

**MLK Crystallography and the Design of Family Selective Mixed Lineage Kinase (MLK) Inhibitors.** Robert L. Hudkins, Cephalon, Inc. West Chester PA, USA.

Several lines of evidence indicate that neuronal apoptosis may be an important mechanism contributing to the progression of disability in Parkinson's (PD) and Alzheimer's diseases (AD). Although the few available therapies afford some degree of symptomatic relief, none prevents the progression of the disease or delays the pathological neuronal cell death associated with the disease. Activation of the c-jun-N-terminal kinase (JNK) pathway, which is critical for naturally occurring neuronal cell death in development, has been shown to mediate apoptotic neuronal death in response to a variety of stimuli and may be important for the pathological cell death in neurodegenerative diseases.

The JNKs (JNK1-3) are stress activated protein kinases (SAPK) that belong to the mitogen activated protein kinase (MAPK) superfamily and are the only kinases that phosphorylate the transcription factor cJun on Ser<sup>63</sup> and Ser<sup>73</sup>, an early initiating event in the cell death process. An important upstream activating component of the JNK signaling cascade are the mixed-lineage kinases (MLK) which function to phosphorylate MAPK kinases, MKK4 and MKK7, of the cascade. The MLKs function as serine/threonine kinases although their catalytic domains have features of both tyrosine and serine/threonine kinases. Overexpression of MLKs results in apoptotic cell death in PC12 cells and primary sympathetic neurons. Conversely, expression of kinase-dead MLKs blocks apoptosis induced by trophic factor withdrawal. Inhibitors of MLKs, and subsequently JNK/cJun, have the potential for not only slowing the progression of the neurodegenerative diseases but also for improving the function of surviving neurons. Our research has focused on the design of potent, selective inhibitors of MLKs for the treatment of AD and PD. CEP-1347 was the first compound from this program to advance into clinical evaluation for Parkinson's disease. Preclinical pharmacology demonstrated that CEP-1347 promoted neuronal survival in several *in vitro* and *in vivo* models. CEP-1347 attenuated the loss of tyrosine hydroxylase activity and dopamine transporter density after administration of 1-methyl-4-phenyl-tetrahydropyridine (MPTP) in mice and reduced the development of neurologic dysfunction in monkeys treated with

MPTP. CEP-1347 is a semi-synthetic 3,9-bis-ethylthiomethyl derivative of the indolocarbazole natural product (+)K-252a. (+)K-252a is a non-selective ATP competitive inhibitor of numerous tyrosine and serine/threonine kinases. One of the drawbacks of CEP-1347 is the requirement for fermentation of the natural product starting material (originally fermented from the culture broth of *Nocardiosis sp*). An ensuing medicinal chemistry program was initiated to identify smaller, fully synthetic MLK inhibitors as second generation compounds. The initial objective was to define the minimum pharmacophore and structural features of (+)K-252a required for MLK activity in order to identify a starting core. Presented will be MLK1 crystallography around this effort and SAR studies of family selective, synthetic second generation MLK inhibitors with improved properties for advancement.