DEVELOPING TOPICS: S4-04 NOVEL APPROACHES TO APP THERAPEUTIC MODULATION

S4-04-01 CSF AMYLOID LOWERING IN HUMAN VOLUNTEERS AFTER 14 DAYS' ORAL ADMINISTRATION OF THE NOVEL BACE1 INHIBITOR E2609

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Background: E2609 is a novel, small chemical BACE1 inhibitor that is involved in production of amyloid beta (A b) leading to the deposition of amyloid plaques considered the hallmark of Alzheimer's disease (AD). In healthy volunteers, single oral doses of 5-800 mg E2609 demonstrated prolonged dose-related decreases in plasma amyloid (i.e., A b 1-x). We now present the effects of 2-week dosing of E2609 on plasma and CSF amyloid. **Methods:** In this single-center, randomized, double-blind, placebo-controlled study, healthy adult subjects aged 50 to 85 years are randomized to receive E2609 or placebo in 5 cohorts of 10 subjects each (8 E2609: 2 Placebo). E2609 doses of 25, 50, 100, 200 and 400 mg are given once daily for 14 days. Blood samples are collected at multiple times prior to, during and following the 14 day treatment period for both PK and PD assessments. CSF is collected via time-matched lumbar puncture 2 days prior to dosing and after administration of the final dose on Day 14. Primary PD endpoint is plasma and CSF A b 1-x. Other A b peptides (including: A b 1-40, A b x-40,A b 1-42,A b x-42) and sAPP± and sAPPβ are also being assessed. **Results:** The 25, 50 and 100 mg dose cohorts have been completed. E2609 administered for 14 days has been well-tolerated. Plasma exposure is approximately dose proportional. Steady state was achieved with some accumulation of the parent drug and measured metabolites after approximately 7 days. E2609 showed pronounced and sustained decreases in plasma A b 1-x at all doses compared with placebo, with nearly maximal effects (>85% reduction from baseline) observed at the lowest dose (25 mg). All subjects treated with E2609 showed significant reductions in CSF A b 1-x from baseline (Figure 1). Compared with placebo, statistically significant mean treatment reductions were 43.6, 59.4 and 71.3% (Table 1). Significant changes were also observed for the other CSF A b proteins examined (Figure 2). **Conclusions:** These preliminary results suggest that E2609 is a potent BACE1 inhibitor that results in consistent and sustained significant reductions of human plasma A b 1-x and dose-related decreases of amyloid peptides in CSF. No clinically significant safety concerns have been observed to date following repeated oral dosing of E2609.

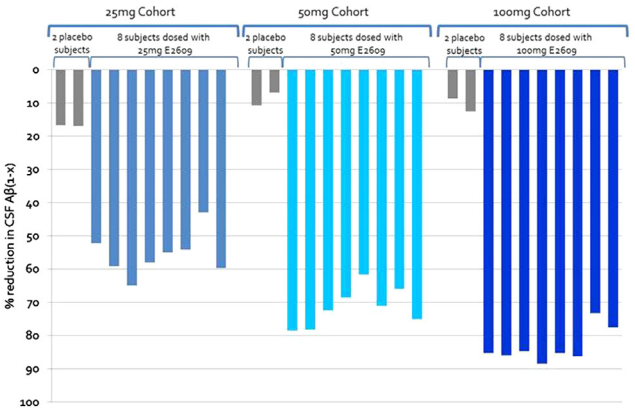

Figure 1. Reduction in Aβ(1-x) from baseline after 14 days dosing in individual subjects

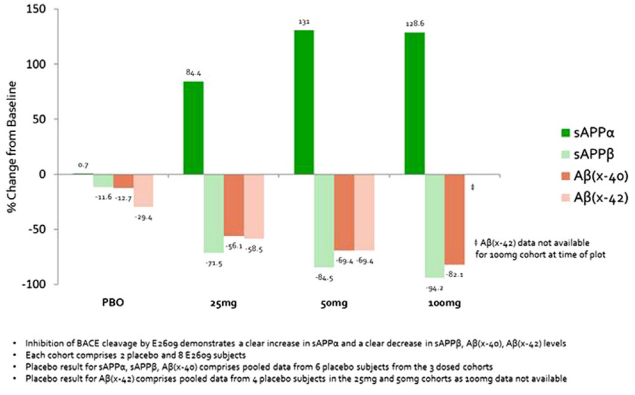

Figure 2. Change in sAPPα, sAPPβ, Aβ(x-40) and Aβ(x-42) compared to baseline after 14 days dosing

S4-04-02 APP-MEDIATED REGULATION OF NEURONAL CHOLESTEROL TURNOVER AND SYNAPTIC ACTIVITY: A NEW THERAPEUTIC TARGET FOR THE TREATMENT OF ALZHEIMER'S DISEASE

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Background: Processing of amyloid precursor protein (APP) by b - and g -secretase leads to production of A b, which accumulates in senile plaques found in brains of patients with Alzheimer disease (AD). The neuronal function of APP remains unclear. Genome wide association studies on AD confirmed ApoE4 allele as a major risk factor and identified other susceptibility loci encompassing clusterin and ABCA7 genes, further supporting the hypothesis that perturbation of lipids metabolism favors progression of AD. We wondered whether APP was able to modulate synthesis of cholesterol. **Methods:** Expression of human APP (hAPP) in primary cultures of neurons and astrocytes or down regulation of endogenous APP expression was performed using recombinant viruses encoding hAPP cDNA or APP shRNA, respectively. Cholesterol biosynthesis was measured by incorporation of 14 C acetate. Expression of enzymes involved in the mevalonate pathway was measured using qRT-PCR. Proteins were quantified by western blotting, and localized using immunofluorescence. Neuronal activity was measured by calcium oscillations. In vivo experiments were performed in 5xFAD transgenic mice in which long-term potentiation (LTP) was measured. **Results:** Expression of human APP

Table 1
Mean percentage decrease in CSF Aβ(1-x) after 14 days dosing compared to baseline at day -2

	% Decrease in CSF Aβ(1-x) Mean % ± S.D.			
Cohort and Dose	E2609 N = 8 / Group	PLACEBO* Pooled N = 6	Treatment Difference (E2609 – Placebo)	95% Confidence Interval
Cohort 1: 25 mg	55.7 % ± 6.6	12.1 % ± 4.2	43.6 %**	(37.32, 49.98)
Cohort 2: 50 mg	71.4 % ± 5.9	12.1 % ± 4.2	59.4 %**	(53.49, 65.23)
Cohort 3: 100 mg	83.4 % ± 5.2	12.1 % ± 4.2	71.3 %**	(65.87, 76.75)

*Result calculated from pooled placebo data
**$p<0.001$

(hAPP) in rat cortical neurons decreased HMG-CoA reductase (HMGCR)-mediated cholesterol biosynthesis, as well as SREBP-1 and -2 mRNA levels, while down regulation of endogenous APP expression had opposite effects. In neurons, APP and SREBP1 co-immunoprecipitated and co-localized in the Golgi. This interaction prevented Site-2 protease-mediated release of mature SREBP, leading to inhibition of transcription of its target genes. In cultured astrocytes, APP and SREBP1 did not interact nor did APP affect cholesterol biosynthesis. Therefore, we further analyzed the mevalonate pathway specifically in neurons. Neuronal expression of hAPP not only decreased HMGCR-mediated cholesterol biosynthesis, but also decreased cholesterol 24-hydroxylase mRNA levels and consequently cholesterol hydroxylation, leading to inhibition of cholesterol turnover. Such an inhibition dramatically decreased synaptic activity both in vitro and in vivo. Reactivation of the mevalonate pathway rescued synaptic activity in cultured neurons, as well as LTP in 5xFAD transgenic mice. **Conclusions:** Our results demonstrate that APP controls neuronal cholesterol turnover needed for synaptic activity and further emphasize the stimulation of neuronal cholesterol turnover as a possible target for the treatment of AD.

S4-04-03 AN APP TRANSLATION MODULATOR ARN2966 REDUCES BETA-AMYLOID DEPOSITION AND PREVENTS MEMORY DEFICITS IN ALZHEIMER'S DISEASE TRANSGENIC MICE

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Background: Accumulation of amyloid-Aß (Aß) peptide in the brain is central to the pathogenesis of Alzheimer's disease (AD). Development of anti-Aß therapies have been advocated for AD prevention and for the treatment during early stages of the disease when Aß toxicity still significantly influences neurodegenerative cascade including synaptotoxicity, neurofibrillary pathology, and neuronal loss. Aß production can be decreased by modulating processing or translation of the amyloid precursor protein (APP). While development of APP secretases inhibitors and anti-Aß immunization approaches are widely pursued, very few molecules capable of modulating APP expression and showing drug development potential have been identified thus far. **Results:** ARN2966 showed dose dependant inhibition of Aß40 and Aß42 production in APP_{751SW} transfected CHO cells (IC_{50}<1µM for Aß40), along with lowering the level of APP and C83, and C99 peptides, while the level of APP mRNA was unaffected. The inhibitory effect of ARN2966 on APP translation was directly confirmed in ^{35}S-Methionine labeling experiments. ARN2966 showed BBB penetration after oral and intravenous dosing. ARN2966 treatment prevented memory deficit inAPP_{SW}/$PS1_{dE9}$ mice, without producing any appreciable signs of toxicity. The absolute brain levels of Aß40 and Aß42 were reduced by 82% and 56% in ARN2966 vs vehicle treated animals, respectively ($p<0.001$). The level of Aß oligomers was reduced by 20.5% ($p<0.05$) while the load of Thioflavin-S positive Aß plaques was reduced by 21.9% ($p<0.001$). **Conclusions:** Our studies demonstrate potential of using APP translation modulator as a therapeutic approach for AD and characterize a novel, BBB permeable therapeutic compound with strong potential for further preclinical development.

S4-04-04 THE APP LIGAND NETRIN-1 IMPROVES MEMORY AND BIOMARKERS AFTER ICV ADMINISTRATION IN PD APP MICE

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Background: The axon guidance and trophic factor netrin-1 (N1) has been shown to bind the amyloid precursor protein (APP) and modulate APP-mediated signaling (Lourenco et al., 2009). Therefore, we tested N1 as a potential therapeutic in a transgenic mouse model of AD. **Methods:** In biodistribution (BD) studies, mice were injected with 5ul of 1ug/ul rmNetrin-1 (R&D Systems) in saline with 0.1% BSA into the right lateral ventricle and euthanized at 1, 6, and 24 hours. For chronic BD, rats were cannulated intraventricularly and received 24 ug/day rmNetrin-1 from subcutaneous Alzet pumps (ICV-P) for 6 days. BD was analyzed immunohistochemically (IHC). In a 14-day efficacy study, J20 PDAPP model mice were used and received either 2.64 ug/day rmNetrin-1 or vehicle by ICV-P (N=3). Working memory was assessed using the Novel Object Recognition (NOR) task. Tissue was analyzed for sAPPa (AlphaLISA, Perkin-Elmer), Ab1-42 and 1-40 (ELISA, Invitrogen). In a 28-day study, J20 mice received either rmNetrin-1 or vehicle, and non-transgenic mice received vehicle only (N=15/group). NOR was performed at Day 14 and 28. Biochemical analysis was performed as above. IHC analysis included anti-Iba1, anti-GFAP, anti-Netrin, and endogenous mouse IgG labeling. **Results:** Good BD of N1 was seen in both acute and chronic studies, predominantly on the cannulated side. N1 treatment resulted in a significant 35% novelty preference (NP) in the 14-day study. Significant decreases in Ab1-40 and Ab1-42 occurred, but no change in sAPPa. In the 28-day study, significant 30% (equal to control) and 14% NP was seen in N1-treated J20 mice at 14 and 28 days, respectively. Astrocytic gliosis, microglial activation, and high IgG levels were seen adjacent to the cannulation tract; inflamed tissue was excluded from biochemical analysis. sAPPa was significantly (t-test, two-tailed) increased by 9% on the left, cannulated side, and Ab1-40 and 1-42 were reduced, the latter by almost 20%, but not significantly due to individual variation. **Conclusions:** These studies support netrin-1 as a potential therapeutic for AD. However, the studies also reveal a risk of cannula-site inflammation and the need to address this risk in follow-up studies.

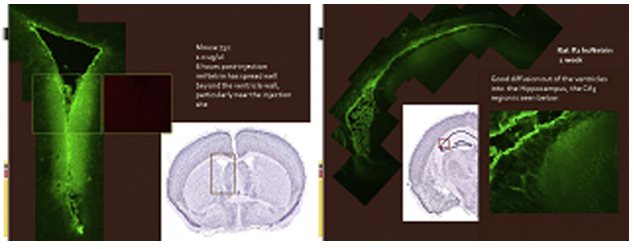

Fig. 1. *Acute and chronic biodistribution*. At 6 hours after acute injection, protein exiting the ventride was the most apparent (A). It was seen lining the ventricle with chronic infusion and in areas posterior to the cannulasite (B)

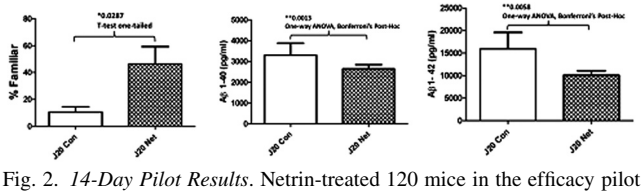

Fig. 2. *14-Day Pilot Results*. Netrin-treated 120 mice in the efficacy pilot showed preference for object novelty(A), no change in sAppα and reductions in Aβ1-40 and 1-42 (B,C)

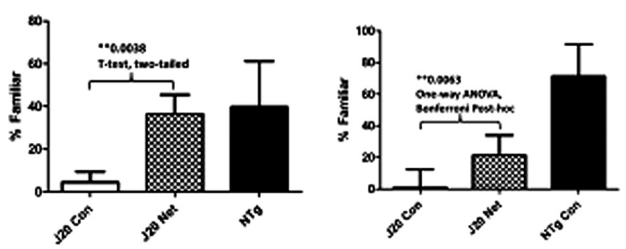

Fig. 3. *NOR in 28-day Study.* At 14 days, Netrin-treated mice showed dear novelty preference (A). This preference was less at 28 days (B).