**Supplementary Material**

**S1: Inclusion Criteria**

To be eligible for the study all of the following criteria defined in the protocol had to have been fulfilled:

* Patient may be of either gender and must be supervised by a carer who is competent to ensure compliance with the medication and who is willing to participate in completing the various assessments.
* Patients must be able to give written informed consent to participate in this study. Patients who lack capacity to consent may not be entered.
* Competent carer must be available and must provide written consent to his or her own participation in the study.
* Clinical diagnosis of dementia of the Alzheimer type determined by Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria [1] and a diagnosis of Probable Alzheimer's Disease determined by the National Institute of Neurological and Communicative Disorders and Stroke - Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria [2]. Information to support the diagnosis will include that derived from:
  + An abbreviated Cambridge Mental Disorders of the Elderly Examination (short CAMDEX) schedule, performed within six weeks prior to the baseline visit (Visit 0)
  + Computerized tomography or magnetic resonance imaging, with no time limit on previous scans. In centers conducting single photon emission computed tomography (SPECT) or positron emission tomography scans as part of their routine practice or as part of the study, these may be used to inform the NINCDS-ADRDA diagnosis
* Patient must have mild or moderate dementia as determined by:
  + Mini-Mental State Examination (MMSE) [3] value at screening of between 10 and 26 inclusive
  + Clinical Dementia Rating (CDR) [4] at screening of Stage 1 or Stage 2.

**S2: Exclusion Criteria:**

Patients were not to be eligible to participate in the study in any of the following circumstances:

* Patient has a known sensitivity to methylthioninium chloride (MTC), similar agents or any of the excipients used.
* Screening blood sample shows that the patient has glucose-6-phosphate dehydrogenase deficiency.
* Patient has known hereditary methemoglobinemia, has been known to have suffered an attack of acquired methemoglobinemia or has a blood level of methemoglobin at screening which is above the upper limit of normal for age and laboratory.
* Patient has significant impairment of renal, hepatic or hematological function for the age of the patient.
* Patient is currently taking other anti-dementia drugs (e.g., memantine, cholinesterase inhibitors) or has taken these within the previous six weeks.
* It is anticipated that there will be a definite indication for the commencement of other licensed anti-dementia drug treatment within the 24-week treatment period of the trial.
* Patient has started taking other medication known to have an effect on mood or cognition (e.g., anticholinergics, hypnotics, sedatives, anxiolytics, neuroleptics, antidepressants, antiepileptics) within the previous six weeks, or has changed their dose of these medications within the previous six weeks.
* Patient has started taking 'alternative therapy' for Alzheimer’s disease (e.g., vitamin E, folic acid, hormone replacement therapy, *Ginkgo biloba*) within the previous six weeks or has changed their dose of these treatments within the previous six weeks.
* Patient is receiving warfarin or digitalis or any other medication that has a narrow margin between effective dose and toxic dose or between effective dose and ineffective dose, where the subject would be at risk if the levels were elevated or fell due to interaction with MTC.
* Patients who are unlikely to comply with trial visit schedule or with trial medication.
* Significant intercurrent illness which may compromise safety of the patient/validity of the data.
* Females with the potential of childbearing and are not using adequate contraception or females who are breastfeeding.
* Patients with a history of alcohol and/or drug abuse, defined as meeting DSM-IV criteria for substance dependence. This applies to alcohol and/or any illicit drug, including cannabis within the last six months.
* Patient has participated in a clinical investigation of a medication or device within the previous three months.

**S3: Secondary Outcome Measures**

Secondary efficacy outcomes were the change from baseline in cognition (MMSE) [3], Clinical Dementia Severity (CDR-SB), daily living activities (Bristol Activities of Daily Living Scale, BADLS [5], and the Alzheimer’s Disease Functional Assessment and Change Scale, ADFACS [6]), behavioral and psychological symptoms (Neuropsychiatric Inventory, NPI) [7], global clinical state (Alzheimer’s Disease Cooperative Study Clinical Global Impression of Change, ADCS-CGIC [8], and dementia ‘caseness’, short CAMDEX [9]). Additional secondary outcomes investigated included the safety and tolerability of MTC, and the effect of MTC compared with placebo on cerebral perfusion as assessed by hexamethylpropylamine oxime (HMPAO) SPECT scan.

Following screening and randomization at baseline, visits were scheduled at 2, 6, 12, 18, 24, 28, 37, and 50 weeks. ADAS-Cog assessments were made at baseline and at 6, 12, 18, 24, 37, and 50 weeks. MMSE, short CAMDEX, BADLS, and NPI assessment were conducted at baseline and at 12, 24, 37, and 50 weeks; CDR-SB, ADFACS, and ADCS-CGIC assessments were made at baseline and at 12 and 24 weeks. Adverse events (AEs) were to be determined at all study visits and were coded using the MedDRA dictionary (version 9.1); blood for laboratory parameters (see below) was collected at screening (or within 4 weeks prior to scheduled baseline visit), at 6, 12, and 24 weeks, and every 3 months throughout E1.

Hematology Parameters

|  |  |  |
| --- | --- | --- |
| **Measured at screening** | **Measured at baseline1, and 6, 12, and 24 weeks** | **Measured at treatment extension and  every 3 months** |
| * RBC count * Reticulocytes * WBC count with differential * Platelets * Hgb * Hct * MCV * MetHb | * RBC count * Reticulocytes * WBC with differential * Platelets * Hgb * Hct * MCV * MetHb (Visit 5 only) | * RBC count * Reticulocytes * WBC with differential * Platelets * Hgb * Hct * MCV * MetHb (Visits 9 and 13) |

1 or within the prior 4 weeks

RBC, red blood cell; WBC, white blood cell; Hgb, hemoglobin; Hct, hematocrit; MCV, mean cell volume; MetHb, methemoglobin

Serum Chemistry Parameters

|  |  |  |
| --- | --- | --- |
| **Measured at screening** | **Measured at baseline1, and 6, 12, and 24 weeks** | **Measured at treatment extension and  every 3 months** |
| * Sodium * Potassium * Calcium * Urea * Creatinine * Total bilirubin * Alkaline phosphatase * AST * ALT * GGT * Albumin * Glucose * G6PD * TSH * Folate * Vitamin B12 | * Sodium * Potassium * Calcium * Urea * Creatinine * Total bilirubin * Alkaline phosphatase * AST * ALT * GGT * Albumin * Glucose | * Sodium * Potassium * Calcium * Urea * Creatinine * Total bilirubin * Alkaline phosphatase * AST * ALT * GGT * Albumin * Glucose |

1 or within the prior 4 weeks

AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma glutamyl transferase; G6PD, glucose 6 phosphate dehydrogenase; TSH, thyroid stimulating hormone

**S4: Single Photon Emission Computed Tomography (SPECT)**

Baseline images were visually assessed by two experienced Nuclear Medicine experts, blinded to the participant treatment group. The characteristic rCBF SPECT pattern of Alzheimer’s disease is represented by a bilateral or unilateral reduction in the temporoparietal regions. Baseline images were classified as having or not having clear and identifiable temporoparietal reduction, either uni- or bilateral. In addition, images consistent with deficits of a vascular origin of dementia were identified.

*Image acquisition and reconstruction*

The multicenter nature of this study meant that the acquisition protocol at each center was slightly different due to equipment differences. However, each imaging site was sent the same protocol and asked to adhere to it as closely as possible. Each patient was allowed to rest in a darkened room before intravenous injection of 500 MBq of 99Tcm hexamethylpropylamine oxime (HMPAO). Using a dual headed gamma camera and low energy high resolution collimator 128 views over 360° (pixel size=3.38 mm) were acquired. The data were transferred to the main trial center and reconstructed using filtered back projection and corrected for attenuation using a first order Chang correction.

*Region of interest*

Standardized, three-dimensional regions of interest were used to calculate the mean rCBF in the left and right frontal, parietal, temporal, and occipital lobes, using the Wake Forest University ROI analysis tool (http://fmri.wfubmc.edu/cms/software). The data were count normalized to the mean cerebellar uptake [10].

*Statistical parametric mapping*

Using the SPM2 package (University College, Queens Square, London), images were spatially normalized to the standard statistical parametric mapping SPECT template, re-sampled at 4 x 4 x 4 mm voxels, and then smoothed with an 8 mm Gaussian kernel.The images were then intensity normalized to the mean cerebellar uptake [10].

**S5: Adverse Events: 24-Week Study**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **MedDRA System Organ Class**  **MedDRA Preferred Term** | **Placebo**  **(n = 92)**  **n (%)** | **MTC** | | |
| **MTC 30 mg tid**  **(n = 59)**  **n (%)** | **MTC 60 mg tid**  **(n = 80)**  **n (%)** | **MTC 100 mg tid**  **(n = 90)**  **n (%)** |
| Number of Patients with at Least One TEAE | 61 (66.3%) | 43 (72.9%) | 67 (83.7%) | 70 (77.8%) |
| **Gastrointestinal Disorders** | | | | |
| Diarrhea | 5 (5.4%) | 8 (13.6%) | 26 (32.5%) | 28 (31.1%) |
| Nausea | 6 (6.5%) | 3 (5.1%) | 6 (7.5%) | 3 (3.3%) |
| Vomiting | 0 | 3 (5.1%) | 7 (8.7%) | 4 (4.4%) |
| **Infections and Infestations** | | | | |
| Lower Respiratory Tract Infection | 2 (2.2%) | 7 (11.9%) | 5 (6.3%) | 1 (1.1%) |
| Upper Respiratory Tract Infection | 1 (1.1%) | 4 (6.8%) | 0 | 0 |
| **Injury, Poisoning and Procedural Complications** | | | | |
| Fall | 1 (1.1%) | 5 (8.5%) | 4 (5.0%) | 5 (5.6%) |
| **Nervous System Disorders** | | | | |
| Dizziness | 3 (3.3%) | 4 (6.8%) | 2 (2.5%) | 6 (6.7%) |
| Headache | 5 (5.4%) | 1 (1.7%) | 0 | 4 (4.4%) |
| Syncope | 0 | 3 (5.1%) | 0 | 2 (2.2%) |
| **Renal and Urinary Disorders** | | | | |
| Dysuria | 1 (1.1%) | 4 (6.8%) | 8 (10.0%) | 5 (5.6%) |
| Micturition Urgency | 1 (1.1%) | 4 (6.8%) | 5 (6.3%) | 7 (7.8%) |
| Pollakiuria | 2 (2.2%) | 9 (15.3%) | 5 (6.3%) | 13 (14.4%) |
| Urinary Incontinence | 1 (1.1%) | 3 (5.1%) | 4 (5.0%) | 3 (3.3%) |
| Urinary Tract Infection | 7 (7.6%) | 0 | 4 (5.0%) | 4 (4.4%) |
| **Skin and Subcutaneous Tissue Disorders** | | | | |
| Rash | 1 (1.1%) | 4 (6.8%) | 2 (2.5%) | 3 (3.3%) |
|  | | | | |

Number, (n, %) of patients with at least one treatment emergent adverse event with an incidence of ≥ 5% of patients in any treatment Group – Placebo-controlled safety population (n = total). TEAE, treatment-emergent adverse event

**S6: Red-Blood Cell Parameters: 24-Week Study**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Visit (Week)** | **Randomized treatment** | | | | | | | |
| **Placebo** | | **MTC 30 mg** | | **MTC 60 mg** | | **MTC 100 mg** | |
| **n and Mean ± SD RBC (×1012/L)** | | | | | | | | |
| Visit 0 (Baseline) | 92 | 4.53 ± 0.50 | 59 | 4.60 ± 0.37 | 80 | 4.58 ± 0.45 | 90 | 4.55 ± 0.46 |
| Visit 2 (Week 6) | 88 | 0.00 ± 0.21 | 53 | -0.06 ± 0.21 | 69 | -0.21 ± 0.33 | 83 | -0.31 ± 0.34 |
| Visit 3 (Week 12) | 86 | -0.01 ± 0.26 | 54 | -0.05 ± 0.31 | 64 | -0.27 ± 0.28 | 73 | -0.40 ± 0.32 |
| Visit 5 (Week 24) | 83 | 0.03 ± 0.26 | 52 | -0.10 ± 0.23 | 58 | -0.23 ± 0.35 | 75 | -0.33 ± 0.34 |
| **n and Mean ± SD Reticulocytes (%)** | | | | | | | | |
| Visit 0 (Baseline) | 92 | 1.40 ± 0.43 | 59 | 1.48 ± 0.40 | 80 | 1.48 ± 0.44 | 90 | 1.42 ± 0.39 |
| Visit 2 (Week 6) | 88 | 0.06 ± 0.38 | 53 | 0.06 ± 0.41 | 69 | 0.17 ± 0.38 | 83 | 0.50 ± 0.68 |
| Visit 3 (Week 12) | 86 | 0.09 ± 0.38 | 54 | 0.09 ± 0.39 | 64 | 0.26 ± 0.46 | 73 | 0.46 ± 0.55 |
| Visit 5 (Week 24) | 83 | 0.08 ± 0.52 | 52 | 0.13 ± 0.43 | 58 | 0.24 ± 0.57 | 75 | 0.32 ± 0.65 |
| **n and Mean ± SD Hemoglobin (g/dL)** | | | | | | | | |
| Visit 0 (Baseline) | 92 | 13.72 ± 1.41 | 59 | 13.79 ± 1.25 | 80 | 13.67 ± 1.47 | 90 | 13.82 ± 1.36 |
| Visit 2 (Week 6) | 88 | -0.05 ± 0.59 | 53 | -0.24 ± 0.74 | 69 | -0.63 ± 1.01 | 83 | -1.04 ± 0.97 |
| Visit 3 (Week 12) | 86 | -0.05 ± 0.72 | 54 | -0.22 ± 0.82 | 64 | -0.76 ± 0.92 | 73 | -1.19 ± 0.89 |
| Visit 5 (Week 24) | 83 | -0.06 ± 0.81 | 52 | -0.36 ± 0.72 | 58 | -0.68 ± 0.92 | 75 | -1.08 ± 1.06 |
| **n and Mean ± SD Hematocrit (L/L)** | | | | | | | | |
| Visit 0 (Baseline) | 92 | 0.43 ± 0.04 | 59 | 0.43 ± 0.04 | 80 | 0.42 ± 0.04 | 90 | 0.43 ± 0.04 |
| Visit 2 (Week 6) | 88 | 0.00 ± 0.02 | 53 | -0.01 ± 0.02 | 69 | -0.02 ± 0.03 | 83 | -0.03 ± 0.03 |
| Visit 3 (Week 12) | 86 | 0.00 ± 0.03 | 54 | -0.01 ± 0.03 | 64 | -0.02 ± 0.03 | 73 | -0.03 ± 0.03 |
| Visit 5 (Week 24) | 83 | 0.00 ± 0.03 | 52 | -0.01 ± 0.02 | 58 | -0.02 ± 0.03 | 75 | -0.02 ± 0.04 |
| **n and Mean ± SD Mean Cell Volume (fL)** | | | | | | | | |
| Visit 0 (Baseline) | 92 | 94.26 ± 6.22 | 59 | 93.33 ± 4.27 | 80 | 92.13 ± 5.00 | 90 | 93.58 ± 4.60 |
| Visit 2 (Week 6) | 88 | -0.40 ± 3.51 | 53 | -0.44 ± 3.10 | 69 | 0.88 ± 2.65 | 83 | 1.00 ± 3.25 |
| Visit 3 (Week 12) | 86 | -0.46 ± 3.42 | 54 | -0.31 ± 3.26 | 64 | 1.41 ± 4.41 | 73 | 2.66 ± 3.80 |
| Visit 5 (Week 24) | 83 | -1.30 ± 5.27 | 52 | -0.66 ± 3.38 | 58 | 0.47 ± 4.71 | 75 | 1.59 ± 4.11 |

Descriptive statistics for baseline values and change from baseline for red-blood cell (RBC) indices – 24‑week placebo-controlled study phase

**S7: White Blood Cell Parameters: 24-Week Study**

| **Visit (Week)** | **Randomized treatment** | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Placebo**  **(n = 92)** | | **MTC 30 mg**  **(n = 59)** | | **MTC 60 mg**  **(n = 80)** | | **MTC 100 mg**  **(n = 90)** | |
| **n and Mean ± SD WBC Count (×109/L)** | | | | | | | | |
| Visit 0 (Baseline) | 92 | 6.74 ± 1.75 | 59 | 6.67 ± 1.64 | 80 | 6.68 ± 1.81 | 90 | 6.68 ± 1.67 |
| Vist 2 (Week 6) | 88 | 0.05 ± 1.28 | 53 | -0.07 ± 1.36 | 69 | -0.04 ± 1.37 | 83 | -0.25 ± 1.63 |
| Visit 3 (Week 12) | 86 | -0.21 ± 1.34 | 54 | -0.11 ± 1.27 | 64 | -0.33 ± 1.33 | 73 | -0.29 ± 1.14 |
| Visit 5 (Week 24) | 83 | -0.02 ± 1.58 | 52 | -0.14 ± 1.32 | 58 | -0.34 ± 1.79 | 75 | -0.76 ± 1.35 |
| **n and Mean ± SD Neutrophils (×109/L)** | | | | | | | | |
| Visit 0 (Baseline) | 92 | 4.32 ± 1.38 | 59 | 4.44 ± 1.46 | 80 | 4.35 ± 1.42 | 90 | 4.25 ± 1.33 |
| Vist 2 (Week 6) | 88 | 0.05 ± 1.20 | 53 | -0.03 ± 1.25 | 69 | -0.06 ± 1.18 | 83 | -0.10 ± 1.44 |
| Visit 3 (Week 12) | 86 | -0.12 ± 1.20 | 54 | -0.09 ± 1.22 | 64 | -0.27 ± 1.11 | 73 | -0.03 ± 1.07 |
| Visit 5 (Week 24) | 83 | -0.02 ± 1.36 | 51 | -0.04 ± 1.22 | 58 | -0.27 ± 1.16 | 74 | -0.35 ± 1.16 |
| **n and Mean ± SD Lymphocytes (×109/L)** | | | | | | | | |
| Visit 0 (Baseline) | 92 | 1.61 ± 0.62 | 59 | 1.51 ± 0.42 | 80 | 1.58 ± 0.60 | 90 | 1.69 ± 0.52 |
| Visit 2 (Week 6) | 88 | 0.04 ± 0.35 | 53 | -0.06 ± 0.30 | 69 | -0.06 ± 0.61 | 83 | -0.19 ± 0.40 |
| Visit 3 (Week 12) | 86 | -0.05 ± 0.35 | 54 | -0.07 ± 0.29 | 64 | -0.08 ± 0.50 | 73 | -0.24 ± 0.40 |
| Visit 5 (Week 24) | 83 | 0.02 ± 0.48 | 51 | -0.09 ± 0.31 | 58 | -0.05 ± 1.18 | 74 | -0.32 ± 0.39 |
| **n and Mean ± SD Monocytes (×109/L)** | | | | | | | | |
| Visit 0 (Baseline) | 92 | 0.40 ± 0.14 | 59 | 0.39 ± 0.14 | 80 | 0.39 ± 0.15 | 90 | 0.37 ± 0.13 |
| Visit 2 (Week 6) | 88 | -0.01 ± 0.10 | 53 | 0.01 ± 0.09 | 69 | 0.02 ± 0.13 | 83 | 0.00 ± 0.11 |
| Visit 3 (Week 12) | 86 | -0.01 ± 0.12 | 54 | 0.01 ± 0.11 | 64 | -0.01 ± 0.10 | 73 | 0.01 ± 0.12 |
| Visit 5 (Week 24) | 83 | 0.01 ± 0.15 | 51 | -0.02 ± 0.10 | 58 | -0.01 ± 0.09 | 74 | -0.03 ± 0.11 |
| **n and Mean ± SD Eosinophils (×109/L)** | | | | | | | | |
| Visit 0 (Baseline) | 92 | 0.22 ± 0.19 | 59 | 0.16 ± 0.10 | 80 | 0.19 ± 0.12 | 90 | 0.18 ± 0.10 |
| Visit 2 (Week 6) | 88 | -0.03 ± 0.11 | 53 | 0.01 ± 0.09 | 69 | 0.04 ± 0.17 | 83 | 0.05 ± 0.14 |
| Visit 3 (Week 12) | 86 | -0.04 ± 0.10 | 54 | 0.02 ± 0.18 | 64 | 0.02 ± 0.12 | 73 | -0.02 ± 0.10 |
| Visit 5 (Week 24) | 83 | -0.03 ± 0.13 | 51 | 0.02 ± 0.15 | 58 | 0.01 ± 0.09 | 74 | -0.01 ± 0.11 |
| **n and Mean ± SD Basophils (×109/L)** | | | | | | | | |
| Visit 0 (Baseline) | 92 | 0.07 ± 0.06 | 59 | 0.06 ± 0.04 | 80 | 0.06 ± 0.03 | 90 | 0.07 ± 0.05 |
| Visit 2 (Week 6) | 88 | -0.01 ± 0.06 | 53 | 0.01 ± 0.05 | 69 | 0.01 ± 0.06 | 83 | 0.00 ± 0.05 |
| Visit 3 (Week 12) | 86 | 0.00 ± 0.08 | 54 | 0.01 ± 0.07 | 64 | 0.00 ± 0.05 | 73 | -0.01 ± 0.05 |
| Visit 5 (Week 24) | 83 | 0.00 ± 0.06 | 51 | 0.00 ± 0.06 | 58 | 0.00 ± 0.05 | 74 | 0.00 ± 0.05 |

Descriptive statistics for baseline values and change from baseline for white-blood cell (WBC) indices – 24 week placebo-controlled study phase. As part of the automated differential count, any cells that could not be identified using the instrument algorithms were counted as large unclassified cells. Mean/median baseline values were within normal limits (0.08 to 0.3 × 109 cells/L) and small, clinically insignificant mean changes from baseline to each on-treatment visit.

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