A Neurometabolic Approach to Treating Alzheimer's Disease: Hypothesis Testing in a Phase 2a Exploratory Clinical Trial with the New Chemical Entity T3D-959

John Didsbury, PhD, Warren Strittmatter, MD, Hoda Gabriel, PMP, and Stan Chamberlain, PhD.
T3D Therapeutics Inc, Research Triangle Park, North Carolina

ABSTRACT

A major test of the neurometabolic hypothesis of Alzheimer's disease (AD) pathophysiology [i.e. dysfunctional glucose and lipid metabolism being key drivers of disease pathologies] relied upon a Phase 3 clinical study of the PPAR gamma-selective agonist **rosiglitazone**. This trial 'failed' to demonstrate statistically significant improvements in cognitive and functional tests and factored into the pursuit of other approaches to AD drug development, in particular, preventing or reversing structural events, beta amyloid plaques and tau tangles. We contend that the neurometabolic hypothesis cannot be appropriately tested with this agent (or with **pioglitazone**). Restricted PPAR gamma target expression in the brain, poor blood brain barrier penetration and high metabolism of **rosiglitazone** prevents *bona fide* hypothesis testing. The need exists to test the neurometabolic hypothesis with an agent superior to **rosiglitazone**. T3D-959 is an investigational new drug product in Phase 2 clinical development as a potential disease-remedial therapy to slow, stop or reverse the course of AD. This chemical compound is orally delivered and administered once-a-day. T3D-959 is the first PPAR delta-activating compound (agonist) to be developed for the treatment of AD. Uniquely, this drug also activates PPAR gamma (dual agonist) at 15-fold lower potency, which may provide potential additive or synergistic effects in regulating dysfunctional brain glucose and lipid metabolism in AD. Numerous attributes of this agent make it a superior choice to **rosiglitazone** (and **pioglitazone**) to truly test the neurometabolic hypothesis. These include good penetration of the blood brain barrier with indirect clinical evidence of cerebral target engagement, a drug metabolism profile which, in pre-clinical and Phase 1 clinical studies, demonstrates a high likelihood of achieving multiples of its EC_{50} in the brain, from an oral dose, a good therapeutic index and, ubiquitous high brain expression of the primary PPAR delta drug target. An exploratory / feasibility Phase 2a clinical trial [ClinicalTrials.gov (NCT02560753)] in 36 subjects with mild-to-moderate AD (MMSE= 14-26, average = 19.9) has been completed. The purpose of the Phase 2a clinical study was to demonstrate that T3D-959 could produce desired changes in cerebral glucose metabolism (primary outcome measure – FDG-PET) that may indicate potential for cognitive improvement (secondary/exploratory measures ADAS-cog11 and DSST). The therapeutic approach was based on the hypothesis that correcting insulin resistance in the brain (highly correlated with AD and potential key driver of AD pathophysiology) may be disease remedial. The study was a multi-center (3), randomized, parallel, 4-dose design in subjects with mild-to-moderate Alzheimer's disease. Thirty-six subjects were randomized to one of 4 doses of T3D-959 (3 mg, 10 mg, 30 mg or 90 mg). T3D-959 was taken once daily for 14 days. Subjects were evaluated for changes from baseline in **relative cerebral metabolic rate of glucose** (FDG-PET imaging), functional connectivity (BOLD-fMRI), cognitive function (ADAS-Cog11 and DSST) and plasma metabolomics, as well as assessed for safety and tolerability to T3D-959. In this study, plasma drug levels and metabolic analysis support typical systemic PPAR delta and gamma pharmacology. Exploratory FDG-PET neuroimaging outcomes indirectly support dose-dependent brain penetration by T3D-959, and directly demonstrate improvements in relative cerebral rates of glucose metabolism in multiple brain regions. Comparisons to rosiglitazone indicate this agent to be a superior molecule for assessing the contributions of dysfunctional metabolism to AD pathogenesis. This study was supported in part by grant AG-049510 from the NIH.

PHASE 2A CLINICAL TRIAL (ClinicalTrials.gov Identifier NCT02560753)

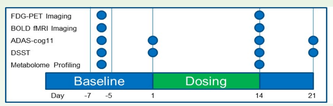
"Phase 2a Feasibility Study of T3D-959 in Subjects with Mild to Moderate Alzheimer's Disease"

TRIAL OBJECTIVES (Partial List)

- **Confirm Phase 1 clinical exposures in AD subjects with single time point pharmacokinetics at EOT**
- **Evaluate T3D-959 related systemic changes in the metabolome with increasing doses of T3D-959**
  - Observe changes in any AD (brain) related metabolites or biomarkers
- **Use FDG-PET to evaluate brain effects of increasing doses of T3D-959**
  - Indirect measure of T3D-959 brain penetration in AD subjects
  - Measure of PPAR delta agonist pharmacology in the brain
- **Evaluate the Safety and Tolerability of increasing doses of T3D-959**

TRIAL DESIGN

- Randomized, parallel, 4-dose design in subjects with mild-to-moderate AD
- Subjects randomized to one of 4 doses of T3D-959 (3, 10, 30, 90mg), no placebo
- T3D-959 taken orally once daily for 14 days.



Selected Results from Pre-Clinical, Phase 1 and Phase 2a Clinical Trials

- Single time point PK data in T3D-959 -201 Study Subjects, taken 3-4 hours after last dose (day 14)
- T_{max} from Phase 1 trial was 3-5 hours; half life ($T_{1/2}$) ranged from 14.8-19.9 hours in 7 day PK
- Rat brain to plasma levels (B/P) are 35% at 1 h and at 12 h post dose
- Plasma Concentration below are close to the C_{max} values
- **T3D-959 PPAR δ EC_{50} = 19 nM ; PPAR γ IC_{50} = 297 nM (Rosiglitazone and Pioglitazone are PPAR γ agonists)**

Extrapolated Human Brain Levels Based on Clinical PK & Rat Data

	3 mg (N=9)	10 mg (N=9)	30 mg (N=10)	90 mg (N=8)
Plasma Concentration of T3D959 after 14 Days of Dosing in nM				
N	8	9	9	8
Mean (STD)	**86.5 (57.87)**	**289.9 (128.14)**	**763.1 (447.08)**	**2765.0 (1454.36)**
Median	65.9	244.0	540.0	2360.0
Min – Max	16 - 174	128 - 464	282 - 1650	1120 - 4970
Estimated Brain Conc of T3D959 after 14 Days of Dosing in nM				
Mean	**30**	**101**	**267**	**968**
Median	23	85	189	826
Min – Max	5-61	45-162	98-577	392-1739
Fold above PPAR δ EC_{50}				
Mean	**1.5**	**5.3**	**14**	**50**
Median	1.2	4.4	9.9	43
Min – Max	0.3-3.2	2.4-8.5	5-30	20-91

Phase 2a Safety Results

- **Phase 2a exposure data in AD patients consistent with Phase 1 data in healthy subjects**
- Dose dependent plasma exposures – multiples of EC_{50} even at 3 mg
- One drug-related AE (subject 1001 (30mg) – Self-limited, resolved within 1-day)
- No changes in clinical labs
- No changes in physical and neurological exams
- No changes in ECGs
- No respiratory rate or orthostatic blood pressure and heart rate changes
- No potential bone marrow effects as monitored with hematology testing
- **No potential increases in plasma volume as assessed by the presence or absence of edema**
- No weight gain
- No tolerability issues

Metabolomics Analysis Demonstrates Systemic Exposure and Possible Brain Effects

Metabolomics Poster Presented at 2017 ASENT Meeting

Systemic Lipid Metabolism

INCREASE in a wide array of fatty acid-derived acylcarnitine species, ranging from the end product C2 (acetyl) carnitine through the even-chain medium (C4 to C12) and long chain (C14-C22) species. This profile is consistent with increased flux of fatty acids into the beta-oxidation pathway.

Systemic Glucose Metabolism and Insulin Sensitivity

DECREASE in all three Branched Chain Amino Acids (BCAA) are by higher doses of T3D-959. BCAAs are positively correlated with insulin resistance and diabetes

Glycine levels are INCREASED by high dose T3D-959. Glycine is negatively correlated with insulin resistance and diabetes, and has been shown to increase in response to dietary restriction of branched-chain amino acids (BCAA)

Possible Brain Pharmacology Observed

NAA (N-Acetylaspartate), a known biomarker of AD, is INCREASED in all four T3D-959 dosing groups. It has been reported that NAA levels are decreased in the brain of Alzheimer's patients

(F^{18}) Fluoro Deoxyglucose - Positron Emission Tomography (FDG-PET) Study with T3D-959 Data

- FDG-PET scans were obtained at baseline and after completion of dosing for each dose group: 3mg, 10mg, 30mg or 90mg
- 30 minutes after (F^{18}) FDG is dosed, radioactivity is measured for each voxel of the brain
- Subjects lay supine with eyes open, for 30 minute scans, **as per ADNI protocol**
- **Relative** Cerebral Metabolic Rates for Glucose or **R CMR_{gl}** a unitless measures, as opposed to absolute CMRgl (mass/time/volume)
- **R CMR_{gl}** values (relative to a reference region, **RR**): FDG-PET scans are shorter, less technically challenging, easier on subjects
- Initial FDG-PET analysis was done using **Whole Brain** as the **RR**; second analysis done using brain **White Matter** as **RR**
- Major outcomes: Statistical Region of Interest (**sROI**), prespecified anatomical **ROIs**, and Voxel Wise Analysis of whole Brain
- **Two reference regions, multiple outcomes, four doses: Additional supportive FDG-PET data not shown, available upon request.**

FDG-PET Neuroimaging Results

T3D-959 has expected pharmacological effect of increasing regional glucose metabolism in multiple brain regions (relative to WB)

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

FDG-PET Data from Voxel Wise Analysis and sROI Analysis

- Positive Δ R CMRgl (EOL – BL) values with WB as RR
- Composite of all doses, but mainly driven by high dose
- P values are uncorrected

Observations:

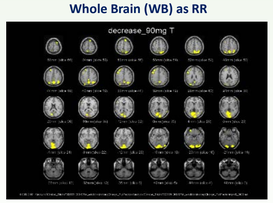
- Multiple Brain Regions with increased glucose metabolism relative to Whole Brain
- Greatest increase in glucose metabolism seen with the two highest doses (30mg & 90mg)
- At higher doses, these regions respond better than the average whole brain
- Putamen shows largest **Δ R CMRgl;** survives FWE correction

Conclusion:

- Multiple Brain regions show relative increases in cerebral glucose metabolism even with **NORMALIZATION** by the RR; Glucose metabolism in RRs may also be increased by T3D-959

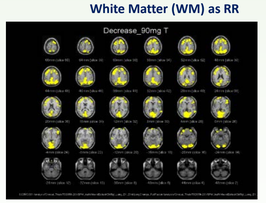
Spatial Extent of ROSDs from Voxel-Wise Analysis Depend on which Reference Region is used

Whole Brain (WB) as RR



ROSDs with a statistically significant Negative **Δ R(WB) CMRgl** at 90 mg

White Matter (WM) as RR



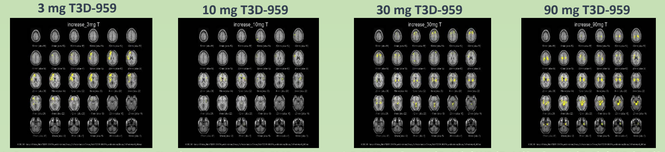
ROSDs with a statistically significant Negative **Δ R(WM) CMRgl** at 90 mg

- **Δ R CMRgl** is the change in Relative Cerebral Metabolic Rate (EOT-BL); **Δ R CMRgl** values can be Positive or Negative
- **ROSD** is Regions of Statistically significant Differences in **Δ R CMRgl (EOT-BL) p = 0.005**
- The negative **ROSDs** identified with **WB** are in similar brain regions as those identified with **WM**
- White Matter (WM) is corpus callosum and centrum semiovale
- The spatial extent of the negative **ROSD** with **WM** as the RR is 9655 voxels
- The spatial extent of the negative **ROSD** with **WB** as the RR is 2357 voxels
- The spatial extent of the positive **ROSDS** with **WB** were greater than positive ROSDs with **WM** (data not shown)
- ROSDs highlighted in the left panel respond exactly the same to T3D-959 as those in the right image, the difference in the two images is entirely due to how the respective Reference Regions respond.
- We postulate that White Matter is more responsive to higher doses of T3D-959 than the average Whole Brain

Dose Dependent Increase in the Spatial Extent of Positive ROSDs from Voxel-Wise Analysis

Whole Brain (WB) as RR

3 mg T3D-959 | 10 mg T3D-959 | 30 mg T3D-959 | 90 mg T3D-959



- Brain Regions Showing Statistically Significant **Positive Δ R CMR_{gl}** where: **Δ R CMR_{gl} = [voxel$_i$ (T=14) / WB (T=14)] – [voxel$_i$ (T=0) / WB (T=0)] > 0**
- The yellow regions (**ROSDs**) in the figures are the voxels which show a statistically significant (p <0.005 uncorrected for multiple comps) difference (EOT-BL)
- Images **do not** show increases in ***absolute*** **CMR_{gl}** for the yellow regions, instead show increases in the spatial extent of the pos **ROSDs** relative to **WB**
- From 10 mg to 90 mg we see a dose dependent increase in the spatial extent of pos **ROSD** (yellow area). **There is a significant trend (R^2=0.998, p=0.026) from 10 mg (70 voxels), to 30 mg (518 voxels), to 90 mg (2136 voxels). Similar dose dependency in negative Δ R CMR_{gl} results (data not shown).**
- **Even at the low dose (3 mg) positive ROSDs (p=0.005, uncorrected for multiple comparisons) are observed**

FDG PET Results for Pre-specified Anatomical ROIs

Evidence for Dose Dependent Effects of T3D-959; Negative Δ R (WM) CMR_{gl} Values Observed Indicate they are less responsive to T3D-959 than WM

Pre-specified ROI	Stats	3 mg	10 mg	30 mg	90 mg
Posterior Cingulate Δ R CMRgl-PC	N	8	9	9	8
	Mean	0.0081	-0.0038	-0.0102	-0.0228
	(SD)	(0.01840)	(0.01726)	(0.02531)	(0.02916)
	Median	-0.0126	-0.0082	-0.0016	-0.0112
Precuneus Δ R CMRgl-PreC	N	8	9	9	8
	Mean	**0.0060**	**-0.0043**	**-0.0133**	**-0.0427**
	(SD)	(0.02332)	(0.01789)	(0.02249)	(0.04695)
	Median	-0.0104	-0.0099	-0.0223	-0.0335
Bilateral Middle Temporal Gyrus Δ R CMgl-BMTG	N	8	9	9	8
	Mean	-0.0011	0.0074	-0.0319	-0.0292
	(SD)	(0.05229)	(0.03433)	(0.03162)	(0.04042)
	Median	0.0042	0.0052	-0.0291	-0.0300
Right Inferior Parietal Lobule Δ R CMgl-RIPL	N	8	9	9	8
	Mean	-0.0149	0.0036	-0.0363	-0.0287
	(SD)	(0.03365)	(0.02266)	(0.03626)	(0.03306)
	Median	-0.0235	0.0039	-0.0364	-0.0153

- Evidence of dose dependency in analysis of ROIs with WM as RR
- Precuneus negative Δ R(WM) CMRgl values correlate well to increasing doses of T3D-959 (p=0.0068)
- Same calculations with Whole Brain as RR show little change (Δ R(WB) CMRgl ~ 0) for all doses of T3D-959 (data not shown)
- Changes observed here are ***negative***: Δ R(WM) CMRgl = [ROI(T=14) / WM(T=14)] – [ROI(T=0) / WM (T=0)] < 0
- One possible interpretation is that WM responds better to T3D-959 than the pre-specified ROIs (which is responding at about the rate of the average whole brain), and ROI/(T=14)/WM(T=14) ratios are decreasing as the dose of T3D-959 increase
- These pre-specified AD sensitive regions have the highest Amyloid Beta plaque levels, and the greatest initial hypometabolism, perhaps it is not surprising that they are less responsive in a 2 wk study

sROI[2] Values from T3D-959 Two Week Phase 2a Clinical Trial

Dose dependent changes in Δ sROI values observed

sROI changes in 2 week high dose T3D-959 Study are similar to CMR_{ratio} changes from 12 month Rosiglitazone Study (see below)

Outcome	Statistics	3 mg (N=9)	10 mg (N=9)	30 mg (N=10)	90 mg (N=8)
Δ sROI "AD spared region" as RR	N	8	9	9	8
	Mean	0.0015	0.0034	-0.0204	-0.0293
	(SD)	(0.02057)	(0.01671)	(0.01952)	(0.01744)
	Median	0.0064	-0.0019	-0.0253	-0.0323
Δ sROI White Matter as RR	N	8	9	9	8
	Mean	0.0016	0.0053	-0.0200	-0.0355
	(SD)	(0.04008)	(0.02471)	(0.01979)	(0.02303)
	Median	0.0081	0.0009	-0.0101	-0.0363

- The **sROI** is statistically determined region of the brain, identified from ADNI data, which shows statistically significant decreases in **Δ R CMRgl** over 12 months in AD patients relative to a second statistically determined "**AD spared**" reference region.
- The **sROI index** is the ratio of the average bq/voxel for the **sROI** divided by the average bq/voxel for the "**AD spared**" region for a particular subject.
- **Δ sROI** is defined as the change in **sROI index** of the treatment period (EOT-BL)
- **Possible dose dependent Δ sROI values for both "AD spared region" and WM RRs**
- **R^2 = 0.753 for median Δ sROI (spared region) vs dose, and R^2 = 0.987 for median Δ sROI (WM)**
- Changes observed at high dose are negative: Δ sROI = [sROI(T=14) / RR(T=14)] – [sROI(T=0) / RR (T=0)] < 0, and similar in value to those obtained in 12 month Rosiglitazone study (see below)

FDG PET Data from Rosiglitazone 12 Month Phase 3 Clinical Trial[1]

Small, non-significant, decreases in the decrease in *absolute* CMRgl observed over the 12 month trial (left panel)

sROI = CMR_{ratio} when "AD Spared Region" is used as RR; CMR_{ratio} results are inconsistent with *absolute* CMR_{gl} *index* results (right panel)

CMR_{gl}^{index} (μg·cm^{-3}·min^{-1}), adjusted 12 month mean change from baseline in ROIs and global grey matter with CI(95%) and p-values (uncorrected). Percentage change from baseline is given in parentheses. Abbreviations: CI, confidence interval; RSG, rosiglitazone; ROI, region of interest; Cing, cingulate; Temp, temporal; Post, posterior; Med, medial

	RSG-XR	Placebo	Difference (RSG-XR-Placebo)	Difference CI(95%)	Difference p-value
ROI					
Post Cing Gyrus	−2.480 (−5.2%)	−6.369 (−13.5%)	3.889 (8.3%)	(−0.844, 8.623)	0.11
Med Temp Lobe	−1.591 (−5.0%)	−4.020 (−12.5%)	2.431 (7.6%)	(−0.856, 5.716)	0.14
Parietal Lobe	−3.296 (−7.5%)	−6.331 (−14.3%)	3.036 (6.9%)	(−1.276, 7.349)	0.16
Post Temp Lobe	−3.056 (−7.4%)	−5.853 (−14.2%)	2.798 (6.8%)	(−1.047, 6.642)	0.15
Cerebellum	−1.593 (−3.7%)	−4.567 (−10.5%)	2.976 (6.8%)	(−1.660, 7.609)	0.20
Frontal Lobe	−2.767 (−6.9%)	−6.436 (−13.7%)	3.169 (6.7%)	(−1.536, 7.873)	0.18
Global Grey Matter	−2.758 (−6.3%)	−5.724 (−13.1%)	2.964 (6.8%)	(−1.296, 7.224)	0.17

Adjusted 12 month mean change from baseline in global grey matter for CMR_{glu}^{index} (μg·cm^{-3}·min^{-1}) or the CMR_{ratio} (a unitless ratio) with CI(95%) and p-values (uncorrected), for the subgroup of patients of E3/5 and E3/4 genotype. Percentage change from baseline is given in parentheses. Abbreviations: CI, confidence interval; RSG, rosiglitazone

Genotype	Index	RSG-XR	Placebo	Difference (RSG-XR-Placebo)	Difference CI(95%)	Difference p-value
E3/5	CMR_{glu}^{index}	−1.607 (−3.6%)	−4.413 (−10.0%)	2.807 (6.4%)	(−7.767, 13.380)	0.69
	CMR_{ratio}	−0.0691 (−6.2%)	−0.0192 (−1.7%)	−0.0499 (−4.3%)	(−0.0935, −0.0063)	0.03
E3/4	CMR_{glu}^{index}	−5.842 (−13.2%)	−5.742 (−13.0%)	−0.100 (−0.2%)	(−8.627, 8.427)	0.98
	CMR_{ratio}	−0.0578 (−3.4%)	−0.0709(−6.3%)	0.0330 (2.9%)	(−0.0027, 0.0688)	0.07

CONCLUSIONS FROM FDG-PET RESULTS

- T3D-959 appears to have the expected pharmacological effect of increasing regional glucose metabolism; From Ad hoc analysis of regions of the brain with positive Δ R CMRgl(EOT-BL) values and Voxel-Wise Analysis;
- The reference regions Whole Brain (WB) and White Matter (WM) are also affected by T3D-959; From the voxel-wise analysis and pre-specified ROI outcomes
- Voxel-Wise analysis shows dose dependent effects in the spatial extent positive ROSDs (and negative ROSDs –data not shown)
- T3D-959 gets into the brain, even at the lowest dose; from Voxel-Wise analysis (V-WA) and from Apo E genotype correlation (data not shown)
- Anatomical ROIs show dose dependent effects; Observed negative Δ R (WM) CMRgl values may indicate they are less responsive to T3D-959 than WM
- Rosiglitazone FDG PET data shows small, statistically insignificant, improvements in absolute CMRgl relative to placebo over 12 months of treatment
- Rosiglitazone CMR_{index} (same as our sROI) is inconsistent with Rosiglitazone absolute CMRgl results and similar in value to our 2 week study

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