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SM07883, a novel DYRK1A inhibitor, reduced Tau pathology – discovery and preclinical development of a potential therapeutic for Alzheimer's disease

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Background: Dual-specificity tyrosine phosphorylation-regulated kinase-1A (DYRK1A) overexpression in Alzheimer's Disease (AD) is correlated to tau hyperphosphorylation, formation of oligomers, and neurofibrillary tangle (NFT) formation. Elevated cellular stress signals such as Aβ and TNFα have been shown to induce DYRK1A activity, which in turn contributes to tau phosphorylation leading to tau pathology. Samumed is developing SM07883, a novel, orally bioavailable, small molecule, DYRK1A inhibitor as a potential therapeutic for AD or other chronic tauopathies.

Objectives:

- Assess the potential of SM07883 to inhibit tau hyperphosphorylation, aggregation, and NFT formation in mouse tau transgenic models.
- Measure the effects of SM07883 on neuroinflammation.
- Evaluate the effects of SM07883 on tau-associated functional phenotypes.
- Establish the safety profile of SM07883 in toxicology studies to enable clinical trials.

Methods: SM07883 selectivity and potency were evaluated in kinase panels, and inhibition of tau phosphorylation (pTau) was measured in cell-based assays. Increases in pTau and DYRK1A activity after treatment with $A\beta_{42}$, $A\beta_{25-35}$ or TNFα were measured in primary cortical neurons or microglial cells. The effects of SM07883 on LPS-induced TNF-α secretion were measured in cultured microglial cells and after intraperitoneal (IP) or intracerebroventricular (ICV) injections in mice. Cytokines were measured in plasma and brain lysates by electrochemiluminescence. SM07883 pharmacodynamics was measured in wild-type (WT) mice. To assess long-term efficacy, pTau and oligomeric and aggregated Tau were biochemically quantified in brain stems and spinal cords from ten-month-old JNPL3 mice (P301L human Tau overexpression mutation) orally administered SM07883 or vehicle (3 mg/kg, QD, 3 months). NFT containing cells with tau-positive inclusions were detected and quantified by immunostaining. Astrocyte activation was assessed using glial fibrillary associated protein (GFAP) staining with Western Blot quantification, and activated microglia were identified by Iba1 staining. Motor coordination was evaluated biweekly for 14 weeks after treatment initiation using a wire hang test. General tolerability was assessed by monitoring weight, morbidity, and mortality. GLP-compliant, 28-day, repeat-dose oral toxicity studies in both mice and monkeys were conducted with in-life, clinical pathology, histopathology, and toxicokinetic evaluations.

Results: SM07883 selectively and potently inhibited DYRK1A kinase activity (IC_{50} = 2 nM). Overexpression of both DYRK1A and the tau gene (HEK293T cells) increased tau

phosphorylation. In these cells, treatment with SM07883 reduced pTau at multiple sites including Thr212, AT8, Thr181, and Ser396 (EC_{50} 16, 69, 127, and 200 nM, respectively). In pharmacokinetic studies, SM07883 was orally bioavailable across multiple species while crossing the blood brain barrier (brain to plasma ratios > 2 in rodents). Compared to vehicle, WT mice showed a dose-dependent reduction of transiently induced brain pTau in a pharmacodynamic model starting with a single, 1.25 mg/kg SM07883 dose (47%, $p<0.001$). JNPL3 mice treated with SM07883 demonstrated significant ($p<0.05$) reductions in Tau hyperphosphorylation, sarkosyl-insoluble tau fragments, aggregated Tau, and significantly lower tau-positive inclusions (NFTs) compared to vehicle. GFAP and Iba1 immunoreactivity were reduced and decreased GFAP immunoreactivity was confirmed by Western Blot (37%, $p=0.001$). Motor function in the wire hang test was significantly improved in SM07883-treated JNPL3 mice compared to vehicle ($p=0.034$) starting 5 weeks after treatment initiation. SM07883 was well tolerated with significant weight gain ($p<0.001$) over the 3-month treatment period, and reduced morbidity and mortality were observed in treated animals. Additionally, SM07883 inhibited LPS-induced TNF-α secretion in microglial cells (EC_{50} = 71 nM) and decreased proinflammatory cytokines (IL-6, TNFα, IFNγ) in both plasma and brain lysates after IP or ICV injections in WT mice compared to vehicle. The no-observed-adverse-effect-levels (NOAELs) were established in toxicology studies up to the highest dose tested in mice and the middle dose in monkeys (30x and 5x higher AUC than the minimum efficacious dose, respectively).

Conclusion: SM07883, a selective and potent, oral, brain-penetrant, DYRK1A inhibitor significantly reduced tau phosphorylation and decreased the effects of pathological tau overexpression and neuroinflammation resulting in improved functional endpoints compared to vehicle in mice. IND enabling, 28-day, repeat-dose toxicological studies demonstrated acceptable safety profiles with a sufficient therapeutic margin. The biochemical profile of SM07883 established in these studies, including the effects on IL-6 and GFAP, provide a basis for potential biomarker profiles for clinical trials. SM07883 is a potential treatment for AD. A phase 1 clinical trial is planned.