Title: Translational anti-amyloid efficacy In Down syndrome therapy; implication of therapeutically limiting APP expression

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Over-expression of the amyloid precursor protein (APP) is linked to Alzheimer-like (AD-like) pathology of amyloidosis and dementia in Down Syndrome children as they reach adulthood. We reported that APP has a key role in the ferroportin-dependent efflux of iron when overloaded into neurons. The RNA-binding repressor protein iron-regulatory protein-1 (IRP1) controls iron-dependent levels of APP in neurons. We are beginning a new project to pharmacologically recapitulate the therapeutic strategy applied to Down syndrome mice to reduce APP levels by one-third and thus to find a drug that can recover many of the developmental neural deficits in these DS mouse models; this would include a restoration of damage to the L. Ceorulus centered on fear conditioned responses as found in Down syndrome mice.

Our published data showed an "on target" mechanism of JTR-009, where the drug irreversibly replaced IRP1 binding to the APP 5'UTR specific sequences in cultured neural cell lines and primary neurons. We determined the Kd of reversible binding of the repressor IRP1 to the 5'UTR specific APP IRE stem loop to be 30 pM to then block ribosome attachment and the onset of APP translation in conditions of iron deficit. The planar nature of JTR-009, and that of key analogs under development, may permit irreversible intercalation into the RNA helix of the APP stem loop to then inhibit APP translation. To explain co-inhibition with expression of the neurofibrillary microtubule protein tau, our sequence alignments revealed that a core AGU tri-loop and potential IRP1 binding site existed in the 5'UTR tau mRNA where tau, like APP, is known has a role in iron efflux. Five of ten APP blocker analogs of JR-009 were administered to cultured neurons, where we found the fourth in this series limited both APP translation and the appearance of phosphorylated tau. We observed no neurotoxicity relative to the well-tolerated APP blocker drug pospiphen, as measured by MTT assay.

The "break new ground" strategy is to set up a bilateral Harvard/Stanford collaboration to target the endogenous RNA structure of the 5'untranslated region of APP mRNA. This "RNA therapeutics" approach, for example, is the basis by which common antibiotics prevent translation in bacterial infections to cause bacteriostasis by targeting and inactivating the 23S ribosome to block microbial translation. Our facility will culture human stem cell neurons from AD/DS patient. We will identify which of 20 medicinally optimal benzimidazole analogs of APP blocker-9 can provide not only a breakthrough anti-amyloid efficacy by targeting this RNA structure, nevertheless we seek also to limit APP sufficient to ameliorate developmental lesions associated with Down syndrome even before the onset of amyloidosis. Our labs will adopt a bench-to-bedside approach to culture human progenitor stem cells and to test efficacy in DS mice expressing three copies of the APP gene; we thus seek to limit APP translation back to normal diploid content and also to prevent Aβ peptide amyloidosis in these models. We will establish the off-label activity of FDA analogs in human stem cells of DS individuals and identify any FDA approved activity to developmentally restore developmental lesions in the etiology of Down syndrome and aging context.

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