Pantothenate-kinase-associated neurodegeneration (PKAN) is an inherited disease caused by \textit{PANK2} gene mutations\(^1\) that are thought to result in the reduction of cellular coenzyme A (CoA). Patients with PKAN exhibit a variety of symptoms, including dystonia, rigidity, bradykinesia, spasticity, difficulty swallowing and speaking, shortened lifespan, and sometimes cognitive and visual impairment.\(^2\) The clinical symptoms are often associated with an accumulation of iron in the brain and postmortem pathology indicates an enrichment of ischemic foci in the globus pallidus,\(^3\) implicating an interruption of oxidative metabolism in the central nervous system (CNS). CoA is cell autonomous and thus any effective PKAN therapy must penetrate both cellular membranes and the blood-brain barrier (BBB). One of the first ideas was to treat patients with pantothenate in an attempt to raise CoA synthesis by increasing substrate supply. Although it remains possible that high-dose pantothenate over extended periods may be useful in reducing the symptoms,\(^4\) the strong feedback inhibition of the pantothenate kinases (PANKs) means that there is little to no increase in tissue CoA levels in animals treated with high-dose pantothenate.\(^5\)

Three different approaches to PKAN therapy have been proposed that are designed to bypass the \textit{PANK2} genetic defect by supplying a CoA biosynthetic pathway intermediate downstream of PANK. Phosphopantetheine is the product of PANK but cannot cross cell membranes due to its charged nature. Fosmetpantotenate was designed as a prodrug to deliver phosphopantetheine to cells and elevate intracellular CoA.\(^6\) The charged moieties on phosphopantothenate are chemically masked by covalent modification with hydrophobic groups to promote penetration across cellular membranes. The synthetic additions to phosphopantothenate are then released by intracellular enzymes (esterases) and the resulting phosphopantothenate bypasses PANK and is converted to CoA. This cellular pathway was established by showing that intact dual-labeled \([^{18}\text{O}]\)phospho\([^{13}\text{C}]\)pantothenate was converted to CoA.\(^7\) Fosmetpantotenate raised liver CoA levels in mice, but an elevation of brain CoA could not be demonstrated.\(^7\) However, the intrastriatal injection of fosmetpantotenate elevated brain CoA,\(^6\) confirming the low to absent BBB penetration when fosmetpantotenate was delivered systemically. Although fosmetpantotenate is a promising bypass drug, inefficient BBB penetration is a significant liability.

Phosphopantetheine is another intermediate in the CoA biosynthetic pathway downstream of \textit{PANK}, and phosphopantetheine itself\(^8\) or its derivative, acetyl-phosphopantetheine,\(^9\) was proposed as another bypass option. There are 2 serious problems with these potential therapeutics. (1) Both compounds are phosphorylated and, like phosphopantetheine, do not diffuse across cell membranes. The isotopic labeling experiment that was used to demonstrate the incorporation of the intact phosphopantetheine into cellular CoA cannot rule out the possibility that it was first degraded to pantetheine or pantotenate and then phosphorylated by \textit{PANK} prior to incorporation into CoA.\(^5\) Pantetheine is an excellent substrate for the \textit{PANK} enzyme.\(^10\) Because pantetheine is readily degraded to pantetheinone by cultured cells and in circulation,\(^11\) degradation prior to incorporation is the likely route to CoA. (2) The HoPan-treated mouse model\(^12\) was used to demonstrate that acetyl-phosphopantetheine reversed the effect of HoPan on liver CoA levels in mice.\(^9\) Because pantetheine alone potently counteracts HoPan-mediated reduction of CoA levels\(^12\) and acetyl-phosphopantetheine is degraded to pantetheinone by digestion,\(^13\)-\(^15\) it is not proven that the therapy bypasses PANK. The HoPan-treated mouse model is also not a representative PKAN model because HoPan treatment can be used to reduce CoA in the liver and kidney, but HoPan does not reduce brain CoA.\(^12\)
Extracellular CoA itself was the third proposed bypass treatment using cultured neuronal cells derived from PKAN patient fibroblasts or Drosophila. The facts that highly charged molecules such as CoA or CoA biosynthetic precursors cannot diffuse into cells and that CoA is digested by an assortment of extracellular enzymes to ultimately yield pantetheine and cysteamine prior to absorption by intestine or other tissues were not considered. There is no direct evidence that the treatment of cells with CoA increases cellular CoA. Extracellular CoA, pantetheine, or pantetheine is not effective in raising CoA levels in human cultured cells (Figure 1A). CoA and pantetheine are completely degraded to pantetheine during cell culture (Figure 1B).

The BBB penetration challenge was addressed by the recent development of a novel drug called PZ-2891. The physicochemical properties of drugs capable of crossing the BBB are established and PZ-2891 was designed to have the properties of polar surface area, number of hydrogen bond donors, molecular weight, and a cLogP value similar to the top 25 CNS drugs (Table 1). By comparison, the properties of fosmetpantotenate provide a clear rationale for the difficulties encountered in elevating brain CoA with this therapy (Table 1). PZ-2891 is very lipophilic and diffuses across membranes to elevate cellular CoA levels by acting as an allosteric activator that prevents feedback inhibition of the PANK enzymes, including PANK1 and PANK3 which are intact activities in the context of mutated PANK2 as found in PKAN patients.

A PKAN mouse model with disrupted brain CoA biosynthesis was derived by specific deletion of the murine PANK1 and PANK2 genes in neurons to serve as a platform to evaluate PKAN therapeutics. Brain CoA levels were reduced significantly in this model and the animals exhibited phenotypic characteristics that resembled PKAN including reduced locomotor activity, growth rate, and lifespan. Oral administration of PZ-2891 elevated brain CoA and substantially resolved the severe locomotor, growth, and lifespan phenotypes. These data show that PZ-2891 penetrates the BBB to elevate

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**Table 1.** Physicochemical properties of PZ-2891, protected phosphopantotenate (RE-024 or fosmetpantotenate), and the top 25 central nervous (CNS) system drugs.

| PROPERTY | MEAN VALUE OF TOP 25 CNS DRUGS | SUGGESTED LIMITS | PREFERRED RANGE | PZ-2891 | RE-024 |
|----------|-------------------------------|------------------|-----------------|---------|--------|
| PSA (Å)  | 47                            | <90              | <70             | 72      | 149    |
| HBD      | 0.8                           | <3               | 0-1             | 0       | 3      |
| cLogP    | 2.8                           | 2-5              | 2-4             | 2.3     | 0.42   |
| MW       | 293                           | <500             | <450            | 349     | 474    |

Abbreviations: HBD, hydrogen bond donor; MW, molecular weight; PSA, polar surface area.

Properties of the top 25 drugs considered critical for blood-brain barrier penetration were taken directly from Hitchcock and Pennington. The properties of PZ-2891 and RE-024, also known as fosmetpantotenate, are provided for comparison.
CoA in CoA-deficient neurons to ameliorate the severe consequences of the CoA deficiency. There remain many challenges in the development of PKAN therapeutics, but the identification of a class of small molecule allosteric PANK activators that efficiently cross the BBB is an important step toward clinical deployment of a safe and effective treatment.

Acknowledgements
The author thanks the members of the St Jude Pantazine team, particularly Charles O Rock, Richard E Lee, and Stephen W White, as well as Lalit K Sharma, Chitra Subramanian, Mi-Kyung Yun, Matthew W Frank, Rajendra Tanggallapally, Anne Edwards, Julie Maier, Katie Creed, Karen Miller, Jina Wang, Lois Richmond, and Ruobing Zhou for their hard work and support during the project.

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SJ conceived and wrote the article and data were provided by the St Jude Pantazine team.

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