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.

Notum Inhibitors

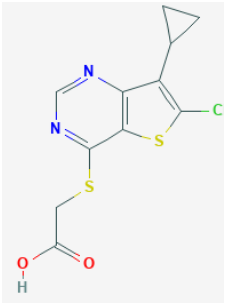
Evidence Summary

Notum is a negative regulator of Wnt, so inhibitors may benefit diseases with reduced Wnt, such as Alzheimer's disease and osteoporosis, but exacerbate conditions with high Wnt, such as cancer and fibrosis.

Neuroprotective Benefit: Decreased Wnt signaling may contribute to synaptic dysfunction and loss in Alzheimer's disease, thus Wnt activators may be neuroprotective. Whether Notum inhibitors would be effective Wnt activators for AD is unclear.

Aging and related health concerns: Due to the pleiotropic, context-dependent nature of Wnt signaling, Notum inhibitors may benefit some age-related diseases, like osteoporosis, while exacerbating others like cancer, osteoarthritis, and fibrosis.

Safety: Wnt activators may increase the risk for cancer and have on-target side effects in a variety of tissues. Aside from fetal malformation, the specific risks for Notum inhibitors have not been established.

Availability: Research use	**Dose**: N/A	LP-922056 **Chemical formula:** $C_{11}H_9ClN2O_2S_2$ **MW**: 300.8 g/mol  Source: PubChem
Half-life: N/A	**BBB**: Varied (LP-922056 not penetrant)	
Clinical trials: None	**Observational studies**: Notum expression is elevated in cancers with deregulated Wnt signaling	

What is it?

Notum is a palmitoleoyl-protein carboxylesterase, which acts as **a negative regulator of Wnt signaling** [1]. Notum is a secreted enzyme that modifies Wnt ligands in the extracellular space. The biological activity of Wnt ligands depends on the presence or absence of particular lipid modifications [2]. Porcupine (PORCN) is an O-acyl transferase enzyme that uses palmitoyl- coA to perform O-fatty acylation of Wnt ligands. These lipid-modified Wnt ligands are in an activated state which allows them to be secreted and bind to extracellular Wnt receptors including Frizzled and LRP5/6, which are the mediators of canonical Wnt signaling. The activation of the canonical pathway ultimately leads to the stabilization and accumulation of the transcription factor β-catenin, which then translocates to the nucleus to mediate gene transcription. The non-canonical pathways involve the binding of Wnt ligands to receptor complexes that do not include LRP5/6 and do not activate β-catenin. Under some contexts, the canonical and non-canonical pathways can have opposing effects. Notum acts in the opposite manner to PORCN, by removing the palmitoyl lipid modifications on Wnt ligands, which prevents them from binding the receptors, and thus inactivates them. **Notum inhibitors have been developed to act as Wnt activators**, and have primarily been tested in preclinical models for osteoporosis.

Neuroprotective Benefit: Decreased Wnt signaling may contribute to synaptic dysfunction and loss in Alzheimer's disease, thus Wnt activators may be neuroprotective. Whether Notum inhibitors would be effective Wnt activators for AD is unclear.

Types of evidence:

- 4 observational studies (Wnt signaling component expression in AD brain)
- Several laboratory studies (None for Notum inhibitors in AD models)

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function?: None

Human research to suggest benefits to patients with dementia: None

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

Alzheimer's disease: POTENTIAL BENEFIT (Preclinical)
Evidence from postmortem human brain tissue suggests that **Wnt signaling is decreased in the context of Alzheimer's disease** (AD), while animal model studies suggest that restoration of Wnt is neuroprotective [3]. Wnt plays critical roles in synaptic formation, maintenance, and function, thus the **decline in Wnt signaling is thought to be a major mediator of synaptic loss and neuronal degeneration in AD.** Dickkopf-related protein 1 (DKK1) acts as a negative regulator of canonical Wnt signaling by binding and sequestering the receptor LRP6. DKK1 is typically expressed at very low levels in the healthy human brain, but has been found to be increased in the AD brain [4]. Meanwhile, LRP6 levels were found to be reduced in postmortem AD brain, compared to age-matched controls, which is associated with increased amyloidogenic processing of amyloid precursor protein (APP) [5]. The decrease in LRP6 is associated with the decrease in β-catenin in the AD brain. Single nucleotide polymorphisms (SNPs) in the LRP6 locus have also been associated with AD [6]. A synonymous SNP in exon 18 (18e, rs1012672; C → T) was associated with AD (P = 0.0092), such that individuals with at least one copy of the minor (T) allele had 69 to 80% greater risk of getting AD compared with major allele (CC) homozygotes. In cell culture, the LRP6 Val-1062 allele (14e, rs2302685; T → C), which was part of a haplotype associated with modified AD risk, reduced activation of β-catenin signaling. These studies suggest that the decline in Wnt signaling may be mediated at the receptor level.

The activity of GSK3β, which acts as a negative regulator of β-catenin, has also been shown to be elevated in the AD brain [7]. While canonical Wnt signaling inactivates GSK3β, it participates in a variety of signaling cascades, thus GSK3β activity is regulated based on the cumulative activity of a variety of cellular activities. Thus, the increase in GSK3β in AD is likely related to the deregulation of multiple signaling pathways [8]. GSK3β is an important driver of AD pathology due to its role in the hyperphosphorylation of tau. Although controversial, Wnt signaling is also thought to be neuroprotective by stimulating neurogenesis. A study examining Wnt component expression in the prefrontal cortex (PFC) found that the altered expression of Wnt components was region specific, with more dysregulation in disease affected areas [7]. While this suggests a connection between impaired Wnt signaling and disease progression, the variability could complicate attempts for therapeutic targeting by deregulating Wnt signaling in regions where it is intact.

Studies in flies indicate that Notum is important for synapse development by regulating trans-synaptic signaling of Wnt [9]. This suggests that Notum inhibition could promote synapse maintenance and formation. However, it is unclear whether the balance of PORCN and Notum is dysregulated in the AD brain. The inhibition of Notum would increase the proportion of Wnt ligands with the capacity to bind and activate receptor complexes, however, if the primary impairment of Wnt occurs downstream of this step, the increased availability of ligand may only be partially effective at restoring Wnt signaling. Since the human data suggests that reduced availability of the LRP6 receptor may be key factor, it may be necessary to combine interventions which boost availability of both Wnt ligands and receptors. Additionally, as is seen in cancer, the extracellular activity of Notum may not be effective in addressing dysfunction to β-catenin levels and signaling that are driven by intracellular processes. **The contribution of Notum deregulation and modulation needs to be further explored** in the AD brain and models.

APOE4 interactions:
ApoE4 has been shown to inhibit canonical Wnt signaling, potentially by indirectly inducing DKK1 [10], suggesting that E4 carriers may preferentially benefit from Wnt activators. It is not known whether E4 status affects Notum expression or activity.

Aging and related health concerns: Due to the pleiotropic, context-dependent nature of Wnt signaling, Notum inhibitors may benefit some age-related diseases, like osteoporosis, while exacerbating others like cancer, osteoarthritis, and fibrosis.

Types of evidence:

- 4 observational studies (Notum expression in cancer or osteoarthritis)
- 1 genetic association study (Notum SNPs and bone mineral density)
- Several laboratory studies

Cancer: UNCLEAR

Due to its roles in promoting cell proliferation and survival, elevated Wnt signaling is an indicator of poor prognosis in a variety of cancers, including colorectal cancer, hepatocellular carcinoma, breast cancer, endometrial cancer, and pancreatic cancer [11]. The expression of Notum tends to be low in most adult tissues, but is sensitive to local changes in Wnt. As part of the negative feedback loop of Wnt signaling, Notum is typically induced in response to an elevation in Wnt signaling, which likely accounts for its increased expression in tumors [12]. In several cancers, including hepatocellular carcinoma and colorectal cancer, the levels of Notum were associated with high levels of intracellular β-catenin, indicative of active canonical Wnt signaling [12; 13]. The sustained elevation in Wnt signaling, despite high levels of the endogenous inhibitor Notum, is indicative of a broader **deregulation of Wnt signaling in the context of cancer**. For example, in colorectal cancer, the expression of the negative regulators notum and glypican-1 were increased to varying degrees, whereas glypican-3 levels decreased in tumors with high Wnt/β-catenin activation [13]. The inhibition of Wnt has been seen as a potentially promising therapeutic strategy for cancer [11], which might suggest that it would be detrimental to inhibit an endogenous Wnt inhibitor. However, due to the deregulation of Wnt components, it is unclear whether Notum contributes to cancer progression in a biologically meaningful way. It has been hypothesized that since Notum acts as an extracellular inhibitor of Wnt signaling by modifying extracellular Wnt ligands, it may be ineffective at curtailing Wnt signaling in cancer that is driven by high levels of intracellular β-catenin [12].

Preclinical models using PORCN inhibitors, have found that the decline in Notum expression can be used as a biomarker of responsiveness to this class of Wnt targeted drugs [14]. PORCN activates Wnt ligands by adding a lipid group (palmitoylation), whereas Notum catalyzes the reverse reaction to inactivate the ligands by removing the lipid moiety. In this case, the decrease in Notum is indicative of a decrease in (palmitoylated) activated Wnt ligands, and is consistent with the notion that inhibiting Wnt is beneficial

for cancer. In cell culture, metastatic colorectal cancer cell proliferation was suppressed by inhibiting Notum [15]. This may indicate that in the context of deregulated Wnt present in some types of cancer, Notum itself may contribute to cancer progression. Consequently, it is currently unclear how the modulation of Notum would affect the progression of cancer, and whether it depends on the particular way in which Wnt signaling is disrupted in a given cancer.

Osteoporosis: POTENTIAL BENEFIT (Preclinical)
Canonical Wnt signaling is a major regulator of bone mass [16]. As a negative regulator of Wnt, **Notum acts a negative regulator of bone formation**. In humans, SNPs in the Notum locus are associated with bone mineral density. The common variant (31%) rs35344256 C allele is associated with reduced bone mineral density (β=−0.0134, P=1.5E−11), whereas the rare (2.9%) rs147901986 G variant is associated with increased bone mineral density (β=0.0386; $p=4.6E^{-11}$) [17]. Notum is primarily secreted by osteoblast lineage cells, and in adult mice, inactivation of Notum increased cortical bone mass by increasing periosteal bone formation [17]. The ability to increase bone formation in response to mechanical loading declines with aging. The bones of aged female mice failed to upregulate Wnt signaling in response to skeletal loading to the same degree as young female mice, suggesting that activation of Wnt may be a therapeutic strategy for osteoporosis [18]. Treatment with the orally available Notum inhibitor LP- 922056 increased cortical bone thickness and strength in the midshaft femur in mice and ovariectomized rats, with similar efficacy to teriparatide, which is a parathyroid hormone currently used to stimulate bone formation [19]. Osteoporosis is considered to be one of the primary peripheral indications for the development of Notum inhibitors.

Osteoarthritis: POTENTIAL HARM (Notum decreased in OA)
Osteoarthritis involves the loss of protective cartilage in the joints, which is sometimes accompanied by the development of bone spurs. Wnt signaling is implicated in the progression of osteoarthritis because it promotes bone formation and inhibits chondrogenesis, the formation of cartilage [20]. The expression of seven Wnt negative regulators was assessed in the peripheral blood from patients with osteoarthritis (n=40) and healthy controls (n=40), and only Notum showed a significant difference between the groups ($Median_{OA}$=0.4451ng/mL vs. $Median_{CONTROL}$=0.8263ng/mL, p=0.0013) [21]. This study suggests that low levels of Notum may contribute to a pathogenic elevation of Wnt in the context of osteoarthritis. However, it has not been established whether a Notum inhibitor would exacerbate osteoarthritis progression.

Regeneration: POTENTIAL MIXED/CONTEXT DEPENDENT (Preclinical)
Due to its role in promoting the maintenance of stem cells, Wnt signaling is generally considered to be pro-regenerative. However, the **effects may be organ type specific and/or context dependent**. The loss of regenerative potential in the intestinal epithelium with age was found to be related to a reduction in Wnt signaling, mediated by an increase in the production of Notum by aged intestinal Paneth cells [22]. The increase in Notum appears to be driven by the inhibition of PPAR-α in Paneth cells. In mice, use of the Notum inhibitor ABC99 improved the regenerative capacity of aged intestinal stem cells. Meanwhile, in the zebrafish heart, which unlike the mammalian heart is capable of regeneration, the regenerative effects involve cross-talk between the Notch and Wnt signaling pathways [23]. The induction of Notch signaling leads to the induction of Wnt inhibitors, including Notum, and in this context, Wnt signaling impairs regeneration by promoting scarring. Consequently, the effects of Notum inhibitors on regeneration and healing may be variable.
Liver fibrosis: POTENTIAL HARM/MIXED/CONTEXT-DEPENDENT (Preclinical)
Excessive Wnt/β-catenin signaling is implicated in fibrosis, which is the excessive accumulation of extracellular matrix components, but as with other aspects of Wnt signaling**, the pro-fibrotic effect may be tissue-type or context dependent**, as in some cases Wnt has been shown to have anti-fibrotic effects [24]. In patients with HBV-mediated liver fibrosis, positive correlations were found between protein expression of NFATc1 and p-JNK and liver fibrotic scores [25]. In HBV-infected hepatocytes, Notum was found to suppress Wnt5a mediated NFATc, JNK, and pro-fibrotic gene expression, suggesting that Notum inhibitors could promote fibrotic signaling in the liver, at least in the context of HBV infection. It is unclear whether Notum inhibitors would have pro-fibrotic effects in other tissues or contexts.

Metabolism: POTENTIAL HARM (Preclinical in rodents)
While Notum expression is generally low in healthy adult tissues, it is relatively higher in the fetal and adult liver in humans. In mice, hepatocyte specific deletion of Notum did not affect liver zonation, but it did impact metabolic function during adulthood, in a sexually dimorphic manner [26]. As the Notum-lacking male mice aged, they had an increased risk of developing obesity, and became glucose intolerant and insulin resistant. However, it is not known whether these effects are specifically related to the loss of Notum in the fetal liver, and whether inhibiting Notum only in adulthood would also influence metabolic regulation. In a study aimed at identifying endocrine network regulators, liver secreted **Notum was found to promote the 'beiging' of adipose tissue in mice**, suggesting that Notum enhances catabolic and brown adipose tissue-like machinery [27]. In adipose cells and mice, Notum treatment enhanced expression of markers of brown adipose tissue (PGC1α and UCP). Due to a heightening in the ratio of brown adipose tissue to white adipose tissue, the Notum treated mice also showed increased

thermogenic capacity and cold tolerance. It is unclear whether Notum affects liver-adipose tissue cross-talk in a similar manner in humans.

Safety: Wnt activators may increase the risk for cancer and have on-target side effects in a variety of tissues. Aside from fetal malformation, the specific risks for Notum inhibitors have not been established.

Types of evidence:

- Several laboratory studies

Notum inhibitors have not been clinically tested, and the few that have been tested *in vivo* have only been tested in relatively short-term preclinical studies [19; 22; 28; 29; 30]. Safety concerns have not been reported in animal studies using available Notum inhibitors conducted thus far, but comprehensive assessments of safety do not appear to have been made in these studies.

Wnt signaling is a challenging therapeutic target because Wnt ligands play important roles in all cell types, and must be tightly regulated to maintain proper tissue homeostasis [24]. Attempts to correct an imbalance in one cell/tissue type through the use of inhibitors or activators could ultimately produce an imbalance in another tissue. In some cases, it may be possible to limit side effects by targeting Wnt components with tissue restricted isoforms. For example, PORCN inhibitors for cancer and other peripheral indications could limit neurological side effects by designing the drug to lack activity for the predominant form found in the brain (isoform D) [2]. Only two potential isoforms have been computationally mapped for Notum (https://www.uniprot.org/uniprot/Q6P988), so it is unclear whether there is a way to target Notum in a tissue specific manner, especially since Notum is a secreted protein.

Notum clearly plays critical roles during development, and thus like other Wnt targeted drugs, Notum modulators could affect fetal development and would need to be contraindicated in pregnant women [24]. The placenta has significantly higher expression of Notum than any other tissue (https://www.proteinatlas.org/ENSG00000185269-NOTUM/tissue), suggesting that Notum may be important for the maintenance of both maternal and fetal health.

Due to the relatively low expression of Notum in most healthy adult tissues, Notum modulators may potentially have less side effects in healthy tissues. However, they could have on-target side effects in

people who have multiple morbidities, as is common in aging, which involve deregulated Wnt signaling. **The primary concern for Wnt activators, such as Notum inhibitors, is increased risk for cancer**, due to its roles in regulating proliferation and differentiation. More extensive safety studies are needed.

Sources and dosing:

Notum inhibitors are still in preclinical development. LP-922056 is an orally available Notum inhibitor developed by Lexicon Pharmaceuticals [29], which may be useful for peripheral indications such as osteoporosis, but is not suitable for CNS diseases, due to its lack of BBB penetrance [31].

Research underway:

Medicinal chemistry efforts are underway to develop novel Notum inhibitors with improved PK properties, metabolic stability, and BBB penetrance [30; 31; 32; 33].

Search terms:

Pubmed, Google: Notum

- Alzheimer's disease, brain, aging, cancer, osteoporosis, osteoarthritis, cardiovascular, diabetes, inhibitor, safety

Websites visited for Notum Inhibitors:

- PubChem (LP-922056)

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