Effects of a PPAR Delta/Gamma Agonist, T3D-959, on Metabolic and Cognitive Functions in Mild to Moderate Alzheimer's Disease Subjects

John Didsbury, PhD[1 †], Hoda Gabriel, PMP[1], Warren Strittmatter, MD[1] and Stan Chamberlain, PhD[1]

(1) T3D Therapeutics, Inc., Research Triangle Park, NC, USA

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Disclosures

John Didsbury, Ph.D.

- Employee of T3D Therapeutics, Inc.
- Shareholder inT3D Therapeutics, Inc.

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Overview of T3D-959

A. Preclinical Studies
B. Early Clinical Development
C. Phase 2a Exploratory Feasibility Study of T3D-959 in Mild to Moderate AD (T3D959-201)
D. Future Clinical Development

T3D-959:

- Orally administered indane acetic acid as a sodium salt
- Brain penetrant, 20h plasma $T_{1/2}$
- PPARδ (delta) / PPARγ (gamma) dual nuclear receptor agonist. Regulation of glucose and lipid metabolism
  - Primary target PPARδ 19nM EC_{50} on human receptor (regulator of energy expenditure)
  - Secondary target PPARγ 297nM EC_{50} on human receptor (regulator of energy storage)

PPAR Agonists in AD – A Brief Summary

PPAR gamma selective agonists - originally developed to treat Type 2 Diabetes by improving *systemic* Insulin Resistance (IR)

PPAR gamma selective agonists were engineered to limit central exposure

PPAR gamma has limited regional expression in the brain

Rosiglitazone – PPAR gamma (γ) selective agonist (thiazolidinedione)

- Poor brain penetration: only 0.0045% of oral dose gets into brain (rat)
- 'Failed' Phase 3 AD trial demonstrated that peripheral PPAR modulation does not provide efficacy in AD

Pioglitazone – PPAR gamma (γ) selective agonist (thiazolidinedione)

- Some success in Phase 2 AD trial in subjects with Type 2 Diabetes co-morbidity
- TOMORROW Phase 3 failure in cognitively normal subjects – failure of drug or failure of genetic algorithm for predicting risk to progression to AD?

T3D-959 - Potent PPAR delta / gamma agonist

- PPAR delta – high ubiquitous brain expression
- Different chemical class than Rosiglitazone or Pioglitazone (equivalent potency on gamma)
- Brain penetrant: rat brain/plasma ratio = 35% at 1 hr and 12 hr time points

T3D-959 Preclinical Studies

- Multiple Type 2 Diabetes Models
- AD model of sporadic AD – i.c. Streptozotocin (STZ) rat model

ACTIVITIES RELATED TO AD

AD Metabolic Events

↓ Insulin resistance

↓ IGF-1 resistance

↑ Reverse cholesterol transport

↓ Triglycerides

↑ HDL

↓ Ceramide Synthase 2 and SMPD3 (↓Ceramide)

AD Structural Events

↓ Aβ peptide

↓ pTau/Tau ratio

↓ GSK3β activation (↓ tau hyperphosphorylation)

↑ neuronal cell survival (in vitro)

Reversal of neuronal cell loss

Reversal of white matter atrophy

AD Stress Events

↓ Oxidative stress (histology)

↓ Inflammatory cytokines (TNFα, IL-1β)

↓ Ubiquitin (involved in protein misfolding – tangles/plaques)

AD Cognitive Impairment Motor Function Impairment

↑ Spatial learning and memory, STZ rat, (also seen in APP/PS1 mice, Tg2576 mice & 3xTg-AD mice w/ other PPAR delta agonists)

Anti-cachexia (↑ lean/fat mass ratio)

Motor function improvement in STZ rat model

T3D-959 Early Clinical Development

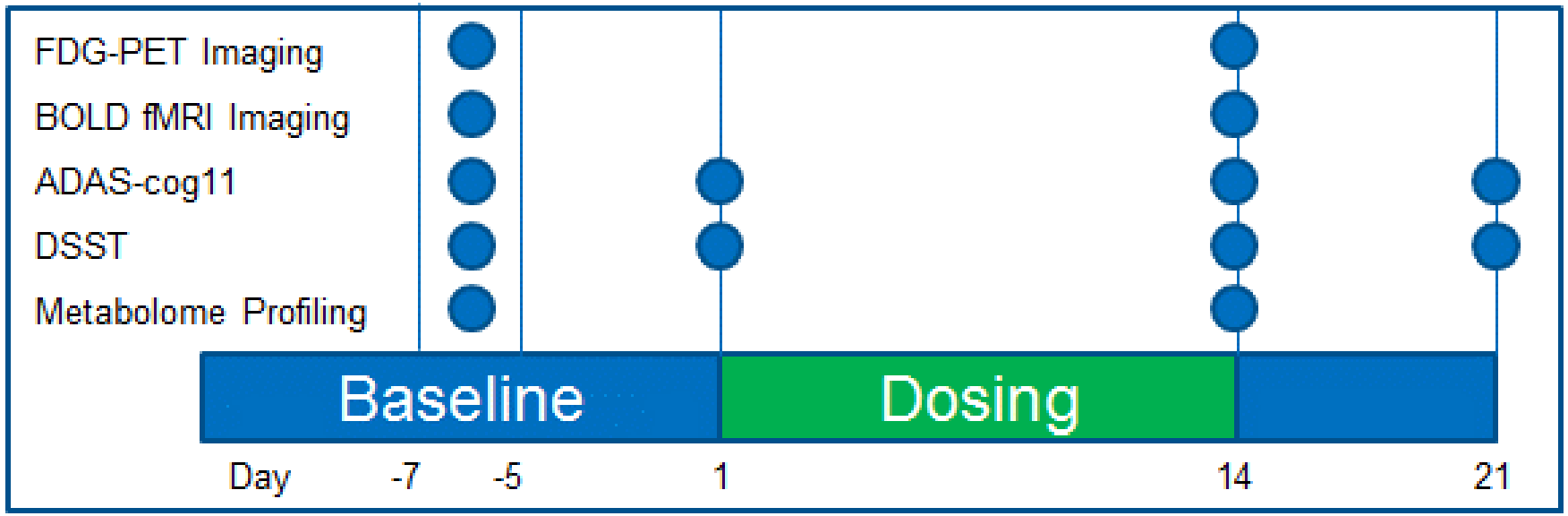
- Healthy Volunteers (n=96)
  - Ascending single dose (QD):
    - safe and well tolerated up to 200mg;
    - no Maximum Tolerated Dose (MTD) reached.
  - Multiple ascending dose, 7-day (QD):
    - safe and well tolerated up to 200mg;
    - no drug-related AEs,
    - no SAEs,
    - no Maximum Tolerated Dose (MTD) reached
  - Pharmacokinetics: $T_{1/2}$=20h, T_{max}=3-4h.

Phase 2a Exploratory Feasibility Study of T3D-959 in Mild to Moderate AD (T3D959-201)

Population	34 participants, 57-90 years, mild/mod. AD MMSE14-26 [17 mild MMSE 20-26, 17 moderate MMSE14-19]
Concurrent AD Medications	28 of 34
ApoE4 genotype	n=17 ApoE4 positive n=17 ApoE4 negative
N Per Dose Per Group	3mg (n=8), 10mg (n=9), 30mg (n=9), 90mg (n=8)
Dosing	Once daily for 14-days
Primary Objectives	FDG-PET – relative brain glucose metabolism (CMRgl) BOLD fMRI – hippocampal functional connectivity (resting state default mode network) ADAS-cog11 DSST Plasma Metabolome Profiling
Secondary Objectives	Safety & Tolerability

T3D959-201

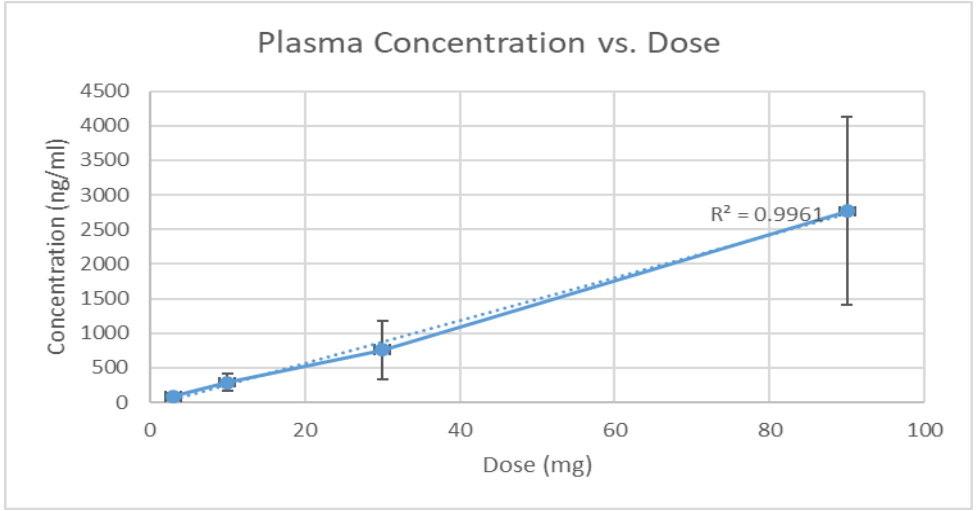
Dose	3mg	10mg	30mg	90mg
N	8	9	9	8
Age (avg.)	73.3	71.4	74.6	75.4
Sex M/F	4 / 4	4 / 5	4 / 5	4 / 4
MMSE (avg.)	19	19.9	21.9	18.8
ApoE4+/ApoE4-	3 / 5	7 / 2	4 / 5	3 / 5



T3D959-201: Pharmacokinetics

Single Point Plasma PK at approximate Tmax (3-4h post dosing at EOT) Consistent with Phase 1 Studies

3mg cohort – 86ng/ml = 195nM
10mg cohort – 290ng/ml = 654nM
30mg cohort – 763ng/ml = 1.72uM
90mg cohort – 2,766ng/ml = 6.24uM



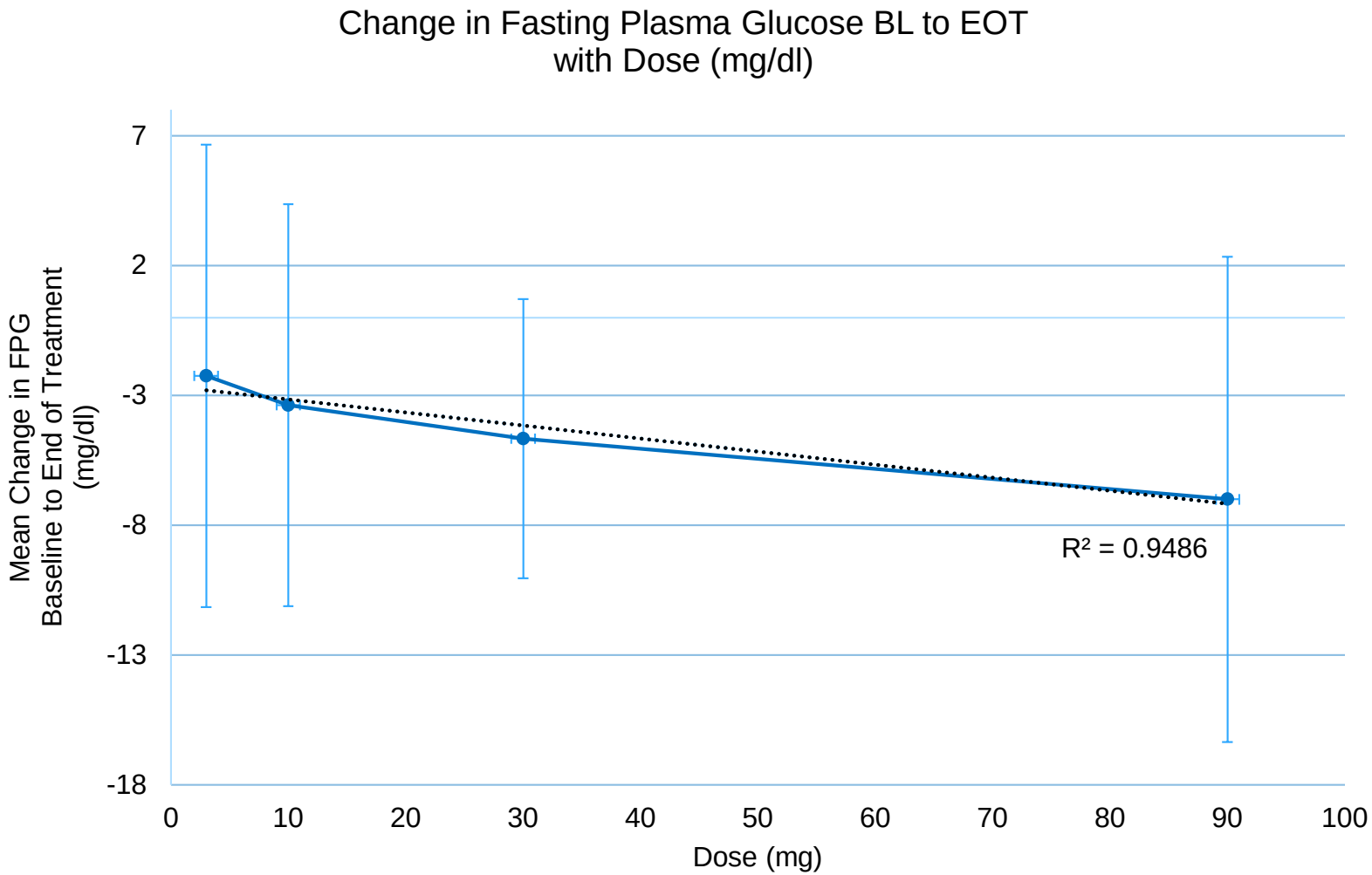
Potential Target Exposure, Assuming 35% Brain Penetration:

ED50 multiples (X)	PPAR delta	PPAR gamma
3mg cohort	3.6X	0.3X
10mg cohort	12.0X	0.8X
30mg cohort	31.7X	2.0X
90mg cohort	114.9X	7.4X

T3D959-201: Safety

Summary of Adverse Events (ITT Population)				
	3mg (N=9)	10mg (N=9)	30mg (N=10)	90mg (N=8)
Subjects with at least 1 AE	2 (22%)	0	2 (20%)	0
Total Number of Events	3	0	5	0
Subjects with at least 1 SAE	0	0	0	0
Total Number of Events	0	0	0	0
Subjects with at least 1 Drug-Related AE	0	0	1 (10%)	0
Total Number of Events	0	0	1	0
Subjects with at least 1 Mild AE	2 (22%)	0	1 (10%)	0
Total Number of Events	2	0	0	0
Subjects with at least 1 Moderate AE	1 (11%)	0	2 (20%)	0
Total Number of Events	1	0	4	0
Subjects with at least 1 Severe AE	0	0	0	0
Total Number of Events	0	0	0	0

T3D959-201: Evidence of Peripheral Drug Activity



T3D959-201: Evidence of Peripheral Drug Activity - Metabolomics

- **Clinical Metabolomic data collection:**
  - Fasted Plasma samples collected at Baseline and End of Treatment for all dose groups
  - Samples analyzed by Metabolon Inc. (Durham); **Over 821 metabolites monitored**

- **Analyses of Metabolomic Data:**
  - The 820 Metabolites analyzed and organized into eight super pathways and over one hundred sub-pathways
  - Four Analyses Done: Dose Groups, Genotype, Gender and Responder (**Dr Chris Newgard, Duke Molecular Physiology Inst.)**

- **<u>Key Observations</u>:**
  - **Lipid Metabolism effects observed with increasing dose**
    - INCREASE in a wide array of fatty acid-derived acylcarnitine species. This profile is consistent with increased flux of fatty acids into the beta-oxidation pathway.
  - **Systemic Glucose Metabolism and Insulin Sensitivity changes with increasing dose**
    - DECREASE in all three Branched Chain Amino Acids (BCAA) by higher doses of T3D-959. BCAAs are positively correlated with insulin resistance and diabetes

T3D959-201: Evidence of Peripheral Drug Activity - Metabolomics

- Several species of ceramides are decreased by T3D-959 30 mg and 90 mg dose groups.
- Ceramides have been implicated as mediators of insulin resistance and metabolic diseases

Ceramides	3mg	10 mg	30 mg	90 mg
ceramide (d16:1/24:1, d18:1/22:1)	1.13	0.95	**0.71**	0.84
ceramide (d18:1/14:0, d16:1/16:0)	**1.1**	1.05	**0.86**	**0.77**
ceramide (d18:1/17:0, d17:1/18:0)	1.03	1.12	0.91	**0.84**
ceramide (d18:1/20:0, d16:1/22:0, d20:1/18:0)	1.12	1.05	**0.83**	**0.84**
N-palmitoyl-sphingosine (d18:1/16:0)	1.06	1.02	**0.89**	**0.89**
N-stearoyl-sphingosine (d18:1/18:0)	1.05	0.98	**0.82**	**0.82**
ceramide (d18:2/24:1, d18:1/24:2)	1.08	0.96	0.92	0.99
Some of these are mixtures of ceramides				
Green means statistically significant (p=0.05) decrease				
ceramide nomenclature: d18:1/14:0 the shingosine first and the N-acyl group second				

T3D959-201: Evidence of Peripheral Drug Activity - Metabolomics

- All three branched chain amino acids (BCAA), Leu, Ile and Val, along with related metabolites, are significantly decreased ($p<0.05$) in the 90 mg T3D-959 group.
- BCAAs are positively correlated with insulin resistance and diabetes.

Metabolite	3 mg	10 mg	30 mg	90 mg
leucine	0.95	0.98	1	0.8
N-acetylleucine	0.98	1	0.91	0.82
isovalerylcarnitine (C5)	1.11	0.97	0.99	0.68
isoleucine	0.93	0.94	0.96	0.84
2-methylbutyrylcarnitine (C5)	0.99	1.02	0.96	0.81
valine	0.93	1.06	0.96	0.79
isobutyrylcarnitine (C4)	0.95	1.01	0.77	0.57

Green means statistically significant (p=0.05) decrease

T3D959-201: Evidence of Peripheral Drug Activity - Metabolomics

- Genotype and Gender differences in T3D-959 Metabolomic Data
- Long Chain Fatty acids increase with T3D-959 in E4 'High Dose' subjects (30 mg and 90 mg)
- Numbers are the ratio of metabolite EOT/BL. Red means p value is <0.05

Lipids - Long Chain FA	Baseline All E4/E3	Low Dose E3 EOT/BL	Low Dose E4 EOT/BL	High Dose E3 EOT/BL	High Dose E4 EOT/BL
laurate (12:0)	1	1.1	0.9	0.83	1.58
5-dodecenoate (12:1n7)	0.99	1.26	0.79	0.94	1.72
myristate (14:0)	0.98	1.19	0.88	0.98	1.54
myristoleate (14:1n5)	1.03	1.2	1.01	1.08	2.23
palmitoleate (16:1n7)	0.81	1.24	0.91	1.38	2.42
nonadecanoate (19:0)	0.84	1.31	0.93	0.86	1.43
10-nonadecenoate (19:1n9)	0.87	1.48	0.9	0.89	1.92
eicosenoate (20:1)	0.9	1.33	0.9	0.98	1.79
stearidonate (18:4n3)	0.89	1.47	0.89	0.88	2.02
eicosapentaenoate (EPA; 20:5n3)	0.88	1.27	0.96	1.03	1.63
linolenate [alpha or gamma; (18:3n3 or 6)]	0.85	1.13	0.87	1.07	1.65
dihomo-linolenate (20:3n3 or n6)	0.92	1.31	0.82	1	1.34
docosapentaenoate (n6 DPA; 22:5n6)	0.93	1.28	0.88	0.97	1.45
dihomo-linoleate (20:2n6)	0.81	1.39	0.82	0.94	1.79

T3D959-201: Exploratory Neuroimaging Outcomes – FDG-PET

METHODOLOGY

Relative Brain Glucose Metabolism measured (not absolute) to reduce patient burden of multiple testing (Δ R CMRgl (EOT-BL)). Measured at baseline (BL) and at end of 14-day dosing (EOT).

Analyses:

1. Exploratory voxel-wise (SPM) analysis of the whole brain to identify Regions of Statistically Significant Differences (ROSD) for Δ R CMRgl (EOT – BL) with uncorrected $p<0.005$..

2. Pre-defined Summary Indices: Addressing Type I error due to multiple regional comparisons.
   a) sROI Statistical Regions of Interest
   b) Four anatomical ROIs: *pre-specified* known AD-affected regions of interest (ROIs): 1) Posterior Cingulate (PC), 2) Precuneus (PreC), 3) Bilateral Middle Temporal Gyrus (BMTG), and 4) Right Inferior Parietal Lobule (RIPL)
   c) Hypometabolic convergence index (HCI)
   d) Longitudinal HCI (MCID) Δ R CMRgl (EOT-BL) for four.

3. sROI Index and the longitudinal change in the sROI index (Δ sROI)

T3D959-201: Exploratory Neuroimaging Outcomes – FDG-PET

OBJECTIVES

Primary Hypotheses Testing - Postulates

1. T3D-959, upon oral delivery, can penetrate the human blood brain barrier (as indicated from rat pharmacokinetic studies).
2. T3D-959's will increase the regional cerebral metabolic rate for glucose (CMRgl) in the brain of AD patients (based on its mechanism of action as an insulin and IGF-1 sensitizer).
3. T3D-959-elicited changes in CMRgl in the brain of AD patients will exhibit a dose dependency.
4. T3D-959 will increase CMRgl, including in AD-vulnerable hypometabolic brain regions.

Exploratory Hypotheses Testing - Postulates

5. T3D-959-elicited changes in CMRgl may be a potential biomarker of the drug's ability to improve cognitive function.
6. ApoE4 carriers and ApoE4 non-carriers will exhibit different T3D-959-elicited changes in glucose metabolism.

T3D959-201: Exploratory Neuroimaging Outcomes – FDG-PET

DATA ANALYSIS – COMPLICATED BY T3D-959 MOA

MOA: PPAR delta, ubiquitous expression in brain, regulator of glucose energy expenditure
Changes in cerebral and cerebellar glucose metabolism anticipated after drug treatment.

Issue: Ubiquitous expression of PPAR δ in the brain and a primary mechanism of action to improve insulin and IGF-1 resistance predicts that glucose metabolism in any Reference Region (RR) would also be affected by drug treatment.

Resolution (partial): Two RR's used and compared, Whole Brain (WB) and White Matter (WM)

Δ (RR) CMRgl is defined as the change in **the ratio of** ROI to Reference Region (EOT minus BL)

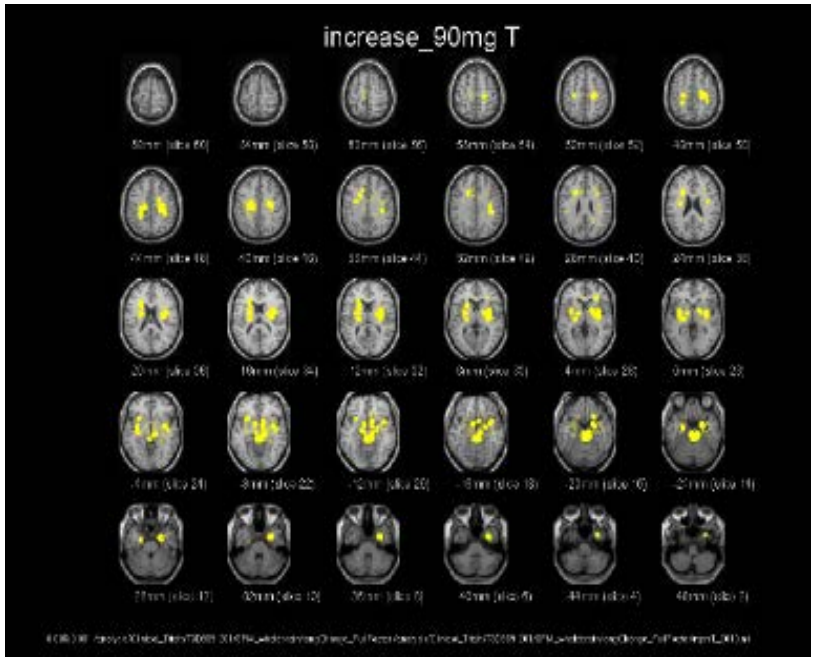
T3D959-201: Exploratory Neuroimaging Outcomes – FDG-PET

Whole Brain (WB) and White Matter (WM) Reference Regions Respond Differently to T3D-959

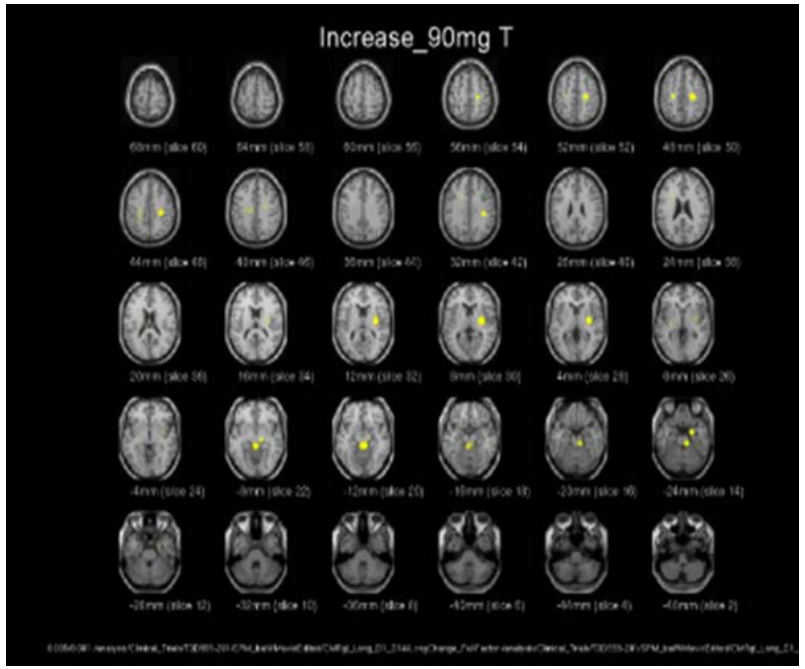
Positive ROSDs : Increased drug treatment-related, regional glucose metabolism greater than increased drug treatment-related, reference region glucose metabolism.

Spatial extent of ROSD is greater when Whole Brain (WB) is used as reference region than when White Matter is RR

Positive Δ R (WB) CMR_{gl} with 90 mg T3D-959



Positive Δ R (WM) CMR_{gl} with 90 mg T3D-959



Observations:

- Regions with positive ROSDs -Portions of multiple brain bilateral regions including the Insula, Hippocampus, Vermis, and the Putamen
- Positive ROSDs calculated with the different RRs are in similar brain regions, but differ in spatial extent of the ROSDs

T3D959-201: Exploratory Neuroimaging Outcomes – FDG-PET

Regions of Special Sensitivity to T3D-959

- Brain Regions with larger increases in glucose metabolism than the average Whole Brain
- Positive Δ R CMRgl (EOT – BL) values with Whole Brain as reference region
- From overlay of voxel wise analysis and sROI analysis
- Composite of all doses

Brain Regions	Δ R CMRgl (EOT-BL)	P-value
Orbital_front_intersection_L	0.03±0.04	3.0E-05
Orbital_front_L	0.01±0.05	1.9E-01
Orbital_front_ intersection _R	0.03±0.03	3.0E-05
Orbital_front_R	0.01±0.05	5.2E-01
Insula_ intersection _L	0.03±0.03	1.0E-6
Insula_L	0.02±0.03	1.1E-04
Insula_ intersection _R	0.03±0.04	2.0E-05
Insula_R	0.02±0.04	1.3E-03
Cingulum_Ant_ intersection _L	0.04±0.05	1.3E-04
Cingulum_Ant_L	0.03±0.04	5.2E-04
Cingulum_Ant_ intersection _R	0.03±0.04	1.9E-04
Cingulum_Ant_R	0.02±0.05	7.9E-03
Putamen_L_ intersection	0.06±0.06	1.0E-05
Putamen_L	0.05±0.07	5.0E-05
Putamen_R_ intersection	0.06±0.06	1.0E-05
Putamen_R	0.05±0.06	3.0E-05

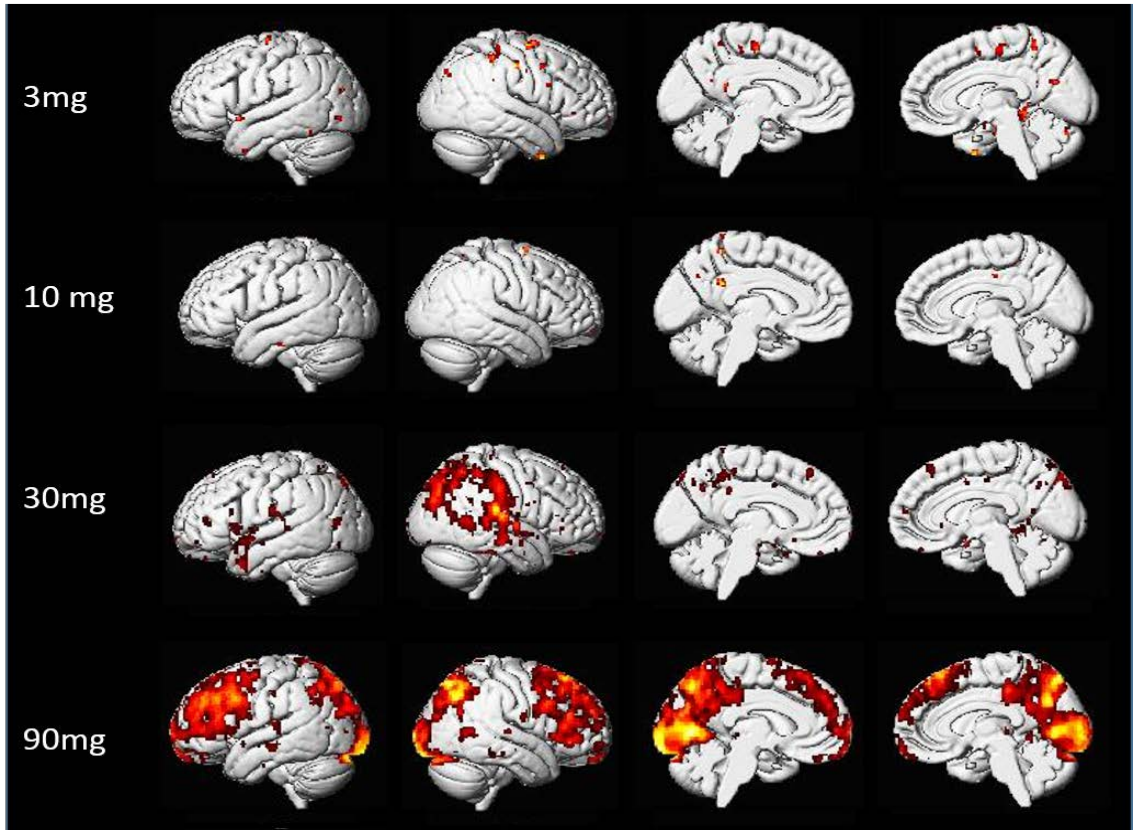
Observations:

- Multiple Brain Regions with positive Δ R CMRgl (EOT – BL) values relative to Whole Brain – p values listed
- Greatest increase in Δ R CMRgl (EOT – BL) observed with the two highest doses (30mg & 90mg) (data not shown)
- Putamen shows largest dose dependent changes and survived Family Wide Error analysis

T3D959-201: Exploratory Neuroimaging Outcomes – FDG-PET

Evidence of Drug Exposure and Activity in the Brain

Dose Dependent Increase in the Spatial Extent of Regions of Statistically Significant Change (ROSD) in Relative CMRgl (EOT-BL)



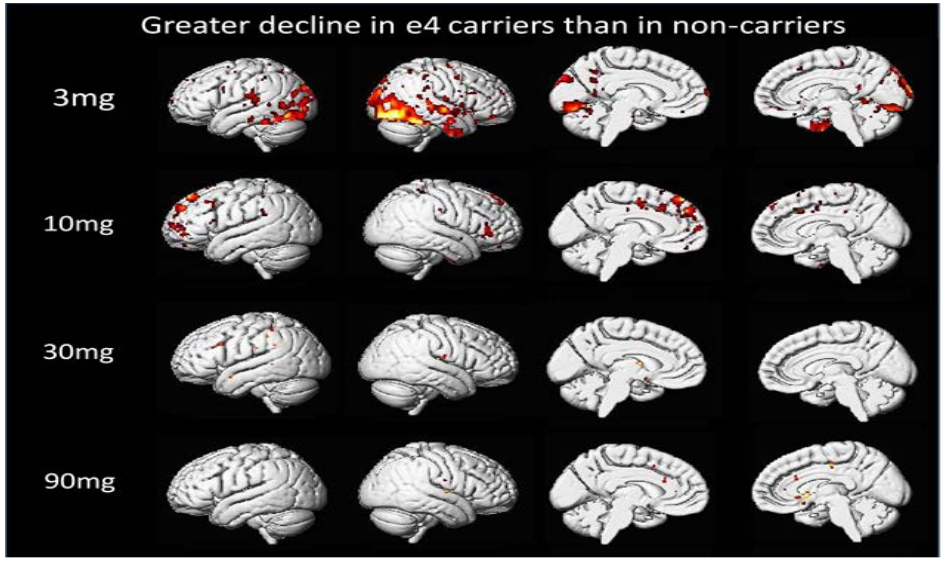
Composite of Trial Subjects by dose group (n=8-9)

Observations

- Dose dependent changes observed in **multiple** FDG-PET Outcomes including voxel-wise analysis on left
- Statistically Significant changes observed even at 3 mg dose (not shown)
- Regional specificities observed – different regions respond to drug differently

T3D959-201: Exploratory Neuroimaging Outcomes – FDG-PET

- A genotype difference in response to a low level of drug was observed as shown in this voxel-wise analysis
- Colored region indicate where difference between Δ R CMRgl^{Carrier} and Δ R CMRgl^{NC} for each voxel reaches statistical significance ($p<0.005$)
- At higher doses genotype-based differences disappear – no colored regions at the higher doses
- Greater decline in ApoE4 carriers means: Δ R CMRgl^{Carrier} **<** Δ R CMRgl$^{Non\text{-}Carrier}$



- Data from average Whole Brain voxel-wise analysis:
- This data is evidence that the low dose (3 mg) dose gets into the brain and engages its target
- We do not know if these are positive or negative Δ R CMRgl values for carriers and non-carriers

These observations are not simply a reflection of the difference in glucose metabolism between ApoE4 Carriers and Non-Carriers, since **Δ R CMRgl** measures the change over the T3D-959 treatment period

T3D959-201: Exploratory Neuroimaging Outcomes – FDG-PET

Main Observations from Relative FDG-PET Clinical Neuroimaging Studies

A) Apparent dose-dependent effect of T3D-959 on cerebral glucose metabolism.

B) T3D-959 alters cerebral glucose metabolism even at the lowest 3 mg dose

C) Reference regions (Whole Brain and White Matter) used to calculate regional relative changes in glucose metabolism, appear to be also affected by T3D-959.

D) AD-affected, and AD-spared brain regions appear to respond equally to the two lower doses of T3D-959, but at the two higher doses, AD-affected regions, do not respond to drug as well as AD-spared brain regions such as brain white matter.

E) Caveat: Interpretation of the FDG-PET results above is dependent on the use of two reference regions, multiple different doses and relative (not absolute) CMRgl data. The possibility that the observed increases/decreases of Δ R CMRgl may be due to subtle effects on brain glucose metabolism not directly related to drug, can not be excluded.

T3D959-201: Exploratory Cognitive Outcomes – Digit Symbol Substitution Test (DSST)

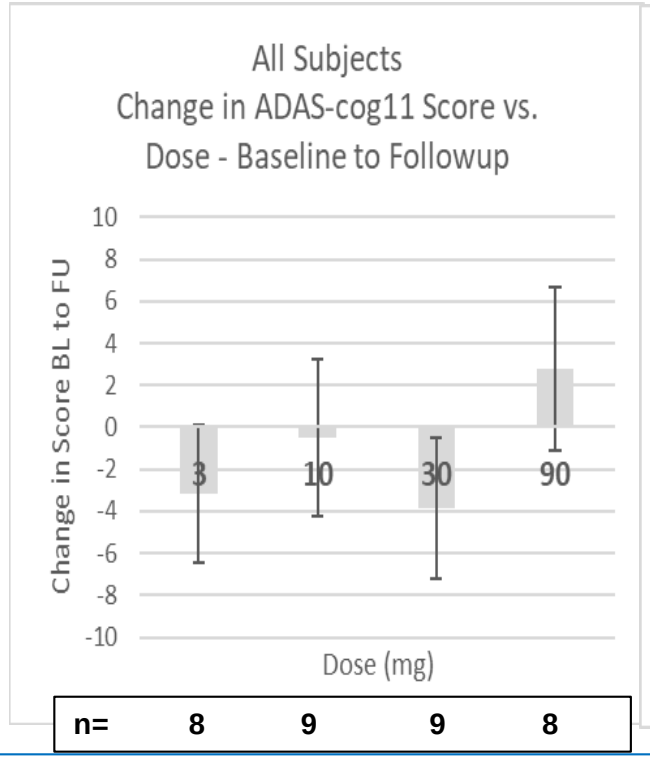
Average Improvement at Followup Day 21 (all doses)		
	Moderate Patients (MMSE=14-19)	**Mild Patients (MMSE=20-26)**
All	**2.4** (+/-6.0) n=17	**7.0** (+/-8.2) n=17
ApoE4-	**1.7** (+/-4.8) n=11	**8.0** (+/-4.7) n=6
ApoE4+	**4.4** (+/-7.6) n=6	**6.5** (+/-9.6) n=11

T3D959-201: Exploratory Cognitive Outcomes – ADAS-cog11

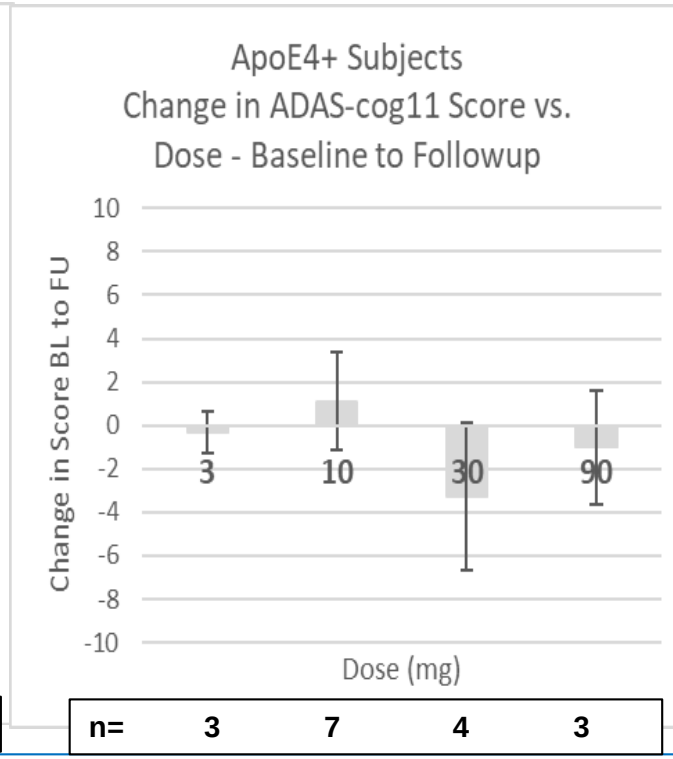
ApoE Genotype Influence on Cognitive Outcomes as Assessed by the ADAS-cog11 Test

- Dose trend analysis – significant genotype effect p=0.004
- 10mg Cohort – skewed distribution of E4 positive subjects
- All subjects E4+ and E4- improved with 30mg dose
- All E4- subjects improved with 3, 10, 30mg doses
- 90mg Cohort E4- subjects – 4 of 5 with moderate disease severity

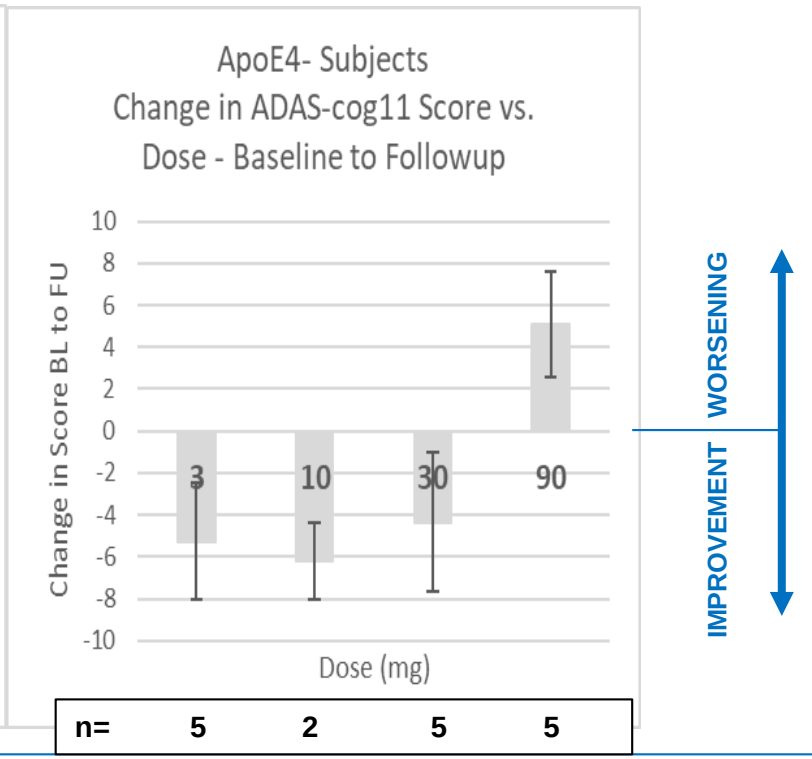
All Subjects



ApoE4+ Subjects



ApoE4- Subjects



T3D959-202: Open Label Extension

4-Subjects who completed T3D959-201, dosed 18-22 weeks, 15mg QD

Subjects – Demographics & Baseline Characteristics

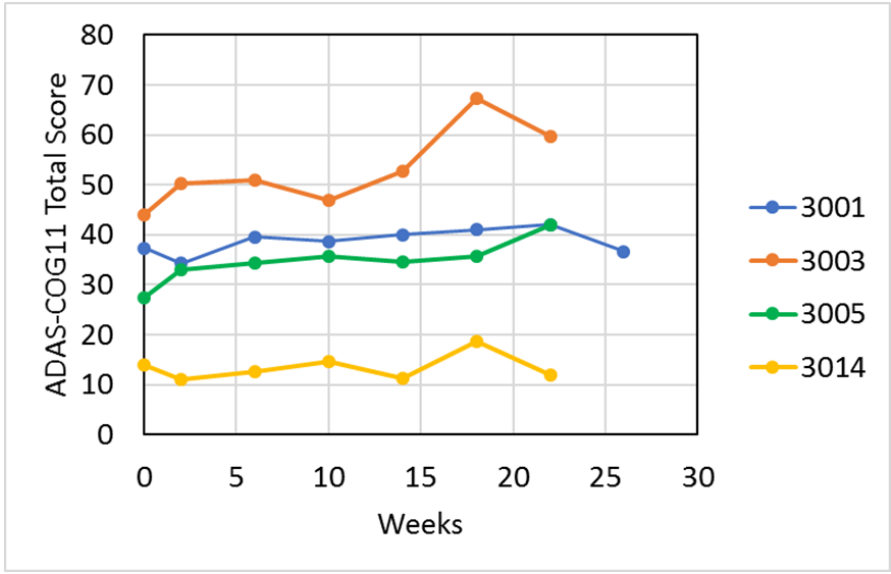
Subject	Sex	Age	ApoE4 Genotype	MMSE	ADAS-cog11	DSST
3001	M	88	4/4	19	37.3	5
3003	M	70	w/4	18	40.0	3
3005	M	71	w/4	22	27.3	5
3014	M	71	w/4	25	14	38

Safety

- No AEs
- No Safety Signals
- No Tolerability Issues

Cognitive Assessments

- CIBIC+ group average = 2.75
  (all at least minimally improved)
- ADAS-cog11

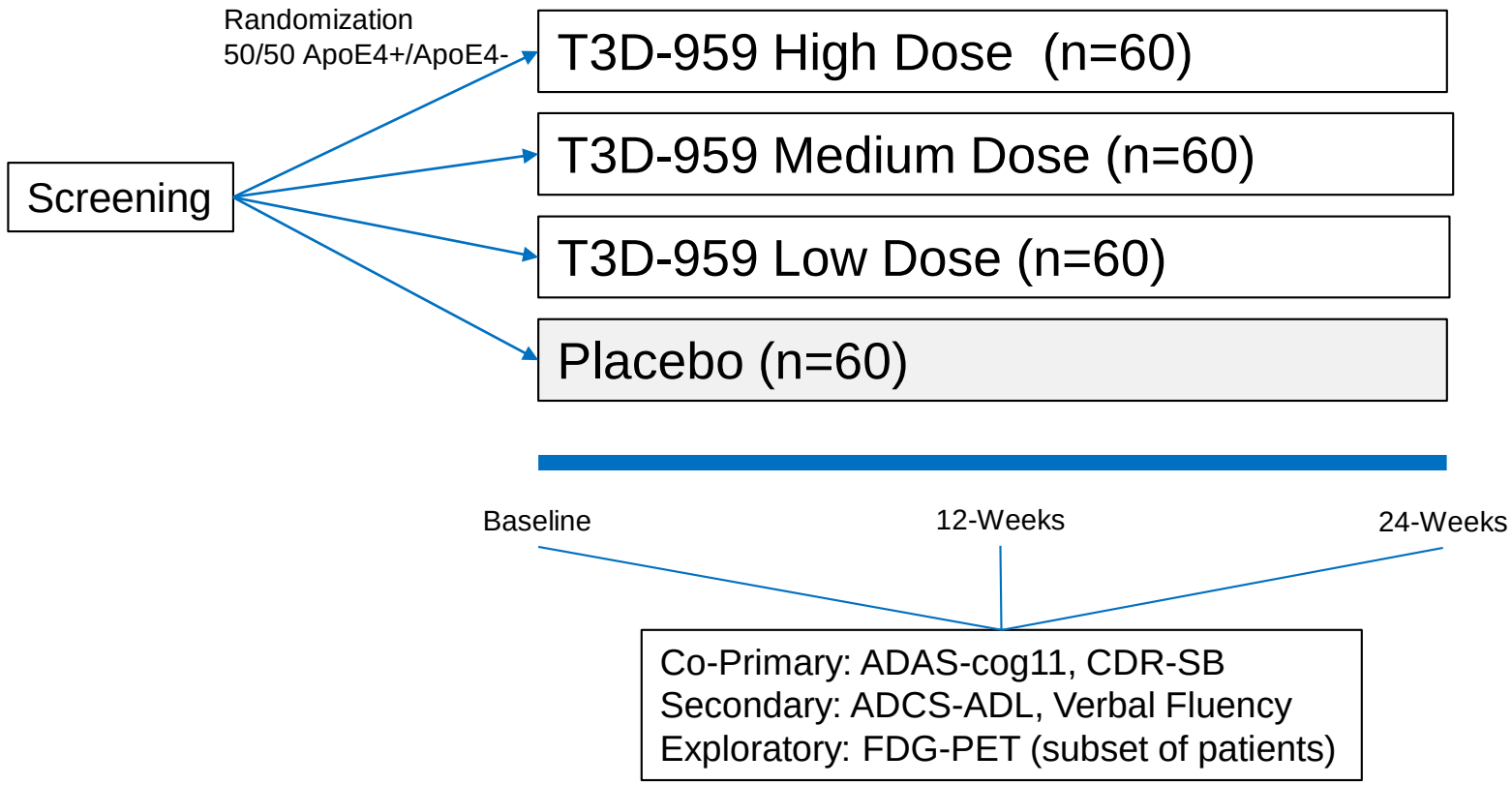


T3D959: Clinical Development: In Progress

- Multi-kilogram Scale GMP Manufacturing of T3D-959 Drug Substance (API) Underway
- 6-Month Rat Chronic Toxicology Study Underway
- 9-Month Monkey Chronic Toxicology Study Underway
- Preparing for Drug Product Campaign to support Phase 2b clinical trial
- Preparing for radiolabeled synthesis of T3D-959 for GLP and GMP mass balance studies

T3D959-203: Clinical Development - Future

Planned Phase 2b Study (Mild-to-Moderate AD)



Conclusions: T3D959-201 Phase 2a Outcomes

- New Chemical Entity T3D-959, a PPAR delta-selective agent is safe and well tolerated across all doses
- Good systemic exposure in Phase 1 confirmed in Phase 2a
- Metabolomics profile verifies expected systemic pharmacology consistent with improving insulin resistance
- FDG-PET outcomes suggest dose-dependent brain penetration and expected pharmacology
- Multiple signals of potential efficacy observed
  - Potential to improve cognitive outcomes
  - Potential ApoE4 genotype association with treatment outcomes (significant differences observed in; (a) plasma metabolome, (b) CMRgl & (c) ADAS-cog11)
  - Potential to change brain glucose metabolism
- Results support continued evaluation in a Phase 2 RCT

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Metabolomics

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Clinical Trial Sites

- New Hope Clinical Research, Charlotte, NC. - Dr. Santosh Gopalakrishanan
- Miami Jewish Hospitals, Miami, FL. - Dr. Marc Agronin
- Brain Matters Research, Delray Beach, FL. - Dr. Mark Brody

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