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.

3K3A-Activated Protein C (3K3A-APC)

Evidence Summary

3K3A-APC is an experimental drug currently in clinical trials for stroke. In Alzheimer's disease, it may have beneficial effects on the vasculature, reducing inflammation, and reducing amyloid.

Neuroprotective Benefit: Clinical studies suggested 3K3A-APC may be beneficial in stroke, and preclinical studies suggest benefits in Alzheimer's disease.

Aging and related health concerns: 3K3A-APC is exclusively studied for stroke and neurodegenerative disorders, and there is little evidence it would be beneficial for aging in general.

Safety: Short clinical studies suggest 3K3A-APC is associated with few adverse events, but long-term side effects are unknown.

Availability: Not currently available – currently in clinical trials for stroke; administered as a bolus injection	**Dose**: Up to 540µg/kg every 12 hours for five doses in stroke	**Sequence**: YGVYTKVSRYLDWIH **MW**: 1900 g/mol
Half life: 0.2-0.3 hours	**BBB**: Yes, in animals	
Clinical trials: Recent phase 2 study completed in January 2018	**Observational studies**: None	

What is it?

3K3A-Activated Protein C (3K3A-APC) is a modified version of activated protein C (APC) in development from ZZ Biotech for treatment of acute stroke. APC is a protease possessing two distinct functions: 1) anticoagulant properties mediated by proteolysis of coagulation factors Va and VIIIa, and 2) cytoprotective effects including antiapoptotic effects, anti-inflammatory effects, and endothelial barrier stabilization. 3K3A-APC is a modified version of APC where 3 lysine residues (191-193) are replaced by 3 alanine residues. This modification reduces the anti-coagulant properties by 90% while retaining the cytoprotective properties, thus diminishing the risk of intracranial bleeding. The cytoprotective properties of 3K3A-APC are mediated by binding to protease-activated receptor 1 (PAR1), and possibly to some extent by binding other receptors such as endothelial protein C receptor (EPCR), sphingosine phosphate 1 receptor 1 (S1P1), integrin Mac-1 (also known as CR3), apoER2, and tunica intima endothelial receptor tyrosine kinase 2 (Tie-2) (Griffin et al, 2018).

PARs contain their own ligands, meaning that cleavage of PAR1 by APC at the N-terminal at Arg46 exposes a cryptic ligand on the receptor that activates an anti-inflammatory, cytoprotective, and endothelial barrier protective pathway through PAR1 signaling. PAR1 is also the target of thrombin. Thrombin cleavage of PAR1 exposes a different cryptic ligand which mediates a pro-inflammatory pathway and the loss of endothelial barrier function. These two opposing effects are thought to occur because thrombin binding to PAR1 promotes receptor internalization and a reduction of signaling

through PAR1. On the other hand, APC binding to PAR1 promotes accumulation of the receptor on the cell surface (Griffin et al, 2018).

Studies have tested both 3K3A-APC and APC. Both molecules signal through PAR1, but 3K3A-APC lacks the anti-coagulant properties of APC (which are mediated by inactivation of factor Va and VIIIa).

Neuroprotective Benefit: Clinical studies suggested 3K3A-APC may be beneficial in stroke, and preclinical studies suggest benefits in Alzheimer's disease.

Types of evidence:

- 1 clinical safety study and 1 clinical study in stroke
- 1 preclinical study in an Alzheimer's animal model
- 8 preclinical studies in animal stroke models
- 2 preclinical studies in an ALS and MS model

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

Human research to suggest benefit in stroke patients
ZZ Biotech announced results of a phase 2 study in stroke patients. Four doses and placebo were tested (120, 240, 360, and 540 µg/kg) in combination with tissue-type plasminogen activator (tPA). All doses were safe and well-tolerated. Hemorrhage incidence was reduced in the 3K3A-APC group (67.4% vs. 86.5% in placebo). Total hemorrhage volume was also reduced (0.8mL vs. 2.1mL in placebo; p=0.066) (Lyden et al, 2019). In a phase 1 dose escalation study, the most common adverse events were headache, nausea, and vomiting reported in 54%, 8%, and 4% of individuals, respectively. 540ug/kg was considered the highest tolerated dose (Lyden et al, 2013).

Mechanism of action from animal studies
Preclinical studies suggest that 3K3A-APC crosses the blood-brain barrier in mice by transport through the endothelial protein C receptor (EPCR) (Deane et al, 2009).

In a mouse model of Alzheimer's disease, 4-month daily treatment with 3K3A-APC reduced amyloid in brain tissue and around the vasculature, reduced inflammation (NF-kB, GFAP, and Iba1 expression), increased cerebral blood flow, and improved cognition (Lazic et al, 2019).

In mice, murine recombinant 3K3A-APC (0.2mg/kg) was administered at 4 hours and 1, 3, 5, and 7 days after middle cerebral artery occlusion (MCAO) and compared with a placebo, tissue-type plasminogen activator alone (tPA – standard of care for stroke), and a combination of the two. Compared to placebo, 3K3A-APC reduced infarct volume and edema volume by 62% and 58%, respectively. tPA, but not 3K3A-APC, increased intracranial bleeding (as measured by an increase in hemoglobin in the brain). tPA in combination with 3K3A-APC did not increase intracranial bleeding but still had beneficial effects on infarct volume, consistent with 3K3A-APC's vasculoprotective features. Behavior also improved. Similar benefits were seen 28 days later, when human recombinant 3K3A-APC was administered 24 hours after experimental stroke and in an embolic stroke model in spontaneous hypertensive rats (Wang et al, 2013; Wang et al, 2012; Thiyagerajan et al, 2008). Similar results were reported in a mouse model of TBI, with administration of murine recombinant 3K3A-APC at 6, 12, 24, and 48 hours after injury leading to a 56% reduction in lesion volume 7 days later and improved behavior (Walker et al, 2010). In addition, in an animal model of stroke, human recombinant 3K3A-APC improved survival of transplanted neural stem cells and promoted differentiation into neurons (rather than astrocytes). These transplanted neurons integrated into the host cortex and sent axons to other brain regions (Wang et al, 2016). Another study reported that the benefits of APC (and 3K3A-APC) in ischemia-reperfusion injury are mediated by inhibition of the NLRP3 inflammasome (Nazir et al, 2017).

Some studies suggest there may be therapeutic differences between human recombinant 3K3A-APC and murine recombinant 3K3A-APC. Guo et al (2009) reported no differences in the anti-coagulant properties of human and murine 3K3A-APC. However, ten times the amount of human 3K3A-APC was required for the same benefit in a mouse stroke model (0.2mg/kg for mouse 3K3A-APC and 2mg/kg of human 3K3A-APC). *In vitro* studies suggested that human 3K3A-APC was five-fold more cytoprotective in an oxygen-glucose deprivation model in human brain endothelial cells whereas mouse 3K3A-APC was 2.5-fold more effective in a mouse neuron model of excitotoxicity. The correct dosing will have to be determined in clinical trials.

In vitro studies suggest that APC acts through PAR-1 and EPCR to prevent apoptosis of hypoxic human brain endothelial cells by transcriptional downregulation of the tumor suppressor protein p53, restoration of the pro-apoptotic Bax/Bcl-2 ratio, and reduction of caspase 3 signaling (Cheng et al, 2003).

A study in a multiple sclerosis mouse model suggested there were beneficial effects on behavior using APC and a different mutated form of APC lacking the anti-coagulant activity, though the effects using

the mutated APC dissipated over time (Han et al, 2008). Another study in an ALS mouse model reported that 5A-APC (another APC analog with diminished anti-coagulant properties) prevented the early breakdown of the blood-spinal cord barrier and delayed the onset of motor symptoms (Winkler et al, 2014).

APOE4 interactions:
None reported

Aging and related health concerns: 3K3A-APC is almost exclusively studied for stroke and neurodegenerative disorders, and there is little evidence it would be beneficial for aging in general.

Types of evidence:

- 1 biomarker study
- 1 preclinical study

3K3A-APC has been almost exclusively studied in CNS disorders. Plasma APC levels were reported to be reduced in diabetic patients (especially in patients with evidence of vascular injury) and inversely correlated with carotid intima-media thickness (Matsumoto et al, 2007). APC reduced cell death and reduced levels of pro-inflammatory markers (such as TNFα and MCP-1) in a mouse model of hepatic ischemia and reperfusion injury (Park et al, 2009). Whether treatment of peripheral cardiovascular diseases with 3K3A-APC or APC alone will be beneficial is unknown.

Safety: Short clinical studies suggest 3K3A-APC is associated with few adverse events, but long-term side effects are unknown.

Types of evidence*:*

- One phase 1 and one phase 2 study

Currently, 3K3A-APC (as opposed to APC) appears safe in clinical trials with only mild side effects such as headache, nausea, and vomiting, though the side effects occur with a similar frequency to a placebo group (Lyden et al, 2013; Lyden et al, 2019). No studies have tested chronic 3K3A-APC dosing.

Drug interactions:
No interactions are known, but theoretically it could interact with other anti-coagulants (since it still has some [~10%] of its anti-coagulant activity).

Sources/dosing:
3K3A-APC is an experimental drug and is not currently available. Clinical studies have used up to 540µg/kg every 12 hours for five doses in stroke.

Research underway:
A phase 2 study was finished in January 2018. There are no other studies listed on clinicaltrials.gov.

Search terms:
3K3A-APC
APC + Alzheimer, cardiovascular