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.

TRIM21-based Degraders

Evidence Summary

TRIM21-based degraders may be useful for reducing levels of some intractable intracellular cytosolic targets, but optimal delivery mechanisms are needed for their clinical utility.

Neuroprotective Benefit: TRIM21-based degraders may be well-suited to target and reduce levels of fibril forming proteins associated with neurodegenerative disease.

Aging and related health concerns: TRIM21-based degraders may be able to target some disease-associated proteins, such as oncoproteins, difficult to target with small molecule approaches, but delivery methods may be a barrier.

Safety: The safety of TRIM21-based degraders has not been established, and will likely depend on the delivery strategy.

Availability: Research use	**Dose**: N/A May depend on protein target and delivery system	**Chemical formula:** N/A **MW**: N/A
Half life: N/A	**BBB**: Will depend on delivery mechanism	
Clinical trials: None	**Observational studies**: None	

What is it?

TRIM21 (Tripartite motif containing 21) is an E3 ubiquitin ligase and cytoplasmic antibody receptor that plays a role in pathogen defense [1]. In the context of a viral infection, TRIM21 is induced by type I interferons, and it binds to the Fc region of antibodies (IgG, IgM, IgA) which have bound to their target viral antigens, and then targets the entire complex for degradation via the ubiquitin-protease system. TRIM21 contains an N-terminal RING-B-box-coiled-coil (RBCC motif) and a C-terminal PRY-SPRY domain, which binds with high affinity to the Fc fragment of immunoglobulins (Ig). The RBCC motif has the E3 ubiquitin ligase activity through the RING domain, and also includes a B-box domain, and a coiled-coil dimerization domain.

The mechanism of TRIM21-based protein degradation has been adopted into new technologies aimed at specifically degrading disease-associated proteins [2]. The development of targeted protein degradation technologies is a very active area of research, and a wide variety of systems have been tested in preclinical models, each with their own set of strengths and weaknesses. Many of these systems can only target proteins which themselves have a known binding site with an E3 ligase, and require the development of novel small molecules for each target [3].

TRIM21-based protein degraders were developed to try to circumvent this issue because TRIM21 binds to the Fc region of the antibody, rather than the targeted protein itself, thus allowing for greater variety in the types of proteins that can be targeted [3]. In theory, all that is needed is a specific, high-affinity antibody toward the protein of interest, which can also potentially be customized to only recognize particular pathogenic forms of the protein. These TRIM21-based protein degradation systems are continuing to be optimized, such that there are numerous different versions.

Further optimization is going to be needed to allow for clinical translation, particularly with respect to the delivery of these degraders to cell types of interest in a reliable, safe, and effective manner. Most of the studies conducted to date have been in cell culture systems, with a focus on targets relevant for cancer and neurodegenerative disease.

TRIM21-based targeted protein-degradation technologies:

Trim-Away: This technology involves the introduction of an antibody targeting a protein of interest as well as exogenous TRIM21 to the cell to allow for TRIM21-mediated degradation of the antibody-protein complex via the proteasome [4]. Various cell delivery methods have been tested, including microinjection, electroporation, and streptolysin O-mediated permeabilization [5]. Theoretically, the system could work using endogenous TRIM21, however, this leads to wide variability in efficacy depending on the TRIM21 levels in a particular cell type of interest. The repertoire of antibodies suitable for this technique is also limited because the antibody needs to be able to bind to its target under physiological (i.e. non-denaturing) conditions [5]. Another limitation of this system is the large size of the antibodies, which can make it difficult to get sufficient amounts of them into the cell to facilitate clearance of the target protein. The levels of the target protein may also pose a challenge, as it may require a very high affinity for low abundance targets or a very high amount of cellular uptake for high abundance targets [6]. A variety of derivatives of this technique have been developed to try to get around these limitations.

TrimTAC: This system is designed to degrade proteins in multimeric complexes/biomolecular condensates [7]. These TRIM21-based PROTACs involve the linkage of acepromazine with a binder of the target protein (such as JQ1, a high affinity binder of BRD4, or a synthetic ligand of FKBP12) via an aliphatic chain. Acepromazine recruits TRIM21, and these constructs were found to specifically degrade condensate forming fusion proteins, but not their soluble counterparts in cell culture.

TRIMbody-Away: This system uses smaller antibody fragments (~15 kDa) to replace full-size antibodies, such as single-domain antibodies, also called VHHs or nanobodies [8]. It involves the creation of a fusion protein involving a truncated form of TRIM21 fused with a nanobody to the protein target of interest. The truncated TRIM21 includes the RING-domain containing N-terminal RBCC domain with E3 ubiquitin ligase activity, but removes the C-terminal Fc-binding region (PRY-SPRY domain), instead replacing it with the nanobody.

ΔTrim-TPD: Various truncated versions of TRIM21 have been tested to improve its degradation efficiency. TRIMbody constructs in which the B-box domain of TRIM21 was deleted were found to have superior degradative performance toward their nanobody-directed protein targets [9]. The B-box domain is involved in the autoregulation of TRIM21, and its deletion increases the catalytic activity of the RING domain. ΔTrim-TPD variants have also been developed to allow for light-inducible (i.e. optogenetic) targeted protein degradation [10]. The small size of these ΔTrim-TPD constructs allows for packaging into viral vectors, such as lentivirus, for *in vivo* delivery. For cell culture studies, these constructs can be delivered as plasmids.

TRIM21–Tn3 fusion proteins: This variant involves the replacement of the TRIM21 C-terminal PRY-SPRY domain with the fibronectin type III domain (FN3) of human tenascin-C (Tn3), which provides a scaffold for target protein binding [6]. The evolved Tn3 domains were derived from naïve yeast-displayed Tn3 libraries against the target proteins of interest. The affinity of the Tn3 domains for the target greatly impacts efficacy, and affinity maturation may be required to effectively target endogenous proteins of interest. The fusion of tandem Tn3 domains allows for more efficient target degradation.

RING-bait: This system is designed to target fibril-forming proteins without requiring an antibody/nanobody for target recognition [11]. Instead, the RING domain of TRIM21 is fused with a 'bait' molecule, which is the protein target of interest, which can then itself be incorporated into actively forming pathological fibrils. The clustering of multiple RING domains in close proximity during fibril elongation will trigger TRIM21 E3 activity and activation of cellular degradation machinery, resulting in the degradation of the entire fibril.

TRIMTECH: This company is developing TRIM21-based degraders (TRIMTACs™), including brain-penetrant degraders. The company was founded by Cambridge Innovation Capital and the Dementia Discovery Fund. Their TRIMTACs™ are bispecific chimeric molecules that bring TRIM21 to proteins of interest, and are specific for oligomeric/aggregated proteins due to the clustering requirement for TRIM21 activity. The TRIMTAC™ technology appears to be similar to the RING-bait system, however, details of their degrader technology have not yet been disclosed, so proprietary differences between the two systems are unclear. (Press release)

A potential limitation that is not addressed by these approaches relates to the cellular localization of the target. This approach is suited for the removal of cytosolic proteins, and thus may be of limited utility for targets associated with intracellular organelles, such as the lysosome or mitochondria [5].

Neuroprotective Benefit: TRIM21-based degraders may be well-suited to target and reduce levels of fibril forming proteins associated with neurodegenerative disease.

Types of evidence:

- Several laboratory studies

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

Targeted protein degradation technologies, such as TRIM21-based degraders are designed to target disease-related proteins of interest [11]. It is currently unclear whether they will be well suited as a clinical strategy for prevention. Theoretically, these could be used to prevent the formation of pathological protein fibrils.

Human research to suggest benefits to patients with dementia:

TRIM21-based degraders have not yet been clinically tested in dementia patients.

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

Current preclinical studies are evaluating the potential of targeted protein degraders for removing toxic, aggregation-prone proteins associated with neurodegenerative disease [12]. Ideally, the degraders would selectively target mutated or modified forms associated with toxicity, while preserving endogenous forms of the protein that play important cellular functions. To date, studies investigating TRIM21-based degraders have primarily focused on targeting pathological tau species.

Tauopathies: POTENTIAL BENEFIT (preclinical)
Pathological proteins that form fibrils, such as tau, may be particularly well-suited to clearance by TRIM21-based degraders due to the requirement of TRIM21 to form clusters in order to engage the cellular degradation machinery [11; 13].
The fusion of the TRIM21 RING domain with a tau-specific nanobody was shown to specifically degrade aggregated tau and prevent seeded tau aggregation in cell culture, in a proteasome-dependent manner [14]. The preference toward aggregated vs monomeric tau was dependent on the affinity of the nanobody, such that the requirement for clustering is stronger for lower-affinity degraders. The nanobodies also specify the type of tau that is preferentially degraded, as they can be developed to target certain structural or mutated forms of tau, such as human 0N4R tau. As a proof-principle, these TRIM21-tau nanobody constructs were shown to be able to degrade 0N4R tau in the brain in the Tg2541 model in which mice express human 0N4R P301S tau under the Thy1 promoter. The TRIM21 degrader was packaged into an AAV vector (AAV9P31) for *in vivo* (i.v.) administration. The degrader was able to reduce the aggregated tau burden at times of both moderate (4 months old) and severe (5.5 months

old) tau pathology. There was, however, also some reduction in soluble tau levels with longer term (two months) treatment.
An alternative degrader has been developed by the same group to try to circumvent the limitations of antibody/nanobody use, particularly with respect to the challenge in achieving sufficient intracellular levels. A TRIM21-based degrader called 'RING-bait' has been shown to effectively degrade aggregated, but not monomeric tau, in preclinical models [11]. The degrader is a chimeric protein in which the RING domain of TRIM21 is fused to the C-terminal end of tau ('bait') to facilitate the incorporation of the construct into actively growing tau fibrils. The incorporation of multiple RING-bait proteins into a tau fibril would facilitate the clustering needed for TRIM21 to induce its E3 ligase activity and activate the cellular degradation machinery. The efficacy of the system depends on the degree of matching between the 'bait' and the form of tau present in the targeted cells. Packaging of the RING-bait construct into AAV vectors allows for its delivery to neurons. Primary neurons from mice expressing P301S tau show a reduction in aggregated and seeded tau in response to AAV(PHP.eB)-RING-bait infection. Similarly, systematic (i.v.) *in vivo* delivery of AAV(9P31) packaged RING-bait expressed under the synapsin promoter slowed the progression of tau pathology in the P301S mouse model. At six months of age, levels of aggregated and hyperphosphorylated tau were significantly reduced in P301S mice treated with the tau RING-bait construct at four months of age, such that they were closer to levels in younger (~four months old) untreated P301S mice at a less advanced stage of disease. Importantly, the use of the TRIM21-based tau degraders did not increase levels of seeding-competent tau, as has been a concern for other tau degrading interventions.
The primary challenge for the clinical translation of this technique is the development of a reliable, safe, and efficient delivery mechanism.

APOE4 interactions: Not established, but the functionality of TRIM21-based degraders is not expected to be affected by ApoE status.

Aging and related health concerns: TRIM21-based degraders may be able to target some disease-associated proteins, such as oncoproteins, difficult to target with small molecule approaches, but delivery methods may be a barrier.

Types of evidence:

- Several laboratory studies

Cancer: POTENTIAL BENFIT (preclinical)

Targeted protein degradation technologies have been considered a potential tool for the specific degradation of cancer-related proteins which have been considered intractable targets to date. Cancer-related proteins have been used as testing and validation targets for many TRIM21-based degraders. Since the vast majority of these have taken place in the context of cell culture models, the translation potential of these techniques for cancer patients remains to be seen, and will likely depend on the specificity of the antibodies/nanobodies and the ability to deliver the constructs to the cell types of interests *in vivo* in a targeted, safe, and reliable manner.

A modified version of TrimAway in which the B-box domain of TRIM21 is deleted (ΔBB) and was administered in conjunction with antibodies toward viral oncoproteins, HPV early protein 6 and early protein 7, was able to promote the degradation of the targeted oncoproteins in the HPV16+ human cervical cancer cell line CaSki [9]. Inducible (optogenetic) Trim-TPD constructs were able to degrade and reduce levels (>80%) of the oncoproteins, c-Myc and KRAS, in Capan-2, CFPAC-1, and A549 cell lines [10]. In a mouse melanoma model using B16F10Luci cells, lentiviral vectors containing OptoΔ2Trim-TPD constructs targeting PD-1 and c-Myc were administered when tumors reached 80–100 mm^3 [10]. Activation of the degraders using blue light significantly prolonged survival and led to an elimination of the tumors in five of the six mice. TRIM21–Tn3 fusion proteins with high affinity to their targeted oncoproteins associated with B-cell lymphomas, MALT1 and EED, were able to promote the degradation of these targets in cell culture [6]. A TRIM21-based bioPROTAC involving a fusion of the TRIM21 RBCC domain with a nanobody to Human antigen R (HuR), an RNA-binding protein overexpressed in high-grade tumors and associated with poor prognosis, had an anti-tumorigenic effect in mouse xenograft models using the HCT116 human colorectal carcinoma cell line [3].

Safety: The safety of TRIM21-based degraders has not been established, and will likely depend on the delivery strategy.

Types of evidence:

- Several laboratory studies

TRIM21-based protein degraders have not yet been clinically tested. The vast majority of the studies investigating these degraders have been done in cell culture, often with the overexpression of exogenous targets, such as EGFP. In general, these constructs have not shown clear evidence of cytotoxicity when used in cell culture experiments [10; 11; 14]. Signs of toxicity have also not been

reported with the *in vivo* administration of TRIM21-based degraders [3; 10; 11; 14], however, these tended to be short-term proof-of-concept studies not designed to thoroughly assess safety outcomes.

The primary safety concern related to the clinical use of TRIM21-based protein degraders stems from the prospective delivery systems, such as viral vectors (i.e. AAVs) or lipid nanoparticles. The ability to deliver these degraders into the relevant cell types of interest for intracellular activity at a therapeutically meaningful level in a safe and reliable manner is the primary challenge and limitation of this technology.

Drug interactions: Not established. Will likely depend on the targeted protein of interest and delivery system used.

Sources and dosing:

TRIM21-based protein degraders are currently in preclinical development from several research groups. Additional work on the optimization of these systems for *in vivo* delivery is needed prior to their translation to clinical use.

Research underway:

Various groups are working on the development of TRIM21-based protein degraders, though efforts are still in the early preclinical stages.

Search terms:
Pubmed, Google: TRIM21-based degraders; protein degradation technologies
Alzheimer's disease, tauopathy, neurodegeneration, cancer, methodology, optimization

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