む）

1. 特許取得

**特許登録**

1. 発明の名称　画像診断支援システム及び方法

特許第5107538号

特許登録日　2012年10月12日

発明者　百瀬 敏光、相馬 努、桜井 晃臣

**特許出願**

1. 発明の名称　脳断層動態画像解析装置

特許出願番号　特願2012-190107

特許出願日　2012年8月30日

発明者　百瀬 敏光、佐藤 友彦、相馬 努、齋藤 大輔、高橋 美和子、大垣 慶介

2. 実用新案登録

なし

3. その他

なし

厚生労働科学研究費補助金(難病・がん等の疾患分野の医療の実用化研究事業研究事業)
(分担)研究年度終了報告書

# 多系統萎縮症の治療に向けたゲノム解析研究

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研究要旨:多系統萎縮症の臨床治験を具体化していく上で必要となる,発症機構の解析とsurrogate markerの検討を進めた.アレル頻度の低いvariantsについては,統計学的な検出力が小さくなる傾向があるために,機能予測などをあわせて対象 variants の絞り込み,症例,健常者のさらなる収集が必要である.

## A 研究目的

多系統萎縮症 (multiple system atrophy, MSA) は,大部分が孤発性で,あるが,一部に多発家系が存在することが知られている.MSA は,小脳失調,パーキンソニズム,自律神経症状などをさまざまな組み合わせで発症する疾患で,病因は未解明である.本研究では,全ゲノム解析/exome 解析に基づき,多系統萎縮症の発症に関わるゲノム要因を明らかにし,解明された病態機序について,疾患の進行を抑制する治療法を開発することを目的としている.これを達成するために,1.患者および健常者のゲノム収集を進めると共に,臨床治験のデザインをする上で必須となる自然歴の情報を収集する,2. 収集された大規模ゲノムリソースを用いて,全ゲノム配列解析,あるいは,exome 解析を実施して,発症に関与するゲノム要因を特定し,発症機構を解明する.3. これらの成果に基づき,本症に対する臨床治験を実施すること,を目的としている.

## B 研究方法

多系統萎縮症のゲノム解析:JAMSAC で収集した,孤発性 MSA 症例および健常対照者,および,MSA 多発家系について次世代シーケンサーを用いたexome解析を実施する.日本人 MSA 症例,健常者から,臨床情報,ゲノム DNA 収集を進めた.

## C 研究結果

日本人 MSA 症例 540 名,健常者 373 名を収集した. MSA 多発家系については,全ゲノム配列解析を実施し,MSA の発症に関与する遺伝子の絞り込み作業を進めた.日本人MSA症例407名,健常者335名について,exome解析を実施し,疾患発症に関連するゲノム上の候補変異を見出した.

D　考察

次世代シーケンサーを駆使したゲノム解析により，多発家系の全ゲノム解析，孤発例，健常者の exome 解析が順調に進んでいる．アレル頻度の低い variants については，統計学的な検出力が小さくなる傾向があるために，機能予測などをあわせて対象 variants の絞り込み，症例，健常者のさらなる収集が必要である．ゲノム解析から見出された発症に関連する可能性が見出された遺伝子について，機能解析，さらに，臨床治験における surrogate marker としての開発を進める．また，上記の成果に基づき，探索的な臨床試験のデザインについての検討を開始し，平成25年度に実施の予定である．

E　結論

MSA 発症に関連する遺伝要因の探索と，surrogate marker の開発を進め，臨床治験に用いることができるようにする．

F　健康危険情報

G　研究発表

1 論文発表

該当なし

2 学会発表

該当なし

H　知的財産権の出願・登録状況

1.　特許取得

該当なし

2.　実用新案登録

該当なし

3.その他

別紙４

研究成果の刊行に関する一覧表レイアウト

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FINAL REPORT

Study Title: Single and 2-Week Repeated Oral Dose Toxicity Study of TAK-070 M-II in Cynomolgus Monkeys

Study Number:

Study Director:

Signatur Date

, Ltd.

Drug Safety Research Laboratories

This Final Report contains 132 pages including the cover page.

Contents

## Tabulated Summary

(Study No.: 0-010)

| | | | |
|---|---|---|---|
| Animal | Cynomolgus Monkey, 3 to 5 years | | |
| Treatment<br>Test article | Orally by gavage<br>TAK-070 M-II | | |
| Dosing period | Single | | |
| Dose level (mg/kg) | 3 | | 30 |
| No. of animals (M:F) | 3:3 | | 3:3 |
| Clinical signs | - | | - |
| Body weight | - | | - |
| Tocicokinetic parameters for TAK-070 M-II (M / F) | | | |
| $T_{max}$ (h) | 1.68 / 1.00 | | 2.00 / 2.00 |
| $C_{max}$ (ng/mL) | / | | / |
| $AUC_{0\text{-}24h}$ (ng·h/mL) | | | |
| $AUC_{0\text{-}inf}$ (ng·h/mL) | | | |
| | | | |
| Dosing period | 2-week | | |
| Dosage level (mg/kg/day) | 0, control [a)] | 10 | 60 |
| No. of animals (M:F) | 3:3 | 3:3 | 3:3 |
| Clinical signs | - | - | - |
| Body weight | - | - | - |
| Food consumption | - | - | - |
| Electrocardiography | - | - | - |
| Blood pressure | - | - | - |
| Respiratory rate | - | - | - |
| Hematology | - | - | - |
| Blood chemistry | - | - | ↑AST#: (M1, F1), ↑ALT#: (M2, F2) |
| Plasma cortisol measurement | - | - | - |
| Necropsy | - | - | - |
| Organ weights | - | - | - |
| Histopathology | - | - | - |
| Tocicokinetic parameters for TAK-070 M-II (M / F) | | | |
| $T_{max}$ (h) | 1st | 2.33 / 1.67 | 2.67 / 2.67 |
| | 14th | 2.33 / 2.67 | 2.00 / 1.67 |
| $C_{max}$ (ng/mL) | 1st | | / |
| | 14th | | |
| $AUC_{0\text{-}24h}$ (ng·h/mL) | 1st | | |
| | 14th | | |
| $AUC_{0\text{-}inf}$ (ng·h/mL) | 1st | | |
| | 14th | | |

-: No treatment-related effects, M: Male, F: Female, ↑: Increased,
AST: aspartate transaminase, ALT: alanine transaminase,
#: Toxicologically significant changes
a): 0.5 w/v% methylcellulose solution

## Summary

A single dose of TAK-070 M-II at dose levels of 3 and 30 mg/kg to cynomolgus monkeys, 3 males and 3 females per group, was conducted to examine toxicity and exposure of TAK-070 M-II. A 2-week repeated dose at 10 and 60 mg/kg was subsequently conducted to examine toxicity and exposure. The following examinations were performed for toxicological evaluation: clinical signs and body weight for single and repeated dosing, and food consumption, electrocardiography, blood pressure, respiration rate, hematology, blood chemistry, plasma cortisol measurement, necropsy, organ weights, and histopathology for repeated dosing.

No test article-related changes were noted in any examination in the single dose study at 3 or 30 mg/kg, or in the repeated dose study at 10 mg/kg.

At the 60 mg/kg in the repeated dose study, increased alanine transaminase activity was noted in 2 males and 2 females at Week 2. Aspartate transaminase activity was also increased in 1 of these males and 1 of these females. No test article-related changes were noted in other examinations.

In toxicokinetics, $C_{max}$ and AUC values after single dosing increased with dose level; a dose proportionality relatioship was observed between 3 and 10 mg/kg; however, both $C_{max}$ and AUC values almost leveled off at 30 and 60 mg/kg. At the dose level ranged 3 to 60 mg/kg, there were no sex differences in any parameters and exposure of TAK-070 M-II seemed not to differ after repeated dosing. The total mean $AUC_{0\text{-}24h}$ values for males and females after single doses at 3, 10, 30, and 60 mg/kg were , and ng·h/mL, respectively.

## 1. Study Objectives

This study was conducted to evaluate the toxicity of TAK-070 M-II when administered orally once or for 2 weeks to cynomolgus monkeys. Systemic exposure was also assessed.

## 2. Regulatory Compliance

This study was not conducted in compliance with the GLP regulations; however, the Final Report was confirmed to accurately reflect the raw data in compliance with the following standards, except for the stability of the test article, which is to be confirmed at a later date:

- Standards for the Reliability of Application Data (Article 43, Enforcement Regulations, Pharmaceutical Affairs Law, March 23, 2005, MHLW, Ordinance No.37)

## 3. Animal Welfare

This study was approved by the Institutional Animal Care and Use Committee (Approval No. IACUC720-010) and was performed in accordance with the animal welfare bylaws of , Ltd., Drug Safety Research Laboratories, which is fully accredited by AAALAC International.

## 4. Sponsor

The University of Tokyo, The University of Tokyo Hospital
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7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
TEL: 81 (0) 3-5800-9083 FAX: 81 (0) 3-5800-9097

## 5. Test Facility

, Ltd.
Drug Safety Research Laboratories
, Japan
TEL: 81 (0) FAX: 81 (0)

## 6. Study Schedule

Study Initiation Date: November 27, 2012

Single Dose Study

(The day before the day of dosing was designated as Day −1. The dosing day was designated as Day 0. The observation period was 3 days.)

| | |
|---|---|
| Initiation of Acclimation: | November 27, 2012 |
| End of Acclimation / Grouping: | November 29, 2012 |
| Day of Dosing: | November 30, 2012 |
| End of Observation: | December 2, 2012 |

2-Week Repeated Dose Study

(The day before the first day of dosing was designated as Day −1 of repeated dosing. The first day of dosing was designated as Day 1 of dosing. The first week of the dosing period was designated as Week 1 of dosing.)

| | |
|---|---|
| Non-dosing Days: | December 3, 2012 to January 8, 2013 |
| First Day of Dosing: | January 9, 2013 |
| Final Day of Dosing: | January 22, 2013 |
| Necropsy: | January 23, 2013 |

Completion of Study: March 27, 2013

## 7. Materials and Methods

### 7.1 Test and Control Articles (Vehicle)

#### 7.1.1 Test Article

Name: TAK-070 M-II

Supplier: Laboratory,

Lot Number: M070-M1002

Characteristics

(CERTIFICATE OF ANALYSIS issued on November 28, 2012, , Attachment 1)

| | |
|---|---|
| Assay: | 99.94% |
| Description: | White crystalline powder |

Date of Allocation: November 27, 2012

| | |
|---|---|
| Amount Allocated: | Approximately 1360 g |
| Storage Conditions: | Refrigerated and protected from light in a tight container |
| Storage Location: | The Refrigeration Room in the Test Article Depository [actual range from the day of allocation to the day of transfer (November 27, 2012 to March 22, 2013): 3.3°C to 7.2°C, acceptable range: 2°C to 8°C] |
| Handling: | A mask, a cap, gloves, and safety glasses were worn. |
| Remaining Test Article: | All remaining test article was transferred to the Test Article Controller on March 22, 2013. |

### 7.1.2 Control Article (Vehicle)

| | |
|---|---|
| Name: | 0.5 w/v% methylcellulose (MC) solution |
| Preparation Method: | Prepared in the following ratios:<br>0.5 g of methylcellulose (Metolose SM-400) was added to approximately 40 mL of hot water for injection, and stirred to thoroughly disperse. Cooled water for injection was then added to dissolve. After complete dissolution, this solution was transferred to a measuring cylinder and water for injection was added to make 100 mL. The solution was divided into 1-day aliquots. |
| Manufacturers | |
| MC: | Shin-Etsu Chemical Co., Ltd. |
| Water for Injection: | Otsuka Pharmaceutical Factory Inc. |
| Lot Numbers | |
| MC: | 1065396 |
| Water for Injection: | 2D92 and 2E87 |
| Storage Conditions: | Refrigerated and protected from light |
| Storage Location: | The Refrigeration Room in the Test Article Depository [actual range from the first day of storage to the final day of use (January 8, 2013 to January 22, 2013): 4.0°C to 6.9°C, acceptable range: 2°C to 8°C] |

Confirmation of Concentration:

The vehicle administered on Days 1 and 14 of repeated dosing was analyzed by HPLC. The acceptance criterion was

that no test article peak be detected, and this was confirmed to have been met. Details of the method and results are stated in Attachment 1.

### 7.2 Preparation of Test Article Formulations

Concentrations

Single Dose Study: 0.6 and 6 mg/mL

2-Week Repeated Dose Study:
2 and 12 mg/mL

Conversion Factor: None

Pulverization: The test article was not pulverized before weighing.

Methods

Single Dose Study: Test article was weighed, transferred to an agate mortar, and crushed while 0.5 w/v% MC solution was gradually added to suspend. The preparation was transferred to a measuring cylinder, and 0.5 w/v% MC solution was added to make a 6 mg/mL formulation. The 6 mg/mL formulation was diluted with 0.5 w/v% MC solution to make a 0.6 mg/mL formulation.

2-Week Repeated Dose Study:
The test article was weighed, transferred to an agate mortar, and crushed while 0.5 w/v% MC solution was gradually added to suspend. The preparation was transferred to a measuring cylinder, and 0.5 w/v% MC solution was added to make a 12 mg/mL formulation. The 12 mg/mL formulation was diluted with 0.5 w/v% MC solution to make a 2 mg/mL formulation.

Stability and Homogeneity: The stability and homogeneity of dosing formulations of TAK-070 M-II in 0.5 w/v% MC solution (concentrations of 0.3 and 12 mg/mL) for 7 days when stored refrigerated and protected from light followed by 24 hours at room temperature and protected from light were confirmed (Certificate of Analysis, Certificate No. 720009-2, Attachment 1).

Frequency

Single Dose Study: Before use on dosing day

2-Week Repeated Dose Study:

5 times (dosing formulations were used within 7 days of preparation)

Storage Conditions: Refrigerated and protected from light

Storage Location: The Refrigeration Room in the Test Article Depository [actual range from the day of preparation to the final day of use (January 9, 2013 to January 22, 2013): 4.3°C to 6.4°C, acceptable range: 2°C to 8°C]

Confirmation of Concentrations, Homogeneity, and Particle Size:

The test article formulations for single dosing and for Days 1 and 14 of repeated dosing were analyzed by HPLC to determine concentrations and to confirm homogeneity. The acceptance criterion was a concentration within ±10.0% of the nominal concentration. The acceptance criterion for homogeneity was a coefficient of variance (CV) not exceeding 10.0%. These criteria were confirmed to have been met. Details of the methods and results are stated in Attachment 1.

The 6 mg/mL formulation for single dosing and the 12 mg/mL formulations for Days 1 and 14 of repeated dosing were analyzed to determine particle size. Details of the method and results are stated in Attachment 1. The results were treated as reference data.

The analysis results were as follows: the respective mean concentrations were 99.3% and 101.7% in the 0.6 and 6 mg/mL formulations for single dosing, 101.2% and 102.5% in the 2 and 12 mg/mL formulations on Day 1 of repeated dosing, and 102.3% and 102.2% in the 2 and 12 mg/mL formulations on Day 14 of repeated dosing; the mean particle size (volume under 50%) was 2.9 μm in the 6 mg/mL formulation for single dosing, 2.8 and 2.8 μm in the 2 and 12 mg/mL formulations on Day 1 of repeated

dosing, and and μm in the 2 and 12 mg/mL formulations on Day 14 of repeated dosing.

### 7.3 Dosing of Test and Control Articles

Dosing Route: Oral

Justification for Route: In accordance with the intended clinical route

Dosing Method: Dosing formulations were administered into the stomach via the nasal cavity using a disposable catheter and syringe. The test article formulations were stirred with a stirrer during collection.

Justification for Method: Commonly used method for oral dosing to cynomolgus monkeys

Frequency and Period

Single Dose Study: Once (single dosing)

2-Week Repeated Dose Study:

Once daily, 7 times weekly for 2 weeks (total 14 times)

Justification for Frequency and Period

Single Dose Study: In order to assess toxicity and toxicokinetics after single dosing

2-Week Repeated Dose Study:

In order to assess toxicity after 2-week of repeated dosing

Dose Volume: 5 mL/kg

Individual dose volume was calculated based on the most recent body weight.

Dosing Time

Single Dose Study: Between 12:03 and 12:31

2-Week Repeated Dose Study:

Between 10:01 and 12:16

### 7.4 Test System

Species: Cynomolgus monkey (purpose-bred)

Body Weight

At the Initiation of Acclimation:

Males: 3.40 to 4.71 kg, females: 2.70 to 3.29 kg

| | |
|---|---|
| At Grouping: | Males: 3.78 to 4.62 kg, females: 2.86 to 3.23 kg |
| Age: | 3 to 5 years (at the initiation of acclimation) |
| Origin: | |
| Microbial Status: | Negative in tuberculin test<br>Negative in serum antibody tests for B virus, simian immuno-deficiency virus (SIV), simian retrovirus (SRV), and simian T-lymphotrophic virus (STLV)<br>Negative in fecal microbial tests for *Shigella*, *Salmonella*, *Entamoeba histolytica*, and helminth eggs |
| Dates of Receipt: | August 6, 2011, November 22, 2011, March 20, 2012, April 24, 2012, July 24, 2012, and August 21, 2012 |
| CITES Permit Nos.: | 2011CN/EC0116/GZ, 2011CN/EC0810/GZ, 2012CN/EC0051/GZ, 2012CN/EC0052/GZ, 2012CN/EC0316/GZ, and 2012CN/EC0413/GZ, |
| Date of Allocation: | November 27, 2012 |
| Number of Animals | |
| On Acclimation: | 12 males and 12 females, total 24 animals |
| On Study: | For single dosing: 6 males and 6 females<br>For 2-week repeated dosing: 9 males and 9 females<br>In total: 10 males and 11 females |
| Breeder: | , Ltd.<br>, China |
| Source: | , Ltd. (stock colony)<br>, Japan |

Justification for Selection of the Species:

The monkey (cynomolgus monkey) is a commonly used non-rodent species in toxicity studies, and cynomolgus monkeys had been used for toxicity studies of TAK-070 which is the mother compound of M-II.

### 7.5 Maintenance Conditions

| | |
|---|---|
| Room: | No. |
| Temperature: | Actual range: 24.8°C to 27.8°C,<br>acceptable range: 23°C to 29°C |

Humidity: Actual range: 38% to 80%,
acceptable range: 30% to 70%
Humidity occasionally deviated from the acceptable range due to daily cleaning procedures; however, these deviations were transient (within 120 minutes from the completion of cleaning). Accordingly, they were judged not to have affected the study results.

Ventilation: 15 times/hour

Illumination: 12 hours/day of artificial light (07:00 to 19:00)
The lights were switched on manually for blood sampling and clinical signs observations, as stated in the table below.

| Date | Time |
|---|---|
| November 30, 2012 | 20:02 to 20:37 |
| January 9, 2013 | 18:59 to 19:40 |
| January 22, 2013 | 18:58 to 19:37 |

Animal Cages

Materials: Stainless steel

Cage Size: mm (D) × mm (W) × mm (H)

Number of Animals per Cage:
One

Food: Approximately 108 g (approximately 12 g × 9 pieces) of solid food (HF Primate 5K91 12G 5K9J, Lot Nos. SEP1112 and OCT0212, Purina Mills, LLC) was provided to each animal daily between 14:00 and 16:00. Any remaining food was removed before 11:00 (before dosing during the dosing period) on the following day. On the days of electrocardiography, and blood pressure and respiration rate measurement, food was provided after completion of examinations. On the days before blood sampling (for hematology, blood chemistry, and plasma cortisol measurement) and necropsy, any remaining food was removed at approximately 17:00. The results of analysis of each lot of food by Purina Mills, LLC were obtained and it