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.

Fibronectin-1 Inhibitors

Evidence Summary

The dysregulation of fibronectin may be a key driver of tissue fibrosis. Fibronectin inhibitors help preserve tissue function in preclinical models of fibrosis, but their safety profile has not been established.

Neuroprotective Benefit: Increased deposition of fibronectin may mediate pathology associated with reactive gliosis. Genetic variants that reduce fibronectin deposition are associated with decreased risk for Alzheimer's disease.

Aging and related health concerns: Excessive fibronectin deposition is a feature of fibrotic disease. Preclinical studies suggest that disrupting this accumulation may mitigate disease progression. Fibronectin also plays a role in cancer progression.

Safety: The safety of fibronectin inhibitors has not been established, but could potentially impact wound healing.

Availability: Not available, in research development	**Dose**: N/A	**Chemical formula:** N/A **MW**: N/A
Half life: N/A	**BBB**: N/A	
Clinical trials: None	**Observational studies**: Fibronectin deposition is elevated in a variety of fibrotic diseases, and is associated with poor prognosis in cancer.	

What is it?

Fibronectin-1 is a structural glycoprotein and component of the extracellular matrix [1]. It serves as an important scaffolding protein to direct tissue organization during development and repair. It also helps maintain the structural integrity and function of tissues throughout life. Due to alternative splicing and processing, fibronectin-1 exists in at least 20 different isoforms. Its various domains contain binding sites for other matrix and signaling proteins, thus its structure greatly influences its function. Additionally, fibronectin-1 can be cleaved by a variety of proteases, which can lead to the exposure of cryptic binding sites on the resulting fragments. Despite its ubiquity, the differential processing of fibronectin-1 allows for tissue-specific and context-dependent roles.

There are two major classes of fibronectin-1, plasma and cellular [1]. Plasma fibronectin-1 is primarily synthesized by hepatocytes and released into circulation in a soluble, inactive form largely lacking the EIIIB and EIIIA domains. It forms a complex with fibrin and serves as a major component of the fibrin clot during wound healing. Through interactions with integrins, it stabilizes platelets to promote clot formation. This then triggers the release and deposition of cellular fibronectin, which is important for the establishment and maintenance of tissue architecture. The cellular matrix formed by fibronectin promotes the deposition of other matrix components as well as the sequestration of growth and angiogenic factors.

When this process goes awry, it can lead to pathological tissue remodeling and fibrosis [1]. Excessive fibronectin-1 deposition and dysregulation are common components of a variety of diseases with fibrotic pathology. Genetic variants in fibronectin-1 are also associated with risk for several of these conditions.

Preclinical proof of concept studies provide evidence for a protective effect of fibronectin inhibition, however, the biology is complex, and the optimal way to target fibronectin-1 for clinical efficacy has not been established.

The most used fibronectin-1 inhibitor to date is pUR4, which is a peptide derived from the F1 adhesin of *Streptococcus pyogenes* [2]. Many bacteria contain fibronectin binding proteins, which they use to gain entry into the host cells. The peptide binds with high affinity to the N-terminal 70-kDa region of fibronectin, and acts as an inhibitor of fibronectin polymerization, while preserving the function of its integrin binding domain. The peptide sequence is GSKDQSPLAGESGETEYITEVYGNQQNPVDIDKKLPNETGFSGNMVETEDTKLN.

A dual-action, fully human, single chain variable fragment antibody targeting cryptic sites in fibronectin, Fn52RGDS, prevents its polymerization and downstream cell signaling via interaction with integrins (via the RGD) [3]. It has been tested in some models of fibrotic eye disease.

Neuroprotective Benefit: Increased deposition of fibronectin may mediate pathology associated with reactive gliosis. Genetic variants that reduce fibronectin deposition are associated with decreased risk for Alzheimer's disease.

Types of evidence:

- 2 gene association studies for protective variants in ApoE4 carriers
- 3 biomarker studies for fibronectin-1 levels in AD
- 2 biomarker studies for fibronectin-1 levels in HIV-associated dementia
- 2 case-control gene association studies for risk variants in MS
- Several laboratory studies

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

Variants in fibronectin-1 were identified as modifiers of Alzheimer's disease (AD) risk in carriers of the ApoE4 allele. A gene association study including 3,578 individuals from over 700 non-Hispanic white and Caribbean Hispanic families with a history of AD identified rare coding variants associated with neuroprotection, many of which encoded for proteins involved in the extracellular matrix [4]. Two of the

most highly represented genes were fibronectin-1 and collagen type VI alpha 2 chain (COL6A2). The frequency of the fibronectin-1 protective variants, rs116558455 and rs140926439, in these cohorts were 1.85% and 3.3%, respectively. The prevalence of the alleles varied based on ethnicity, as the rs116558455 variant was primarily found in the Hispanic cohort, and was very rare in non-Hispanic whites. The protective nature of the rs140926439 variant was validated in an independent cohort of 7,185 ApoE4 homozygous carriers of primarily non-Hispanic white European descent. The rs140926439 variant is located at position chr2:215424292, and results in a single nucleotide missense change from C > T [5]. The rs140926439 variant was associated with reduced risk for AD in ApoE4 homozygotes (Odds Ratio [OR]: 0.29, 95% Confidence Interval [CI] 0.11 to 0.78; P = 0.014), and for those who went on to develop AD, the presence of a single copy of the allele delayed the age of onset by 3.4 years (beta: 3.37, 95% CI 0.42 to 6.32; P = 0.025) [4]. A separate study including 413,127 participants in the UK Biobank, a cohort that is predominantly (95%) non-Hispanic white, similarly found a protective effect for the rs140926439 variant specifically in ApoE4 carriers [5]. In the presence of this variant, rates of AD in ApoE4 carriers (0.1%) were similar to those of individuals with ApoE2/E3, who are not at heightened risk for AD, while the prevalence of AD in ApoE4 carriers without this variant (0.4%) was significantly higher. The presence of ApoE4 is associated with increased blood-brain-barrier (BBB) leakiness, and the expression of these proteins at the BBB suggests that these variants may protect against ApoE4-related barrier dysfunction. Levels of fibronectin-1 and COL6A2 are elevated in the BBB of ApoE4 carriers without these protective variants, which may drive pathogenic gliosis and vascular remodeling [2]. The effect was dosage dependent, as those with one copy of ApoE4 had an 8.1% increase in fibronectin expression, while those with two copies had an increase in fibronectin of 26.6%. Examination of postmortem brain tissue indicated that cognitively unimpaired ApoE4/E4 homozygotes with these protective variants had a lower degree of fibronectin-1 deposition and gliosis at the BBB, which was similar to what is observed in cognitively unimpaired individuals without ApoE4 (ApoE3/E3 genotype) [6].

Human research to suggest benefits to patients with dementia:

Fibronectin-1 inhibitors have not been tested in dementia patients.

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

Biomarker and gene association studies

Alzheimer's disease: A retrospective multi-omics biomarker study aimed at identifying senescence-associated secretory phenotypes (SASPs) in peripheral blood, identified fibronectin-1 as one of five upregulated plasma proteins associated with AD [7].
A multi-omics study examining the metabolomic and proteomic signatures of plasma samples from 80 individuals with AD, mild cognitive impairment (MCI), or normal cognition, identified an enrichment of extracellular matrix proteins in those differentially expressed across cognitive groups [8]. Levels of plasma fibronectin-1 progressively decreased from controls to MCI to AD. As a biomarker, fibronectin-1 had an AUC of 0.92, a sensitivity of 80%, and a specificity of 90%. Meanwhile, expression of fibronectin-1 within the frontal and temporal cortices has been found to be increased in AD patients, showing associations with amyloid pathology [9]. The difference between circulating and tissue expression of fibronectin-1 has been observed in the context of various pathological conditions, and may be a reflection of different physiological sources and functions. Additionally, the associations with circulating levels may be confounded by a lack of specificity in the detection of particular isoforms or modifications of fibronectin-1 which may confer distinct functions and downstream effects on pathophysiology. While total levels may decrease, there could be a shift in the overall composition of fibronectin isoforms/fragments in a disease state which exacerbates pathology. For example, plasma levels of high molecular weight fibronectin-1 were increased in AD patients, relative to controls, despite a reduction in fibronectin-1 levels as a whole [8]. A separate observational study including 110 patients with early or moderate AD and 60 controls found that serum fibronectin-1 levels were elevated in AD patients (687.99 ± 225.08 µg/mL) relative to controls (252.29 ± 60.77 µg/mL) [10]. However, it should be noted that controls were, on average, 20 years younger, and fibronectin-1 levels may be affected by age. Caution should be warranted in the interpretation of these biomarker studies. Since the mechanisms underlying the changes seen in circulating levels of fibronectin-1 are not well understood, these changes do not necessarily provide information about the prospective role of fibronectin-1 in AD progression. As an extracellular matrix protein, localization, and the presence (or absence) of interacting partners are critical factors in determining the function of fibronectin-1. As a result, changes in expression alone are insufficient to understand the physiological consequences.

AIDS-related dementia: Circulating levels of fibronectin have been found to be reduced in the CSF of HIV-infected patients with AIDS-related neurological disorders (n=41) relative to controls (n=20) [11]. The most profound decreases were observed in those with AIDS dementia complex (2.0 ± 1.1 ug/mL vs controls 6.1 ±1.1 ug/mL). The decrease may stem from the binding of fibronectin-1 to HIV proteins and increased utilization for wound repair responses from ongoing tissue damage. A gene and protein expression analysis of AIDS patients with HIV-associated encephalitis and dementia identified

fibronectin-1 as a hub node which interacts with structural and regulatory proteins of HIV, and may moderate the association with host endothelial cells and immune responses [12].

Multiple sclerosis: Genetic variants in fibronectin-1 have been associated with MS risk in case-control studies from Greek populations including 389 MS cases and 336 controls. One study found that the minor allele of the rs1250249 variant in fibronectin-1 was associated with a dose dependent reduction in the age of disease onset [13]. Another study found that rs1250258 variant was associated with MS risk (OR: 0.6427, 95% CI 0.5155 to 0.8013) [14].

Mechanistic studies

Gliosis: A common feature of studies examining the effect of fibronectin-1 in neurodegenerative diseases involves its role in reactive gliosis, especially astrocytosis. Astrocytes become activated in response to injury within the CNS to engage in repair processes [15]. However, excessive activation can result in glial scar formation, which tends to impede neuronal regeneration. Fibronectin-1 is a major component of these fibrotic glial scars.
In MS models of inflammatory demyelination, fibronectin accumulates in regions of demyelination and serves as an impediment to successful remyelination [16]. Fibronectin appears to be secreted by reactive astrocytes in response to inflammatory stimuli. Fibronectin is lost from regions that successfully remyelinate, while it persists in chronically demyelinated lesions. Inflammatory stimulus (TNFα)-triggered gliosis enhances astrocytic secretion of fibronectin, which in turn, promotes pro-inflammatory NF-kB signaling through integrin-mediated interactions [17]. Inhibiting fibronectin with the pUR4 peptide attenuates fibronectin-associated neuroinflammation in cellular and animal models [17; 18].
The mechanism underlying the protective fibronectin variant in ApoE4 carriers also appears to involve the modulation of astrocytic reactivity. Postmortem brain tissue from ApoE4 carriers with the rs140926439 variant shows lower levels of both reactive gliosis and fibronectin deposition at the BBB [4]. Studies in a zebrafish model indicate that the presence of fibronectin-1 enhances gliovascular contact and impairs amyloid clearance [4].
Together these studies suggest that the excessive accumulation of fibronectin is a feature of pathological astrocytosis which further exacerbates neuroinflammation and neuropathology. As such, attenuating the level of fibronectin deposition may be a mechanism to promote productive neural repair. This type of approach is likely to be most effective when used in combination with agents to dampen the inflammatory processes that contribute to the induction of reactive gliosis in the first place.

APOE4 interactions: Rare variants in fibronectin-1 suggest that fibronectin inhibitors may preferentially benefit ApoE4 carriers [4; 5].

Aging and related health concerns: Excessive fibronectin deposition is a feature of fibrotic disease. Preclinical studies suggest that disrupting this accumulation may mitigate disease progression. Fibronectin also plays a role in cancer progression.

Types of evidence:

- 1 meta-analyses of studies examining fibronectin-1 as a diagnostic biomarker for cancer
- 6 biomarker studies of fibronectin-1 in fibrosis-related conditions
- 5 biomarker studies of fibronectin-1 in cancer prognosis
- 2 gene association studies of fibronectin variants in cardiovascular disease
- Numerous laboratory studies

Fibrotic disease: POTENTIAL BENEFIT (Preclinical)
Fibronectin-1 is best understood in relation to its roles in wound healing and fibrotic disease [19]. Fibrosis is a consequence of dysregulated wound healing processes, thus the dysregulation of proteins involved in wound healing is a common feature of fibrotic diseases. This dual role also makes these pathways difficult to target. Ideal targets would be altered forms of these proteins that drive pathological fibrosis but have limited impact on productive wound healing processes.
The pathological upregulation of fibronectin-1 has been observed in the context of fibrosis in a variety of organ systems, such as the liver, kidney, heart, retina, and lung. Osteoarthritis is another condition that lies at the intersection of inflammation and fibrosis, in which joint stiffness is associated with excessive deposition of fibronectin.
Fibronectin inhibitors have been shown to mitigate fibrosis in preclinical models. The high-affinity fibronectin-binding peptide, pUR4, decreased fibrotic tissue and preserved liver function in the CCl_4 and dimethylnitrosamine-induced mouse models of liver fibrosis through the inhibition of extracellular matrix accumulation [20]. A pegylated version of pUR4, showing better tissue penetration than the un-modified version, was protective in the mouse unilateral ureteral obstruction model of kidney disease. Treatment with pegylated-pUR4 (12.5 mg/kg) starting three days post-surgery, decreased fibronectin-1 deposition in the kidney by ~70% and collagen deposition by ~60%, which was accompanied by a preservation of tubular structure [21]. The antibody Fn52, targeting the N-terminal 30 kDa region of fibronectin, which is a major site for its self-association, has shown anti-fibrotic effects in models of

proliferative vitreoretionopathy [22]. However, the potential clinical utility of inhibitors that target fibronectin-1 broadly, rather than specific forms/fragments is unclear.

Emerging observational biomarker studies are identifying forms of fibronection-1 that may be particularly relevant for fibrotic disease, which may serve as potential therapeutic targets. Primary lung fibroblasts from patients with idiopathic pulmonary fibrosis show elevated levels of fibronectin containing an extra A domain (EDA) [23]. The presence of this domain has been found to promote the differentiation of pro-fibrotic myofibroblasts in response to TGFβ [24]. An ELISA was developed to detect serum levels of a fibronectin fragment implicated in fibrosis [25]. Inflammation can trigger the fragmentation of fibronection-1 through the induction of various proteases, including matrix metalloproteases (MMPs), which as their name implies, are responsible for the degradation of extracellular matrix proteins. Levels of fibronectin containing an extra B domain (EDB) were found to be elevated in the serum of patients with idiopathic pulmonary fibrosis (median 31.38, IQR 25.79 to 46.84 ng/mL), relative to controls (median 28.05, IQR 21.58 to 33.88 ng/mL) [25]. The assay is designed to detect a fragment of EDB-containing fibronectin-1 cleaved by MMP-8. Future studies may provide insight into its potential relevance in promoting fibrosis.

The balance of extracellular matrix production and degradation is essential for proper tissue function. Excessive activity on either end will disrupt tissue structure and trigger a continual process of tissue remodeling that can ultimately result in fibrosis and a loss of function [26]. The transformation of fibonectin-1 from a facilitator of tissue repair to a promoter of pathological fibrosis typically involves the interplay of chronic inflammation. Activated immune cells can alter and damage the structure of the extracellular matrix through the induction of MMPs. Elevated activity of MMPs resulting from chronic inflammation can lead to excessive degradation of extracellular matrix proteins, which then triggers a wound repair process that can drive increased production of these matrix proteins, eventually devolving into a futile cycle. With continual damage, the deposition of these matrix proteins becomes progressively higher, altering the structural integrity of the tissue and ultimately leading to further declines in function.
This suggests that targeting the inflammation-related fragmentation of fibronectin may have utility in halting this cycle of pathology, however, it is not yet clear whether this can be achieved without interfering with normal wound healing processes.

Cancer: POTENTIAL STAGE-DEPENDENT BENEFIT (Preclinical)
The role of fibronectin-1 in cancer appears to be multifaceted, with evidence to support functions as both a tumor suppressor and as a driver of metastasis, depending on the timing and location of its expression [27]. As a feature associated with senescent cells, the induction of fibronection-1 may prevent cells with oncogenic potential from becoming cancerous. However, once cells have become cancerous, the re-expression of fibronectin-1, particularly under hypoxic conditions, may promote malignant progression. The expression of fibronectin-1 in the tumor microenvironment may alter the cellular adhesion and signaling landscape in a manner that allows tumor cells to evade immune detection and facilitates intravasation.
This suggests that the type and utility of a fibronectin-1 targeted therapeutic may depend on the stage of disease [27]. At the earliest stages, induction of fibronectin-1 may promote a senescent phenotype and halt tumor growth, whereas at later stages, the inhibition of fibronectin-1 may slow progression. Further studies are needed to determine the stages and types of cancer for which fibronectin-1 inhibitors may be best suited.

Currently, the greatest potential utility of fibronectin-1 for cancer is as a prognostic and progression biomarker.
Bladder cancer: A meta-analysis of eight studies including 744 bladder cancer patients assessed the efficacy of urine fibronectin as a non-invasive diagnostic biomarker [28]. The pooled sensitivity of the biomarker was 0.80 (95% CI 0.77 to 0.83) and the pooled specificity was 0.79 (95% CI 0.73 to 0.84). It had a diagnostic odds ratio of 15.18 (95% CI 10.07 to 22.87) and a diagnostic score of 2.72 (95% CI 2.31 to 3.13). Although, the combination of urine fibronectin with cytology was more sensitive 0.86 (95% CI 0.82 to 0.90) than fibronectin alone. Tumor expression of fibronectin-1 was identified as a marker of reduced survival and more extensive metastasis in muscle-invasive bladder cancer [29]. It was also found to be associated with subsets of tumor-infiltrating immune cells and the expression of immune checkpoints, such that high fibronectin expression is associated with better clinical response to immune checkpoint inhibitor therapy.
Ovarian cancer: Fibronectin-1 was found to be associated with the invasiveness and migratory potential of ovarian cancer cell lines [30]. It was also found to be elevated in tumor samples from patients with ovarian cancer, with the highest levels found in the most advanced stages of disease, suggesting an association with disease progression [30].
Gastric cancer: An in-silico biomarker study using data from the Human Protein Atlas identified five genes with theranostic potential for gastric cancer [31]. Cancerous tissue shows increased expression of

fibronectin-1, and its expression increases with tumor progression. While it tracks well with progression, it is elevated in a wide range of malignancies, and thus is not a specific diagnostic biomarker.
Glioblastoma: An analysis of seven microarray datasets including 409 glioblastoma samples found an association with the upregulation of extracellular matrix-related genes [32]. Fibronectin-1 exhibited the greatest number of interactions, and its elevation was associated with poor prognosis. Higher expression of fibronectin-1 was associated with shorter overall survival (HR: 1.5).

Cardiovascular disease: POTENTIAL BENEFIT (Preclinical)
Fibronectin-1 has been identified as both a biomarker and a potential causal factor in aspects of cardiovascular health and disease. The directionality of the associations has not been consistent, which may be related to differences in the functionality of fibronectin isoforms or cleavage products, which are often not distinguished from one another in biomarker studies. The source and location of the fibronectin may also influence its effects, such that circulating levels may not necessarily reflect changes in fibronectin expression and function in specific tissues of interest. Additionally, the effects of fibronectin-1 are context dependent, such that differences in associations between conditions of health and disease may reflect how the functionality of fibronectin-1 can be altered based on the cellular environment.

Biomarker studies: The upregulation of fibronectin-1 was found to be a marker of aortic valve calcification [33]. It was the top connected hub gene in a protein-protein interaction network, and showed good diagnostic potential, with an AUC of 0.833. It was associated with the nature of the immune response, such that it had positive associations with levels of memory B cells, M0 macrophages, and activated mast cells in the aortic tissue. It has been hypothesized that fibronectin-1 may promote osteogenesis in calcified aortic valve disease through the induction of WNT/β-catenin signaling [33]. The upregulation of cellular fibronectin-1 in fibrotic cardiac and vascular tissue is a common finding, while associations with circulating forms show greater variability.
Plasma fibronectin is a soluble form that differs from the cellular form found in tissues due to its lack of the EIIIA and EIIIB segments [34]. It is primarily released from hepatocytes and vascular endothelial cells, thus may be an indicator of vascular damage. The association between plasma fibronectin and coronary heart disease (CHD) has been mixed across observational studies. The largest study to date, which included 1,644 patients who underwent selective coronary angiography, found that patients with CHD had lower levels of plasma fibronectin [34]. There was a 1.39-fold (95% CI 1.22 to 1.59) increase in risk for CHD for every standard deviation decrease in plasma fibronectin, with a cutoff point of 183 mg/L. However, there were no clear associations between plasma fibronectin levels and CHD severity. The

mechanism underlying this association is unclear, but may be related to increased consumption of plasma fibronectin in atherosclerotic vessels in the context of heightened vascular remodeling.
An alternatively spliced form of fibronectin-1 containing an extra A domain (FN-EDA) promotes inflammatory responses and has been found to be elevated in the context of pathological cardiac remodeling [35]. FN-EDA can activate toll-like receptor 4 (TLR4) on immune cell subsets, leading to the induction of pro-inflammatory signaling cascades and matrix metalloproteases (MMPs). An ELISA assay developed to detect FN-EDA in serum found that serum FN-EDA was elevated in 114 heart failure patients (5.58 ± 5.97 µg/mL) relative to 12 controls (0.52 ± 0.26 µg/mL) [35]. Similar to what was observed with plasma fibronectin, serum FN-EDA was not related to disease severity.
These studies highlight that circulating levels of fibronectin-1 can have complex relationships with the degree of fibronectin-1 deposition in tissues, and may differ depending on the form of fibronectin.

GWAS and MR studies: Genetic variants in the fibronectin-1 gene have been associated with coronary artery disease (CAD) risk [36]. The CAD-linked haplotype region, which includes the SNPs rs1250229 and rs1250259, was not associated with changes in fibronectin-1 gene expression, but rather, may affect its levels or activity through post-transcriptional mechanisms [37]. The protective rs1250258-T allele is linked to the rs1250259-A allele, which produces leucine instead of glutamine at position 15. Cell culture analysis indicates the rs1250259-A variant is more readily secreted by coronary artery smooth muscle cells, but does not appear to affect plasma levels. It also shows differential glycosylation. Increased glycosylation may help protect against the cleavage of fibronectin into fragments with inflammation-inducing activity. This suggests that the functionality of fibronectin may be more relevant for its influence on cardiovascular risk than the absolute levels of fibronectin.
A Mendelian randomization analysis aimed at linking blood biomarkers with blood pressure included 4,147 participants from the ORIGIN biomarker study and was validated using samples from the UK Biobank [6]. The study identified an association between cellular fibronectin-1 and pulse pressure. Elevated pulse pressure is an indication of vascular stiffness. The variants linked to pulse pressure (rs1250247, rs1250259, and rs34923683) are associated with lower serum cellular fibronectin-1, which is opposite of what is observed in epidemiological models that show a positive association.
These genetic associations suggest a protective effect of fibronectin-1 in cardiovascular tissue under normal conditions, but these protective effects may no longer be apparent in a pathological context.

Preclinical studies suggest that increased tissue deposition of fibronectin-1 contributes to immune infiltration and adverse cardiac remodeling, which may be mitigated through the use of fibronectin inhibitors.

A study assessing extracellular matrix proteins in the context of vascular aging found that extracellular matrix regulatory proteins were downregulated in conjunction with elevated oxidative stress with vascular aging (REF). This shift resulted in an increase in vascular thickness, perhaps mediated by the elevated levels of collagen and fibronectin-1.
In a mouse model of flow-induced vascular remodeling involving the partial ligation of the carotid arteries, periadventitial delivery of the fibronectin-1 inhibitor pUR4 in pluronic gel immediately after surgery attenuated adverse vascular remodeling [38]. Relative to a control peptide (III-11C), treatment with pUR4 led to reduced thickening of the carotid intima (63%), media (27%), and adventitial (40%) tissues, that likely stems from the reduction in smooth muscle cell proliferation and deposition of fibronectin and collagen in the vessel wall. Treatment with the fibronectin inhibitor also attenuated the expression of vascular adhesion molecules (ICAM-1 and VCAM-1) and the infiltration of leukocytes into the vessel wall. Similarly, in a mouse model of heart failure induced by ischemia/reperfusion (I/R)-injury, treatment with pUR4 (25 mg/kg/day i.p.) for seven days immediately following the I/R event reduced the deposition of fibronectin and collagen, cell proliferation, and the activation of cardiac myofibroblasts [39]. This protected against cardiac hypertrophy, fibrosis, and dysfunction, but did not impact infarct size. The cardioprotective effect was timing dependent, as delayed treatment starting four weeks after I/R injury did not significantly impact cardiac function.

Diabetes-related complications: POTENTIAL BENEFIT **(Preclinical)**
Diabetes is associated with changes to the extracellular matrix, generally leading to excessive deposition of extracellular matrix proteins, such as collagen, resulting in tissue stiffness and dysfunction. Cellular fibronectin-1 levels have been shown to be upregulated in diabetic patients [40], which may be a key driver of vascular and endothelial dysfunction. Although direct fibronectin-1 inhibitors have not yet been tested in the context of diabetes, evidence from observational studies and the use of indirect fibronectin inhibitors suggests that they may have utility in mitigating several diabetes-related complications.

Nephropathy: An integrated bioinformatic analysis of expression patterns in glomerular datasets found an enrichment of immune-related genes in patients with diabetic nephropathy [41]. Fibronectin-1 was identified as an immune-related biomarker of diabetic nephropathy (AUC: 0.837), and was inversely associated with the glomerular filtration rate, such that higher levels were associated with worse kidney function. Fibronectin-1 expression was also positively associated with fibrotic M2 macrophages.
Retinopathy: Excessive extracellular matrix protein deposition is associated with vascular leakiness in diabetic retinopathy [42]. In a rat model, intravitreal injection of siRNA targeting fibronectin-1 prevented

retinal basement membrane thickening and the loss of barrier integrity. The retino-protective effects of anti-integrin therapies in development may be related to a reduction in fibronectin deposition, through disruption of integrin-fibronectin interactions.
Ulcers: Fibronectin-1 plays an important role in wound healing by interacting with collagen and proteoglycans to form a provisional matrix that is eventually replaced by new tissue and supports tissue angiogenesis [43]. While the expression of fibronectin-1 is elevated in the context of diabetes, excessive proteolysis impairs its ability to carry out these functions, and instead contributes to a non-productive inflammatory response. While fibronectin inhibitors may not be suited to this indication, agents that target fibronectin fragmentation could potentially help preserve its wound healing functions.

Infectious disease: UNCLEAR BENEFIT (Preclinical)
Many pathogenic bacteria gain entry into animal tissues through interactions with the extracellular matrix of the host [44]. These interactions are facilitated by the bacterial expression of microbial surface components recognizing adhesive matrix molecules (MSCRAMMs). Fibronectin-1 is a common target of these MSCRAMMS. The cleavage of fibronectin-1 exposes many of the microbial binding sites, and thus enhances the potential for infection. Likely as a counter measure, the production of these fibronectin-1 fragments also exposes binding sites that trigger pro-inflammatory processes aimed at combatting the potential infection.
Interfering with the ability of bacteria to bind fibronectin via its binding proteins has been considered as a potential mechanism to protect against bacterial infection. Preclinical studies in rodents have tested fibronectin binding proteins as vaccines, however, the efficacy has been very limited to date [44]. It is possible that the vaccines tested thus far did not use fibronectin binding proteins with sufficient expression during *in vivo* infection or adequate antigenic potential. More work is needed to determine whether this is a viable avenue toward pathogen control.

Safety: The safety of fibronectin inhibitors has not been established, but could potentially impact wound healing.

Types of evidence:

- Several laboratory studies

Fibronectin-1 inhibitors have not yet been clinically tested, and the preclinical studies to date have primarily been proof of concept studies aimed at assessing efficacy, with little emphasis on safety.

Adverse reactions have generally not been observed with the pUR4 peptide, though the preclinical studies have been short in duration [21]. One study testing pUR4 in the context of skin inflammation found that intradermal administration of pUR4 exacerbated T cell accumulation and inflammatory responses in the skin [45]. It is unclear whether these effects were related to experimental design, or could be relevant in other systems.

Fibronectin-1 has ubiquitous expression and context-dependent effects, thus fibronectin inhibitors have the potential for on-target side effects. The main concern relates to its roles in clotting and wound healing, as these roles would want to be preserved in healthy tissue. The prospective safety profile of a given fibronectin-1 inhibitor will depend on the functions that it inhibits, as well as the spectrum of isoforms that it targets.

Drug interactions: Interactions have not been established.

Sources and dosing:

There are currently no fibronectin-1 inhibitors available for clinical use, nor are there any currently in clinical development.

Research underway:

Fibronectin-1 inhibitors are currently in early preclinical development by several academic research groups.

There are several clinical trials assessing fibronectin as a biomarker of response.

Search terms:

Pubmed, Google: Fibronectin-1

- Alzheimer's disease, neurodegeneration, aging, cancer, cardiovascular, inhibitor, biomarker

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