participants, respectively [10]. In a separate unpublished study, another SNP in the 5' region, rs11742646, was associated with cognitive performance on the Stroop test, which is a measure of cognitive flexibility, in schizophrenia in a Finnish population [9]. A genetic analysis of 223 Swedish adolescents stratified for risk for psychiatric disorders identified five SNPs associated with gene expression profiles in the brain reflective of psychiatric disease, including the rs2530223 SNP in HDAC3 [11]. HDAC3 chromatin remodeling was associated with levels of protocadherins, which are cell adhesion molecules important for proper neuronal wiring. A network analysis suggests that the impact of these SNPs to neuropsychiatric conditions and cognitive flexibility is related to their modulation of GABAergic neurotransmission [11].

Human research to suggest benefits to patients with dementia:

HDAC3 inhibitors have not been clinically tested, however, there is evidence from postmortem human brain tissue to suggest that the expression and/or activity profile of HDACs is altered in the context of Alzheimer's disease (AD) [2]. In frontal cortex tissue, protein levels of HDAC 1,2 were decreased by 32%, while levels of HDAC5 and HDAC6 were increased by 47% and 31%, respectively, in AD patients relative to age-matched controls [12]. Similar alterations were seen in retinal tissue. HDAC3 levels were not significantly altered, which is consistent with preclinical models suggesting that HDAC3 is altered at a functional level rather than at the expression level [2]. If this is the case, then it will be critical to determine the pattern of post-translational modifications and other regulatory features that are specifically altered in AD in order to most effectively modulate/ normalize HDAC3 activity.

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

Learning and memory: Through regulation of the acetylation status of histones and non-histone proteins, HDACs regulate the recruitment of transcription factors to DNA, resulting in the activation or repression of gene expression [2]. HDAC3 is the most highly expressed HDAC in the brain and is essential for brain development, such that global knockout of HDAC3 is embryonic lethal in mice [2]. HDAC3 is highly expressed in the hippocampus and has been shown to be involved in the regulation of gene networks critical for learning and memory. This is primarily mediated by regulating the activation of the transcription factor CREB (cAMP Response Element-Binding Protein) and its downstream pathways, most notably the brain derived neurotrophic factor (BDNF)-TrkB signaling pathway [13]. BDNF promotes

neurogenesis and the mechanisms of synaptic plasticity which underlie learning and memory. HDAC3 is a negative regulator of this CREB-mediated learning and memory program, such that excessive HDAC3 activity can impair cognition. Some of the genes involved in memory formation that are repressed by HDAC3 include Per1 and Nr4a2 [2; 13; 14].

There is evidence from preclinical models to suggest that this repressive activity of HDAC3 is enhanced in the context of neurodegenerative disease, including AD [2]. Unlike the other class I HDACs which are exclusively localized to the nucleus, HDAC3 is found in the nucleus, the cytoplasm, and the plasma membrane [2]. Thus, rather than being regulated at the expression level, its activity appears to be heavily influenced by its cellular localization. In neurodegenerative disease models, HDAC3 localization has been shown to be shifted from the cytoplasm to the nucleus, where it can bind to and regulate chromatin [2]. Additionally, HDAC3 forms multiprotein complexes. The presence or absence of its partner proteins provides for the cell-type and context-dependent activity of HDAC3. In the context of neurodegenerative disease, the increased nuclear interaction between HDAC3 and HDAC1 appears to be a key driver of the repression of genes required for neuronal survival and plasticity, ultimately resulting in neurotoxicity [2]. A reduction in neuronal activity, due to stress or damage, may underlie the increased interaction between HDAC1 and HDAC3, as levels of activity-dependent interaction partners decline.

As a result of its role in learning and memory, HDAC3 inhibitors have been tested in preclinical models of AD and cognitive impairment. The nuclear levels of HDAC3 were found to be elevated in the hippocampus of six- and nine-month-old APPswe/PS1dE9 mice, suggesting that HDAC3-mediated repression of learning and memory genes may be increased in these animals [15]. Lentiviral-mediated inhibition of hippocampal HDAC3 attenuated Aβ levels, microglial activation, and spatial memory deficits, based on Morris water maze performance, but did not impact the levels of phosphorylated tau [15]. In the same AD mouse model, treatment with the HDAC3 inhibitor RGFP-966 at a dose of 30 mg/kg i.p. starting at six months of age did not reliability improve contextual fear memory or promote synaptogenesis in a reporter assay, while a broader HDAC 1,2,3 inhibitor (RGFP-963) was more effective on these measures [16]. Meanwhile, in the 3xTg-AD mouse model, treatment with the HDAC3 inhibitor RGFP-966 (10 mg/kg i.p. daily) for three months starting at nine months of age reduced tau phosphorylation at Thr181, Ser396, and Ser202, in a brain-region specific manner [8]. RGFP-966 also decreased tau phosphorylation in AD patient iPSC-derived neurons, though the impact to particular residues was inconsistent across patients. Studies in rodent hippocampal slices have found that RGFP-966 treatment allowed for the preservation of synaptic plasticity (LTP) following exposure to Aβ42 oligomers [17; 18]. RGFP-966 treatment (10 mg/kg s.c.) also attenuated deficits in synaptic plasticity (LTP) in radiation (30 cGy) exposed mice [19]. The protective effect on plasticity appears to involve the

activation of CREB signaling pathways [18]. RGFP-966 did not significantly impact measures of synaptic plasticity (LTP induction) in healthy animals [17]. However, treatment with the HDAC3 inhibitor PT3 (25 mg/kg i.p.) for ten days in adult wildtype (C57Bl/6) male mice did improve performance on the novel object recognition task, suggesting that impacts to synaptic plasticity and cognition may be influenced by the potency and selectivity profile of a given HDAC3 inhibitor [13]. The discrepancies across studies may be attributed to the context-dependent nature of HDAC3, such that differences in experimental models and conditions could lead to different downstream effects. Disparities between selective genetic deletion of HDAC3 and HDAC3 inhibitors would also be expected, since HDAC3 has both enzymatic and non-enzymatic activity. Furthermore, the microbiome has been found to regulate HDAC3 activity via the production of short chain fatty acids (SCFAs) [20]. The SCFA butyrate, which is associated with neuroprotection [21], can inhibit the activity of HDAC3, which may impact cognitive resilience towards Aβ.

Overall, relieving the HDAC3-mediated repression on CREB signaling and BDNF production would be expected to benefit cognition in the context of AD and other neurodegenerative diseases. However, it is unclear whether HDAC3 inhibitors would be the most effective way to achieve this outcome. Due to the pleiotropic effects of HDAC3 across cell types in the body, specifically targeting the mechanism by which repressive HDAC3 activity is increased in neurons, such as by targeting specific post-translational modifications of HDAC3, may be a more therapeutically viable approach.

Neuroinflammation: The immune response towards Aβ appears to be an important determinant of the neurotoxicity of Aβ. Innate immune cells, such as resident microglia or peripheral macrophages can facilitate the clearance of Aβ, or they can instead adopt a pro-inflammatory state that drives further pathology. Preclinical studies indicate that HDAC3 inhibits the alternative activation of macrophages, which is involved in healing, and instead promotes a polarization toward pro-inflammatory cytokine production, such as IL-1β, driven by the activation of NF-kB and NLRP3 signaling. HDAC3 prevents macrophages and microglia from developing tolerance to endotoxins, such as LPS, in response to repeated exposure, resulting in chronically elevated pro-inflammatory signaling [22]. The use of HDAC3 inhibitors, such as RGFP-966 can restore tolerance to LPS in cultured microglia [22]. HDAC3 inhibitors have also been shown to mitigate neurological damage in acute injury models for traumatic brain injury and cerebral ischemia through the mitigation of inflammation-related neuronal damage [23; 24; 25; 26; 27; 28]. Microglial HDAC3 is upregulated in response to injury/stroke, and inhibition of HDAC3 during this period has been shown to reduce levels of inflammatory cytokines (TNFα, IL-6, IL-1β) and neuronal death in these models. The beneficial effect appears to involve the modulation of the balance between activation states, away from the pro-inflammatory state and towards the pro-repair state [24]. The

modulation of macrophage activation state by HDAC3 involves the regulation of their metabolic state [29]. HDAC3-mediated repression restricts the expression of genes involved in the oxidation of fatty acids [4]. In response to NLRP3 activation, HDAC3 translocates to mitochondria to inactivate a key enzyme in fatty acid oxidation (HADHA) [29]. This restricts mitochondrial oxidative phosphorylation, requiring an upregulation of glycolytic activity. In macrophages, these metabolic adaptations are associated with increased production of the pro-inflammatory cytokine IL-1β. The metabolic reprograming by HDAC3 drives macrophages/microglia toward an M1-like pro-inflammatory state, which is associated with neuronal damage. Additionally, the inhibitor of NF-kB, IkBα plays a role in keeping HDAC3 localized within the cytoplasmic compartment [30]. The degradation of IkBα in response to an inflammatory stimulus allows HDAC3 to translocate to the nucleus and associate with chromatin, which can lead to the activation of pro-inflammatory NF-kB signaling. Therefore, the inhibition of HDAC3 in the context of an inflammatory stimulus, such as an injury or exposure to toxic misfolded proteins, like Aβ, can prevent microglia/macrophages from adopting a state that will exacerbate inflammation and drive neuronal damage. This suggests that HDAC3 inhibitors may be particularly suited toward use in acute conditions during a defined window of heightened inflammation.

APOE4 interactions: It has not been established whether ApoE status would impact the efficacy of HDAC3 inhibitors. One study found that ApoE4 was associated with increased nuclear localization of class II HDACs, but not of class I HDACs, such as HDAC3 [31].

Aging and related health concerns: HDAC3 inhibitors likely have the most utility in cancer. Cell type targeted inhibitors could benefit type 2 diabetes and atherosclerosis by modulating lipid metabolism.

Types of evidence:

- 6 studies assessing the role of HDAC3 as a prognostic factor in cancer
- 2 gene association studies for HDAC3 SNPs in diabetes
- Numerous laboratory studies

Lifespan: UNCLEAR
HDAC inhibitors have shown evidence of lifespan extension in animal models, as well as the induction of anti-aging phenotypes in a variety of models [32]. Lifespan extension has not been shown specifically for HDAC3 inhibition, but it has been shown to modulate pathways associated with aging, such as Klotho,

mTOR, and Nrf2 [33; 34], suggesting that HDAC3 activity may help tip the balance between healthy and unhealthy aging.

Cancer: POTENTIAL BENEFIT (Preclinical)
Cancer is the indication for which HDAC3 inhibitors currently have the most potential because the concern regarding off-target effects is generally lower. The epigenetic remodeling of cancer cells contributes to their ability to proliferate and survive [6]. Pan-HDAC inhibitors have been tested for cancer, however, very few have been approved for use in limited contexts due to their high level of side effects/toxicity. More selective HDAC inhibitors are thought to offer an improved therapeutic profile, but thus far, it has been difficult to develop selective inhibitors with good *in vivo* drug-like properties [35]. HDAC3 has emerged as one of the leading targets for this class of drugs, due to its association as a prognostic factor in a variety of cancers. It will likely be most effective as an adjunct, to augment the efficacy of other anti-cancer therapies. The development of HDAC3 inhibitors for cancer is an active area of research, with numerous research groups working on developing selective HDAC3 inhibitors [34; 35; 36].

Breast cancer: ELEVATED HDAC3 IS A PROGNOSTIC FACTOR FOR WORSE SURVIVAL
An analysis of HDAC expression in relation to clinicopathological features of disease progression including tissue from 238 patients with primary breast cancer found that HDAC3 expression was associated with more aggressive tumor type features [37]. These include less differentiated tumors as well as tumors with negative hormone receptor status. High expression of HDAC3 was associated with worse survival (Odds ratio [OR]: 10.752, 95% Confidence Interval [CI]1.211 to 95.500) in a cohort of 145 patients with invasive ductal breast carcinoma [38]. HDAC3 was identified as one of the top 100 prognostic factors for metastatic breast cancer using a machine learning approach [39]. Elevated cytoplasmic HDAC3 expression was found to be a prognostic factor toward worse overall survival (Hazard ratio [HR]: 1.948, 95% CI 1.089 to 3.486) based on tissue expression from 161 patients with breast invasive ductal carcinoma [40]. Additionally, high cytoplasmic HDAC3 was a prognostic factor for worse brain metastasis-free survival (HR: 3.386, 95% CI 1.724 to 6.650).

Gastric cancer: ELEVATED HDAC3 IS A PROGNOSTIC FACTOR FOR WORSE SURVIVAL
Class 1 HDACs, 1, 2, and 3 have been shown to be elevated in gastric cancer tissue [41]. High HDAC3 expression was associated with worse survival in 876 gastric cancer patients from the Probability GSE216326 dataset (HR:1.42, 95% CI 1.19 to 1.68). A similar association was seen in the SurvExpress

database (n=57) [42]. HDAC3 inhibition shows anti-cancer potential in preclinical models of gastric cancer.

Acute myeloid leukemia (AML): ELEVATED HDAC3 IS A PROGNOSTIC FACTOR FOR WORSE SURVIVAL
A multi-dataset mining analysis including the Oncomine, UALCAN, and GEO cancer databases identified HDAC3 as a prognostic factor for survival in AML [43]. High HDAC3 expression was associated with a shorter survival time. The expression of HDAC3 varied across AML types, and those with Flt3 mutations tended to show higher levels. HDAC3 was correlated with the expression of genes associated with cancer cell growth and survival, including SLC25A5, NDUFA2, Cox4I1, and EIF3K. Additionally, HDAC3 was found to indirectly regulate cGMP-PKG signaling, and the expression levels of the tumor suppressor genes p21 and p53. Database analysis suggests that HDAC3 inhibition would inhibit the proliferation of cancer cells in AML.

Diabetes: POTENTIAL BENEFIT DEPENDING ON CELL TYPES MODULATED (Preclinical)
A community-based case-control study (n=568) in China assessed the contribution of SNPs in HDAC3 to the risk for developing type 2 diabetes [44]. The SNPs rs2547547 (G allele) and rs2530223 (C allele), were associated with increased odds ratios, 1.72 (95% CI 1.13 to 2.64) and 1.39 (95% CI 1.01 to 1.91), respectively. These SNPs are expected to result in increased HDAC3 activity. Meanwhile, the rs11741808 SNP (G allele), which may lead to decreased HDAC3 activity, was associated with reduced risk (OR: 0.53, 95% CI 0.35 to 0.81). A similar trend was seen with respect to the risk for ischemic stroke in patients with type 2 diabetes (n=1,726) where the HDAC3 rs2547547 G allele was more common in ischemic stroke cases, and the rs11741808 G allele was less common in cases [45]. The expression and activity of HDAC3 was shown to be significantly elevated in peripheral blood monocular cells (PBMCs) from patients with type 2 diabetes relative to controls (n=50) [46]. Additionally, HDAC3 activity was correlated with clinical metabolic factors, including fasting glucose, insulin resistance (HOMA-IR), and glycated hemoglobin (HbA1c), as well as circulating pro-inflammatory markers (TNFα, IL-6).

The effect of HDAC3 on diabetes risk may be related to its role in the regulation of lipid metabolism and levels of free fatty acids [44]. Though the effect of modulating HDAC3 activity on diabetes is likely to be complex depending on the cell types impacted. The loss of HDAC3 can promote both lipogenesis and PPAR-mediated beta oxidation of fatty acids [5]. In the liver, this tends to lead to an improvement in glucose tolerance in animal models, though the overall effect depends on the balance of these activities [3]. Since HDAC3 repression of these metabolic genes occurs in the context of multiprotein co-repressor complexes, the presence, absence, or modification state of these interacting partners may determine

the balance between lipid production (lipogenesis) and lipid use (beta oxidation) [3]. Insulin signaling has been shown to affect the repressive activity of some of these interacting proteins, thus altered insulin signaling in diabetes likely influences whether reducing HDAC3 activity is metabolically beneficial or harmful [3]. As noted, the effect of modulating HDAC3 also depends on the cell type. In contrast to the liver where the loss of HDAC3 tends to improve glucose tolerance, the loss of HDAC3 in the skeletal muscle results in impaired glucose tolerance and insulin resistance [4].

In practice, HDAC3 inhibitors work systemically, impacting multiple cell types, such that the net effect may be different than what is seen with cell type specific deletion. Indeed, HDAC3 inhibitors have generally not significantly impacted measures of glucose homeostasis in animal models of diabetes [47; 48]. Instead, HDAC3 inhibitors have shown benefits in reducing diabetes-related complications in these models. These effects are primarily mediated via the modulation of inflammatory and antioxidant pathways. Under physiological conditions, HDAC3 is important for the maintenance of endothelial cell integrity and function, in part, by regulating the production of nitric oxide [5]. Under conditions of stress, such as altered blood flow, HDAC3 expression and/or activity is increased in endothelial cells [5]. Blood flow disturbances and endothelial cell dysfunction are common in diabetes, which likely plays a role in the upregulation of HDAC3 in diabetes. However, due to the induction of endothelial nitric oxide synthetase (eNOS) uncoupling under diabetic/high glucose conditions, this normally protective mechanism becomes pathogenic [49]. Instead of producing nitric oxide, eNOS induces oxidative stress through the production of reactive oxygen species (ROS). Under these conditions, inhibiting HDAC3 improves endothelial function by alleviating oxidative stress [50]. HDAC3 deficient cells also appear to be epigenetically primed to cope with higher levels of oxidative stress, through the activation of endogenous antioxidant pathways, such as Nrf2 [5]. In the db/db mouse model, treatment with RGFP-966 (10 mg/kg s.c. every other day for 10 weeks) reduced oxidative stress, pro-inflammatory signaling, and promoted reendothelialization [50]. Similarly, elevated HDAC3 expression was found to be associated with diabetic retinopathy in streptozotocin-induced diabetic mice, and treatment with RGFP-966 (10 mg/kg i.p. every 3 days for 12 weeks) reduced oxidative stress and retinal cell death in this model [47]. RGFP-966 also alleviated oxidative stress and NLRP3-mediated inflammation in mouse retinal ganglion cells under high glucose conditions [51]. RGFP-966 (10 mg/kg i.p. for 10 days) also showed improved endothelial integrity at the BBB resulting in reduced BBB permeability and increased Nrf2 antioxidant capacity in db/db mice [48].

Elevated HDAC3 activity appears to be particularly detrimental in the context of diabetes, such that HDAC3 inhibition shows potential to alleviate a variety of diabetes-associated complications, at least in animal models. Preferential targeting of particular cell types would likely improve the therapeutic utility

of HDAC3 inhibitors for diabetes. Similar to other conditions, targeting the factors that lead to an elevation in HDAC3 in the affected cell types would likely be a safer approach.

Atherosclerosis: POTENTIAL BENEFIT DEPENDING ON CELL TYPES MODULATED (Preclinical)
HDAC3 can influence atherosclerosis through its regulation of gene networks involved in lipid metabolism and of those involved in immune activation [5]. In this way, changes in HDAC3 activity can impact levels of circulating lipids, as well as the immune response toward atherosclerotic plaques. However, due to the cell-type specific activity of HDAC3, the loss of HDAC3 activity in different cell types can result in different physiological effects. This is related to the preferential incorporation of HDAC3 into particular co-repressor complexes in different cell types, as well as differential expression of transcription factors. For example, HDAC3 appears to primarily interact with the nuclear receptor co-repressor (NCoR) complex in liver cells [3]. The loss of HDAC3 results in the induction of lipogenesis, leading to the accumulation of fat within the liver, which can result in hepatic steatosis [3; 4]. In aged mice, reduced HDAC3 chromatin occupancy, leading to de-repression of PPAR and LXR genes may contribute to the development of aging-related hepatic steatosis [3]. The loss of HDAC3 in the heart can also lead to cardiac lipid accumulation [4]. Due to its role in promoting endothelial cell survival, the loss of HDAC3 in endothelial cells may exacerbate atherosclerosis [5].
In contrast, reducing HDAC3 activity in macrophages and intestinal enterocytes may be atheroprotective. Enhanced PPAR-mediated lipid oxidation stemming from the loss of HDAC3 in enterocytes protects mice against high-fat diet-induced obesity and dyslipidemia [4]. HDAC3 also controls the inflammatory response of macrophages, which influences the stability of atherosclerotic plaques [5]. HDAC3 inhibits the TGF1β mediated alternative activation phenotype in macrophages, which is associated with wound healing, and instead promotes a pro-inflammatory state characterized by NF-kB and NLRP3 signaling. TGF1β acts as an anti-atherosclerotic cytokine which promotes an increase in collagen production and plaque stability [52]. Therefore, high HDAC3 shifts the balance of macrophage activation toward a state which destabilizes plaques. In support of the relevance of these preclinical findings, HDAC3 was found to be specifically upregulated in ruptured human atherosclerotic lesions, and was inversely associated with levels of TGF1β in this tissue [53].
These studies suggest that HDAC3 inhibition could potentially benefit dyslipidemia and/or atherosclerosis if done in a targeted manner, whereby the drug was targeted toward particular cell types.

Kidney disease: POTENTIAL BENEFIT IN FIBROTIC RENAL CELLS (Preclinical)
HDAC3 is essential for kidney development, but altered renal HDAC activity is a common feature of kidney disease [54]. The role of HDAC3 in driving kidney fibrosis provides a clear example of the cell type and context-dependent specificity of HDAC3 activity. In the mouse kidney, HDAC3 was found to be elevated in response to TGF1β-mediated pro-fibrotic signaling [33]. Klotho is highly enriched in the kidneys and acts as a renoprotective protein, in part through the mitigation of oxidative stress and inflammatory cell damage. Klotho expression has been shown to be reduced in the context of kidney disease. HDAC3 is a negative regulator of Klotho, such that the elevation of HDAC3 under fibrotic conditions results in the inhibition of Klotho-mediated renoprotection, and the advancement of tissue damage in the kidney [33]. In this fibrotic environment, HDAC3 inhibition using RGFP-966 (10 mg/kg s.c.) has been found to mitigate fibrotic pathology by allowing for the de-repression of Klotho.

Infection: POTENTIAL MIXED EFFECTS DEPENDING ON PATHOGEN AND CELL TYPE (Preclinical)
HDAC3 is an important regulator of innate and adaptive immune cell activation programs. Depending on the pathogen and the cell type, HDAC3 could facilitate a response that promotes either pathogen clearance or maintenance. Type 1 interferons (IFNs) are involved in innate immune responses to clear pathogens. HDAC3 promotes productive antiviral innate immune responses through the production of IFNs, via modulation of several transcription factors, including through the deacetylation of TBK1, which activates Jak/Stat signaling [55; 56]. In mice, the loss of HDAC3 in macrophages allowed for uncontrolled viral replication of the RNA viruses vesicular stomatitis virus, a mouse adapted H1N1 influenza virus (PR8), and the WSN influenza virus [56]. In the adaptive immune system, HDAC3 is involved in regulating the balance between short lived cytotoxic effector CD8 T cells and long-term memory CD8 T cells [57]. HDAC3 promotes the development of long-term memory cells at the expense of short-term effector cells. Consequently, HDAC3 inhibitors, such as RGFP-966 shift this balance in the other direction. While the cytotoxic T cells are important for mediating acute pathogen clearance, the absence of memory cells prevents the body from developing immunity toward that antigen/pathogen upon repeated exposure. However, particular pathogens have been identified for which HDAC3 activity hinders clearance. The hepatitis C virus (HCV) maintains infectivity through the use of lipoviral particles [58]. Apo-A1, a component of HDL particles is a host-derived component of these HCV lipoviral particles. HDAC3 has been implicated as a causal factor in the variation of HDL across mouse strains [59]. In HCV infected cultured liver cells, the inhibition of HDAC3 with RGFP-966 inhibited HCV replication, in part, through the downregulation of Apo-A1 levels [58]. The use of HDAC3 inhibitors has also been proposed as part of a 'shock and kill' strategy towards HIV [60]. Traditional anti-HIV drugs cannot eliminate latent virus. The inhibition of HDAC3 can reactivate latent HIV, allowing it to be targeted by anti-HIV drugs. Of course, in

the absence of an effective anti-viral treatment, the reactivation of latent HIV or other viruses with HDAC3 inhibitors, coupled with the reduction in IFN production following HDAC3 inhibition poses the risk for an uncontrolled active infection.
The microbiome, through the production of SCFAs, may help regulate immune responses to pathogens through the regulation of HDAC3. In mice, the HDAC3 inhibiting SCFA, butyrate, was shown to promote the differentiation of macrophages into a state with enhanced bactericidal capacity [34].
Together these studies speak to the complexity of HDAC3 in the regulation of immune system responses, resulting in highly context-dependent effects.

Arthritis and autoimmunity: POTENTIAL MIXED EFFECTS DEPENDING ON CELL TYPE (Preclinical)
The contribution of HDAC3 to arthritis is unclear based on biomarker data from patients with rheumatoid arthritis and osteoarthritis. Several studies have found an imbalance between HDAC and HAT activity in arthritis patients, though the direction varied across studies [61]. HDAC3 can promote the activation of pro-inflammatory signaling in innate immune cells, but is also involved in the induction of tolerance promoting regulatory T cells [29; 62]. It is possible that increased HDAC3 activity drives increased inflammation from macrophages, while decreased HDAC3 in T cells results in a loss of self-tolerance. Therefore, HDAC3 may mediate multiple, potentially opposing effects, in different cell types, which may account for the discrepancies across studies. An analysis of synovial fluid from 18 patients with rheumatoid arthritis found that HDAC3-mediated type 1 interferon production and downstream Stat1 signaling contributed to joint inflammation [63]. Meanwhile, a study in 96 participants found that HDAC3 activity was reduced in PBMCs from patients with rheumatoid arthritis relative to controls, resulting in an imbalance between HAT and HDAC activity [61].
In a case-control study including 419 participants of Chinese Han descent, the presence of the T allele in the rs2530223 SNP in HDAC3 was associated with 1.472-fold (95% CI 1.100 to 1.969) increased risk for primary immune thrombocytopenia, which is an autoimmune disorder involving the immune-mediated loss of platelets [64].
HDAC3 is implicated in autoimmunity more broadly due to its role in regulating the expression of FoxP3, which promotes the immunosuppressive activity of regulatory T cells by inhibiting their production of IL-2 [62]. As such, the loss of HDAC3 interferes with mechanisms of self-tolerance. HDAC3 also appears to play a role in the maintenance of tolerance to the commensal bacteria that make up the microbiome. In mice, the loss of HDAC3 in epithelial cells resulted in an overproduction of commensal-specific pro-inflammatory Th17 CD4 T cells coupled with a loss of commensal-specific T regulatory cells, ultimately leading to inflammatory bowel disease [65]. HDAC3 may also play a role in the repair of intestinal epithelial cells. The levels of microbiome-produced SCFAs that have HDAC3 activating activity were

found to be reduced in patients with ulcerative colitis [20]. In mice, this induction of HDAC3 was necessary to facilitate repair and recovery to intestinal damage [20]. These studies highlight the importance of HDAC3 activity in intestinal epithelial cells to protect against inflammatory damage, however, in other cell types, such as macrophages, the microbiome-mediated reduction in HDAC3 activity helps prevent inflammation [34]. As such, the therapeutic efficacy of modulating HDAC3 in arthritis, or other autoimmune conditions will likely depend on the cell types that are preferentially impacted.

Safety: Selective HDAC3 inhibitors suitable for clinical use have not been developed. Animal studies suggest acute HDAC3 inhibition is likely safe, but long-term safety is unknown. The safety and efficacy profile will likely vary across drugs in this class.

Types of evidence:

- Numerous laboratory studies

HDAC3 inhibitors have not yet been clinically tested. Most of the HDAC3 inhibitors developed to date have poor pharmacokinetic drug properties and/or lack strong specificity for HDAC3 [7]. The specificity profile is considered to be a key attribute for the prospective safety and tolerability of the drug, as clinically tested pan-HDAC inhibitors have shown high toxicity in cancer patients [36]. RGFP-966, which shows only modest selectivity for HDAC3 has been the most widely used HDAC3 inhibitor for *in vivo* studies to date [2]. While evidence of toxicity has not been reported, most studies use acute treatment for less than one month in duration, and do not include extensive safety measures as part of the study design. Similarly, other relatively specific HDAC3 inhibitors that have been tested *in vivo* in rodents, such as PT3 and 4e, have not shown evidence of organ toxicity with acute administration [13; 35]. These studies suggest that acute use of HDAC3 inhibitors is likely to show better safety and tolerability compared with pan-HDAC inhibitors, however, the safety profile with long-term use has not been established.

Due to the embryonic lethality of HDAC3 knockout mice, genetic studies use conditional deletion strategies, typically in a particular cell type of interest. These studies indicate that the modulation of HDAC3 can have profoundly different effects in different cell types and under different physiological and pathological conditions [4; 5]. This raises the potential for a high degree of on-target side effects with the use of systemic HDAC3 inhibitors. As such, it will be imperative to understand which cell types in a given condition show evidence of elevated/dysfunctional HDAC3 activity. In this way, HDAC3 drugs can

be designed to preferentially impact the cell types of interest, either through the modification of the chemical or binding properties of the drug, the route of administration, inclusion of a cell-targeted delivery vehicle, or through the targeting of specific HDAC3 protein-protein interactions. Alternatively, HDAC3 activity could be modulated in an indirect manner by instead targeting specific proteins that interact with HDAC3 as part of a repressive complex in a given cell type, or those which produce the post-translational modifications that alter HDAC3 localization or activity.

Due to the role of HDAC3 in regulating immunity, chronic HDAC3 inhibition poses the risk for immunosuppression or altered infection control.

Drug interactions: Specific interactions have not been established, since HDAC3 inhibitors have not yet been optimized for clinical testing. However, they may interact with similar classes of drugs as currently available HDAC inhibitors, such as antidepressants, anticoagulants, and other HDAC inhibitors (valproic acid WebMD) (vorinostat FDA label).

Sources and dosing:

HDAC3 inhibitors with good selectivity, specificity, and pharmacokinetic properties suitable for clinical use have not yet been developed, however, this is an active area of research. The HDAC3 inhibitor RGFP-966 is available for research use from commercial suppliers.

Research underway:

Numerous groups are actively working to develop specific HDAC3 inhibitors with the potential for clinical utility. There are efforts to develop these compounds for use in cancer as well as BBB penetrant compounds for use in neurodegenerative disease [13; 35; 66]. HDAC3-specific PROTACs are being developed for cancer [51].

Search terms:
Pubmed, Google: HDAC3 Inhibitors, RGFP-966

- Alzheimer's disease, neurodegeneration, cognition, SNPs, lifespan, aging, cancer, inflammation, diabetes, cardiovascular, microbiome, infection

Websites visited for HDAC3 inhibitors:

- Drugs.com (Voronistat)
- WebMD.com (Valproic acid)
- PubChem (RGFP-966)

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