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

Evidence Summary

3K3A-APC is undergoing a phase 3 trial for acute ischemic stroke. In November 2023, whistleblowers highlighted evidence of potential harm with the drug along with possible research misconduct.

Neuroprotective Benefit: In a phase 2 study in acute ischemic stroke patients, 3K3A-APC did not significantly decrease intracranial hemorrhage. The whistleblowers' dossier suggested that the phase 2 study may have unintentionally favored 3K3A-APC.

Aging and related health concerns: 3K3A-APC has not been studied in age-related diseases beyond those involving the central nervous system.

Safety: In phase 1 and 2 studies, adverse events occurred at similar frequencies to placebo. Based on whistleblowers, 3K3A-APC may have increased deaths in the first week and disability and dependency at 90 days in the acute ischemic stroke phase 2 study.

Availability: In clinical trials	**Dose**: not established; intravenous	**Sequence**: YGVYTKVSRYLDWIH **MW**: 1900 g/mol
Half life: 0.2-0.3 hours	**BBB**: Penetrant in animals	
Clinical trials: A phase 2 study in ischemic stroke patients included 110 participants.	**Observational studies**: None available	

What is it?

3K3A-Activated Protein C (3K3A-APC) is a modified version of activated protein C (APC) in clinical development by ZZ Biotech for the treatment of acute ischemic stroke, amyotrophic lateral sclerosis (ALS), and diabetic wound healing. In June 2020, the FDA designated 3K3A-APC for the treatment of acute ischemic stroke as a Fast Track development program. However, in November 2023, a group of whistleblowers submitted a dossier to the National Institute of Health which highlighted evidence of increased deaths in the first week after treatment with 3K3A-APC in the phase 2 ischemic stroke trial, along with evidence that research articles supporting the drug "contain seemingly doctored data that suggest scientific misconduct"(Piller, 2023).

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) (reviewed in Griffin et al, 2018).

PARs contain their own ligands, such 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: In a phase 2 study in acute ischemic stroke patients, 3K3A-APC did not significantly decrease intracranial hemorrhage. The whistleblowers' dossier suggested that the phase 2 study may have unintentionally favored 3K3A-APC.

Types of evidence:

- 2 clinical trials
- Preclinical studies in models of stroke, Alzheimer's disease, ALS, and multiple sclerosis

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

No studies have tested 3K3A-APC for the prevention of dementia or age-related cognitive decline in humans.

Human research to suggest benefit in stroke patients:

In a phase 2 study (RHAPSODY Trial), four doses of 3K3A-APC (120, 240, 360, and 540 µg/kg in 100 ml intravenous infusion over 15 minutes every 12 hours) versus placebo were tested in 110 moderate to acute ischemic stroke patients receiving intravenous tissue-type plasminogen activator (tPA), intra-arterial mechanical thrombectomy, or both (Lyden et al, 2019). Vasculoprotection was assessed as microbleed and intracranial hemorrhage rates. There was no statistically significant difference in intracranial hemorrhage rates. Exploratory analyses showed that when the treatment arms were combined, 3K3A-APC reduced intracranial hemorrhage rates compared to placebo, from 86.5% to 67.4%

(p=0.046), and total hemorrhage volume showed a trend of reduction from an average of 2.1 ± 5.8 ml in the placebo group to 0.8 ± 2.1 ml in the 3K3A-APC combined treatment arms (p=0.066). The incidence of a favorable outcome, measured by the 90-day modified Rankin Score of 0 or 1, was not statistically significantly different between 3K3A-APC-treated patients (45.2%) compared to the placebo group (62.8%). The incidence of a favorable 90-day Barthel Index (≥90) was not significantly different between 3K3A-APC-treated (76.9%) versus the placebo group (91.9%). There were also no significant differences in the median interquartile range 90-day National Institutes of Health Stroke Scale (NIHSS), which was 1.5 (0.0-4.0; n = 56) in the 3K3A-APC group and 1 (0.0-3.0; n = 37) in the placebo group. Infarct volume (mean ± SD) at 90 days was similar among all 3K3A-APC doses and the placebo group; 26.2 ± 32.6 ml in 3K3A-APC–treated patients (n=56) versus 26.0 ± 42.1 ml in placebo-treated patients (n=37). Using a prespecified volume threshold (0.06 ml) for defining "hemorrhage-positive" scans, hemorrhage occurred in 56% of 3K3A-APC–treated patients versus 68% of placebo-treated patients, though this difference was not statistically significant (p=0.28). Using quantitative volumetry, there were smaller hemorrhages in the 3K3A-APC–treated group, but this difference was not statistically significant (p=0.07).

In November 2023, a group of whistleblowers submitted a dossier to the National Institute of Health which highlighted evidence that the 3K3A-APC treatment "might have actually increased deaths in the first week after treatment" in the phase 2 study described above (reviewed in Piller, 2023). The article noted that "six of the 66 stroke patients who received 3K3A-APC died within this period, compared with one among 44 in the placebo group, although the death rate evened out after a month". Also, stroke patients who received 3K3A-APC treatment "trended toward greater disability and dependency at the end of the trial, 90 days after treatment". The whistleblowers also noted that the phase 2 study "may have unintentionally favored 3K3A-APC". Among patients who received both the intravenous tPA and intra-arterial mechanical thrombectomy, the placebo group received tPA on average, more than 2 hours later than 3K3A-APC-treated patients: 5 hours 39 minutes for the placebo group, and 3 hours 31 minutes for the 3K3A-APC groups (Lyden et al, 2019). Based on the American Stroke Association, intravenous tPA treatment within 4.5 hours of stroke onset is the standard of care for most ischemic stroke patients.

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

The whistleblowers' dossier highlighted evidence that 37 peer-reviewed publications from Dr. Zlokovic's laboratory, including many that provide biological rationale for the use of 3K3A-APC in stroke patients, "contain seemingly doctored data that suggest scientific misconduct" (reviewed in Piller, 2023). This section discusses findings of 3K3A-APC from peer-reviewed publications that are not on the whistleblowers' list.

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 traumatic brain injury (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: Unknown

Aging and related health concerns: 3K3A-APC has not been studied in age-related diseases beyond those involving the central nervous system.

Types of evidence:

- None

No studies have tested 3K3A-APC for age-related diseases other than those involving the central nervous system described in the Neuroprotective Benefit section.

Safety: In phase 1 and 2 studies, adverse events occurred at similar frequencies to placebo. Based on whistleblowers, 3K3A-APC may have increased deaths in the first week and disability and dependency at 90 days in the acute ischemic stroke phase 2 study.

Types of evidence:

- One phase 1 and one phase 2 study

In a phase 1 placebo-controlled dose escalation study in 64 healthy adults, the most common adverse events were headache, nausea, and vomiting reported in 54%, 8%, and 4% of individuals, respectively. Single intravenous doses of 3K3A-APC at 6, 30, 90, 180, 360, 540, or 720 µg/kg, and 5 doses every 12 hours of 3K3A-APC at 90, 180, 360, or 540 µg/kg were examined. The highest tolerated dose was considered to be 540 µg/kg (Lyden et al, 2013).

In a phase 2 study (RHAPSODY Trial), four doses of 3K3A-APC (120, 240, 360, and 540 µg/kg in 100 ml intravenous infusion over 15 minutes every 12 hours) versus placebo were tested in 110 moderate to acute ischemic stroke patients receiving tissue-type plasminogen activator (tPA), intra-arterial mechanical thrombectomy, or both (Lyden et al, 2019). The maximum tolerated dose of 3K3A-APC (the highest dose associated with an estimated toxicity under 10%) was 540 µg/kg, with an estimated toxicity rate of 7%. Dose-limiting toxicity occurred in 4 (9%) placebo-treated patients and in 3 (5%) 3K3A-APC–treated patients. The continual reassessment method (CRM) model estimated the dose-limiting toxicity frequencies to be 3% for the 120 µg/kg dose, 4% for the 240 µg/kg dose, 5% for the 360 µg/kg dose, and 7% for the 540 µg/kg dose. Adverse events, serious adverse events (SAEs), and hemorrhages all occurred with a similar frequency in 3K3A-APC- and placebo-treated groups. Neuroworsening, defined as an increase in the NIHSS by more than 4 points, were seen at similar rates in both groups: 8 (12.1%) 3K3A-APC-treated and 8 (15.9%) placebo-treated individuals. Mean activated partial thromboplastin time after each dose of 3K3A-APC was not statistically significantly elevated. There were no statistically significant increases in hemorrhage rate or volume with 3K3A-APC treatment. Between the placebo and 3K3A-APC treatment groups, there was no difference in the numbers of microbleeds observed within or outside the infarct zone. No studies have tested chronic 3K3A-APC dosing.

As discussed in the Neuroprotection section, a group of whistleblowers submitted a dossier to the National Institute of Health in November 2023, which highlighted evidence that the 3K3A-APC treatment "might have actually increased deaths in the first week after treatment" in the phase 2 study described above (Piller, 2023). The article noted that "6 of the 66 stroke patients who received 3K3A-APC died within this period, compared with 1 among 44 in the placebo group, although the death rate evened out after a month". Also, stroke patients who received 3K3A-APC treatment "trended toward greater disability and dependency at the end of the trial, 90 days after treatment".

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

Sources and dosing:

3K3A-APC is under development by ZZ Biotech for the treatment of stroke, wound healing, and ALS (ZZ Biotech Pipeline). The phase 2 study in moderate to severe acute ischemic stroke patients tested intravenous doses up to 540 µg/kg (15-min infusion) every 12 hours for 5 doses (Lyden et al, 2019).

Research underway:

Based on ClinicalTrials.gov, a phase 3 study is aiming to evaluate the efficacy and safety of intravenous 3K3A-APC for the treatment of acute ischemic stroke following treatment with thrombolysis, mechanical thrombectomy, or both (NCT05484154). This study has an estimated study start date of December 2023 and a planned enrollment of 1,400 participants. The current status of the trial, as of November 2023, is "Not yet recruiting".

Search terms:
Pubmed, Google: 3K3A-APC

Websites visited for 3K3A-APC:

- ClinicalTrials.gov
- Drugs.com (0)
- WebMD.com (0)