

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

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**PHARMACOLOGY REVIEW(S)**

**DEPARTMENT OF HEALTH AND HUMAN SERVICES  
PUBLIC HEALTH SERVICE  
FOOD AND DRUG ADMINISTRATION  
CENTER FOR DRUG EVALUATION AND RESEARCH**

**PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION**

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# 1 Executive Summary

## 1.1 Recommendations

### 1.1.1 Approvability

Yes

### 1.1.2 Additional Non Clinical Recommendations

None

### 1.1.3 Labeling

TBD

## 1.2 Brief Discussion of Nonclinical Findings

Azilsartan medoxomil (TAK-491) is the pro-drug for TAK-536, a competitive reversible antagonist at angiotensin II (All) receptors (AT1). TAK-491 is hydrolyzed rapidly to TAK-536, by the action of aryl esterase primarily in the gastrointestinal tract and/or plasma, during absorption after oral administration. Azilsartan medoxomil was approved in early 2011 under NDA 200796. All pharmacodynamic, pharmacokinetic, ADME, and toxicology concerning azilsartan medoxomil has been reviewed under that NDA. In the current NDA application, the sponsor is combining azilsartan medoxomil with the thiazide-like antihypertensive drug, chlorthalidone. The planned clinical doses are 40 (b) (4) azilsartan medoxomil with 12.5 or 25 mg of chlorthalidone.

Under this NDA, there are 3 pivotal toxicological studies: 1) Preliminary two-week oral gavage toxicity study of TAK-491 plus chlorthalidone or TAK-536 M-II plus chlorthalidone (double combination) in rats; 2) Thirteen-week oral gavage toxicity study of TAK-491 plus TAK-536 M-II plus chlorthalidone (triple combination) in rats and 3) Effects of TAK-491 plus TAK-536 MII plus Chlorthalidone (triple combination) on Embryo-fetal Development in Rats. In brief, key study findings included the following: In study 1: Plasma chlorthalidone levels were increased by dosing in combination with TAK-491, and an additive increase in plasma urea nitrogen and increases in water intake, urine output and plasma total cholesterol were also observed in the TAK-491/chlorthalidone combination groups. No clear combination effects were observed from dosing the combination of TAK-536 MII and chlorthalidone.

In study 2: The double combination administered in this study contains the prodrug TAK-491 with TAK-536 MII. Two groups of rats received this double combination: One group received 100 mg/kg of TAK-491 with 2000 mg/kg of MII

and the second group received 1000 mg/kg TAK-491 with 2000 mg/kg of MII. In this second group, there was a significant decrease in body weight gain and food consumption. This effect was enhanced in all groups receiving the triple combination containing chlorthalidone. Chlorthalidone given by itself at one dose of 300 mg/kg increased BUN and adrenal weight. It also increased the incidence and severity of background renal tubular regeneration. All these effects were enhanced by co-administration with the double combination (TAK-491 and TAK-536 MII). The increase in kidney weight seen with chlorthalidone alone was similar to that seen in the triple combination. APTT prolongation, decreased potassium and increase in incidence and severity of renal cortical mineralization attributable to chlorthalidone were not observed by co-administration with TAK-491 and TAK-536 MII. Increased plasma creatinine, and neutrophil count, decreased uterine weight, hypertrophy of the adrenal zona fasciculata and dilatation of the renal Henle's ascending tubules with dose-dependency of TAK-491 and chlorthalidone in its incidence were observed only in the triple combination group in which plasma chlorthalidone levels were increased compared to the cohort receiving chlorthalidone *per se*.

In the embryo-fetal study # 3: The double combination administered in this study contains the prodrug TAK-491 with the human metabolite TAK-536 MII. The triple combination group was administered TAK-491, TAK-536 MII and chlorthalidone together. The final group received chlorthalidone alone. In reference to embryo-fetal development in rats, severity of general toxicity in dams was increased, fetal growth was retarded and indices of some visceral variations and wavy ribs were increased by the triple combination. Despite maternal toxicity, neither increased fetal mortality nor teratogenicity was observed in the triple combination group in this study.

## 2 Drug Information

### 2.1 Drug

 (b)  
(4)

#### 2.1.1 CAS Registry Number (Optional)

#### 2.1.2 Generic Names

Azilsartan medoxomil potassium /chlorthalidone

### 2.1.3 Code Name

TAK-491/CLD

### 2.1.4 Chemical Names

Azilsartan: (5-methyl-2-oxo-1,3-dioxol-4-yl) methyl 2-ethoxy-1-[[2'-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)biphenyl-4-yl]methyl}1-*H*-benzimidazole-7-carboxylate monopotassium salt

Chlorthalidone: 2-chloro-5(1-hydroxy-3-oxo-1-isoindolinyl) benzenesulfonamide

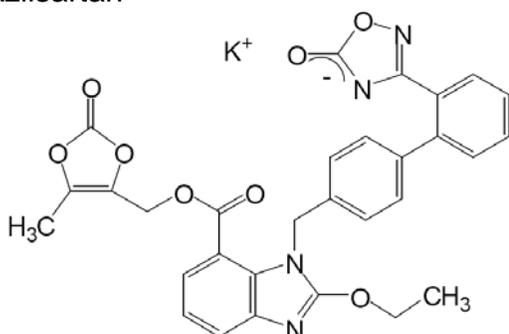
### 2.1.5 Molecular Formulae/Molecular Weights

Azilsartan: C<sub>30</sub>H<sub>23</sub>KN<sub>4</sub>O<sub>8</sub>; 606.62

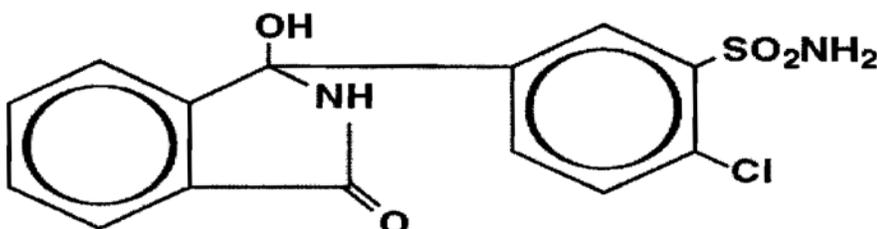
Chlorthalidone: C<sub>14</sub>H<sub>11</sub>ClN<sub>2</sub>O<sub>4</sub>S; 338.76

### 2.1.6 Structures

Azilsartan



Chlorthalidone



### 2.1.7 Pharmacologic class

Angiotensin II A1 Receptor Antagonist/ Diuretic

## 2.2 Relevant IND/s, NDA/s, and DMF/s

IND 77,278 (TAK-491/chlorthalidone)

NDA 200,796 (TAK-491)

**2.3 Clinical Formulation**

See below

**2.3.1 Drug Formulation**

**Composition of TAK-491CLD Tablets, (b) (4) 40mg+12.5mg (b) (4)**

Component	Reference to Quality Standards	Function	Quantity per Tablet (mg)	
			(b) (4)	(b) (4)
			<b>40 mg +12.5mg</b>	
TAK-491 <sup>(1)</sup> (b) (4)	In-house standard	Active ingredient		42.68 <sup>(1)</sup> (40)
Mannitol (b) (4)	Ph.Eur., USP	(b) (4)		(b) (4)
Microcrystalline cellulose (b) (4)	Ph.Eur., NF			
Fumaric Acid	NF			
Sodium Hydroxide	Ph.Eur., NF			
Hydroxypropyl cellulose (b) (4)	Ph.Eur., NF Ph.Eur., USP			
(b) (4)		(b) (4)		
Chlorthalidone	Manufacturer's standard	Active ingredient		
(b) (4)		(b) (4)		
Crospovidone	Ph.Eur., NF	(b) (4)		
(b) (4)		(b) (4)		
Magnesium stearate	Ph.Eur., NF	(b) (4)		
(b) (4)		(b) (4)		

(1) The conversion factor of T-1302593 (TAK-491 salt free form) to TAK-491(potassium salt) is 1.067.  
(b) (4)

Component	Reference to Quality Standards	Function	Quantity per Tablet (mg)	
			(b) (4)	(b) (4)
			40 mg	
			+12.5mg	
		(b) (4)		(b) (4)
Hypromellose 2910	Ph.Eur., USP	(b) (4)		(b) (4)
Talc	Ph.Eur., USP			
Titanium dioxide	Ph.Eur., USP			
Ferric oxide, red	95/45/EC (E172), NF			
(b) (4)	Ph.Eur., USP			
		(b) (4)		
Polyethylene glycol 8000 <sup>(3)</sup>	Ph.Eur., NF	(b) (4)		
(b) (4)	Ph.Eur., USP			
		(b) (4)		
Printing ink Gray F1 <sup>(4)</sup>	Manufacturer's standard	(b) (4)		
(b) (4)	NF			
<i>Tablet weight</i>			370	
		(b) (4)		

(3) The amount of polyethylene glycol 8000 is not included in the total tablet weight.

(4) The components of ink are detailed in Section 3.2.P.4.1.

(5) Quantity sufficient for printing

(b) (4)

**Composition of TAK-491CLD Tablets, (b) (4) 40mg+25mg (b) (4)**

Component	Reference to Quality Standards	Function	Quantity per Tablet (mg)	
			(b) (4)	(b) (4)
			40 mg	
			+25mg	
TAK-491 <sup>(1)</sup>	In-house standard	Active ingredient	42.68 <sup>(1)</sup>	
(b) (4)			(40)	
Mannitol	Ph.Eur., USP			(b) (4)
Microcrystalline cellulose	Ph.Eur., NF			
(b) (4)				
Fumaric Acid	NF			
Sodium Hydroxide	Ph.Eur., NF			
Hydroxypropyl cellulose	Ph.Eur., NF			
(b) (4)	Ph.Eur., USP			
				(b) (4)
				)
Chlorthalidone	Manufacturer's standard	Active ingredient		
				(b) (4)
				)
				(b) (4)
Crospovidone	Ph.Eur., NF			
(b) (4)				(b) (4)
Magnesium stearate	Ph.Eur., NF			
(b) (4)				(b) (4)

(1) The conversion factor of T-1302593 (TAK-491 salt free form) to TAK-491 (potassium salt) is 1.067.

Component	Reference to Quality Standards	Function	Quantity per Tablet (mg)	
			(b) (4)	(b) (4)
				40 mg +25mg
		(b) (4)	(b) (4)	(b) (4)
Hypromellose 2910	Ph.Eur., USP	(b) (4)	(b) (4)	(b) (4)
Talc	Ph.Eur., USP	(b) (4)	(b) (4)	(b) (4)
Titanium dioxide	Ph.Eur., USP	(b) (4)	(b) (4)	(b) (4)
Ferric oxide, red	95/45/EC (E172), NF	(b) (4)	(b) (4)	(b) (4)
		(b) (4)	(b) (4)	(b) (4)
Polyethylene glycol 8000 <sup>(3)</sup>	Ph.Eur., NF	(b) (4)	(b) (4)	(b) (4)
		(b) (4)	(b) (4)	(b) (4)
Printing ink Gray F1 <sup>(4)</sup>	Manufacturer's standard	(b) (4)	(b) (4)	(b) (4)
	NF	(b) (4)	(b) (4)	(b) (4)
<i>Tablet weight</i>		(b) (4)	(b) (4)	(b) (4)

(3) The amount of polyethylene glycol 8000 is not included in the total tablet weight.

(4) The components of ink are detailed in Section 3.2.P.4.1.

(5) Quantity sufficient for printing

(b) (4)

### 2.3.2 Comments on Novel Excipients

There are no novel excipients.

### 2.3.3 Comments on Impurities/Degradants of Concern

(b) (4) an impurity formed in the process of synthesizing the prodrug, azilsartan medoxomil, was tested for toxicity. Results were considered to be negative.

### 2.4 Proposed Clinical Population and Dosing Regimen

The proposed indication for azilsartan medoxomil/ chlothalidone is for the treatment of hypertension, either alone or in combination with other antihypertensive agents. The recommended starting dose in adults is 40 mg/ 12.5 mg taken once daily. The dose may be increased to a maximum of (b) (4) once daily when additional blood pressure reduction is required.

## 2.5 Regulatory Background

Azilsartan medoxomil (TAK-491) is the prodrug of the active moiety (TAK-536) and was approved earlier in 2011.

## 3 Studies Submitted

### 3.1 Studies Reviewed

- a. Preliminary 2-week oral (gavage) toxicity study of TAK-491 plus chlorthalidone (double combination) in rats
- b. 13-week oral (gavage) toxicity study of TAK-491 plus TAK-536 M-II plus chlorthalidone (triple combination) in rats
- c. Effects of TAK-491 plus TAK-536 M-II plus chlorthalidone (triple combination) on embryo-fetal development in rats

### 3.2 Studies Not Reviewed

- a. Single dose toxicokinetic study of TAK-491 plus chlorthalidone or TAK-536 M-II plus chlorthalidone (double combination) in rats
- b. Single dose toxicokinetic study of TAK-491 plus chlorthalidone or TAK-536 M-II plus chlorthalidone (double combination) in rats (supplemental study)
- c. 2-week oral (gavage) toxicokinetic study of TAK-491 plus TAK-536 M-II plus chlorthalidone (triple combination) in rats
- d. Range-finding combinational study for effects of TAK-491 plus TAK-536 M-II plus chlorthalidone on embryo-fetal development in rats

### 3.3 Previous Reviews Referenced

None

## 4 Pharmacology

### 4.1 Primary Pharmacology

No new data for either agent was submitted with this application.

### 4.2 Secondary Pharmacology

No new data for either agent was submitted with this application.

### 4.3 Safety Pharmacology

No new data for either agent was submitted with this application.

## 5 Pharmacokinetics/ADME/Toxicokinetics

### 5.1 PK/ADME

See below

### 5.2 Toxicokinetics

See toxicology studies below.

## 6 General Toxicology

### 6.1 Single-Dose Toxicity

No single dose toxicity studies were submitted.

### 6.2 Repeat-Dose Toxicity

Study title: Preliminary two-week oral gavage toxicity study of TAK-491 plus chlorthalidone or TAK-536 M-II plus chlorthalidone (double combination) in rats

Study no.:	07-198/su
Study report location:	electronic
Conducting laboratory and location:	Developmental Research Center; Takeda Pharmaceutical Company, Ltd., Osaka, Japan
Date of study initiation:	11/19/07
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	TAK-491, lot # M491-015 (99.8%) TAK-536 MII, lot # M117-M1003 (94.7%) Chlorthalidone, lot # MA06-001 (99.7%)

### Key Study Findings

Plasma chlorthalidone levels were increased by dosing in combination with TAK-491, and an additive increase in plasma urea nitrogen and increases in water intake, urine output and plasma total cholesterol were also observed in the TAK-491/chlorthalidone combination groups. No clear

combination effects were observed from dosing the combination of TAK-536 MII and chlorthalidone.

#### Methods

Doses: See table below  
Frequency of dosing: Once/day for 2 weeks  
Route of administration: Oral gavage  
Dose volume: 10 ml  
Formulation/Vehicle: All agents were dissolved in 0.5 w/v% methylcellulose solution containing 0.5 w/v% citric acid  
Species/Strain: F344/Jcl/ rat  
Number/Sex/Group: 4 rats/sex/group for main study  
Age: 6 weeks  
Weight: Males: 99-115 g; females: 80-96 g  
Satellite groups: 3 animals/sex/group for TK satellite groups  
Unique study design: none  
Deviation from study protocol: none

Animals	F344/Jcl rats; Age of animals at dosing commenced: 6 weeks of age, Male: 99-115 g, Female: 80-96 g; Main groups: 4 animals/sex/group (total 88 animals), Satellite groups for toxicokinetics: 4 or 8 animals/sex/group (total 88 animals) The group composition was as follows.											
	Group No. <sup>1)</sup>	1 <sup>2)</sup>	2 (12)	3 (13)	4 (14)	5 (15)	6 (16)	7 (17)	8 (18)	9 (19)	10 (20)	11 (21)
	TAK-491 <sup>3)</sup> (mg/kg/day)	0	1000	0	0	0	100	100	1000	1000	0	0
	TAK-536 M-II (mg/kg/day)	0	0	2000	0	0	0	0	0	0	2000	2000
	CLD (mg/kg/day)	0	0	0	100	300	100	300	100	300	100	300
	Number of animals <sup>4)</sup> (M:F)	4:4	4:4 (4:4)	4:4 (4:4)	4:4 (4:4)	4:4 (8:8 <sup>5)</sup> )	4:4 (4:4)	4:4 (4:4)	4:4 (4:4)	4:4 (4:4)	4:4 (4:4)	4:4 (4:4)
CLD: Chlorthalidone 1) Number in the parenthesis indicates the Group No. for toxicokinetics. 2) Control, 3) As TAK-491F (TAK-491 free acid) 4) Number in the parentheses indicates the numbers of animals for toxicokinetics. 5) 4 animals/sex were used for determination of blood CLD levels.												
Test article	TAK-491 (potassium salt, conversion factor: 1.067, Lot No. M491-015) TAK-536 M-II (Lot No. M117-M1003) Chlorthalidone (CLD; Lot No. MA06-001)											

## Observations and Results

### Summary Table (at end of review) Provided by Sponsor

#### Mortality

Mortality was monitored 3X a day (before dosing, 1 hr after and 4 hr after dosing) during the dosing period. No animals died in any treatment group.

#### Clinical Signs

Clinical signs were monitored 3X a day (before dosing, 1 hr after and 4 hr after dosing) during the dosing period. No abnormal clinical signs were noted in any treatment group.

## Body Weights

Body weight was measured 2X per week. Body weight and body weight gain were not affected in any treatment group.

## Feed Consumption

Food consumption was measured at 7 day intervals. In the TAK-491 only group, food consumption was decreased in both sexes during weeks 1 and 2. In the chlorthalidone only groups, a decrease in food consumption was observed in both sexes only during week 1. In the double combination (TAK-491/chlorthalidone) groups, a decrease in food consumption was observed in both sexes during weeks 1 and 2. In the double combination (TAK-536 MII/chlorthalidone) groups, a decrease in food consumption was observed in all groups only during week 1.

## Ophthalmoscopy

Not performed.

## ECG

Not performed

## Hematology

Hematology was tested at necropsy the day after the last dosing. In the TAK-491 only group, decreases in erythrocyte counts, hematocrit values and hemoglobin concentrations and increases in leukocyte and lymphocyte counts were observed in females in all groups.

## Clinical Chemistry

Blood was collected at necropsy by abdominal aortic puncture the day after the last dosing. In the TAK-491 only group, decreases in ALT and CK were observed in both sexes, an increase in urea nitrogen and a decrease in potassium were observed in males in the low dose group and both sexes in the high dose group. In the TAK-491/chlorthalidone groups, an increase in urea nitrogen and decreases in sodium and CK were observed in both sexes and an increase in total cholesterol and decreases in chloride and AST were observed in males in all the groups. A decrease in ALT was observed in both sexes in all the groups except for males in one group (100/100 mg/kg/day). A decrease in potassium in males (1000/300 mg/kg/day) and increases in total protein and albumin in females (100/300 and 1000/300 mg/kg/day) were observed in 1 or 2 groups. The increase in urea nitrogen in these combination groups was more remarkable than those in the TAK-491 only group and chlorthalidone only groups. In the TAK-536 MII/chlorthalidone combination groups, decreases in potassium and chloride

were observed in both sexes in all the groups. An increase in urea nitrogen in males was observed in 1 group (2000/300 mg/kg/day).

## Urinalysis

Water intake and urine output: No effects were observed in any single test article group. In the TAK-491/chlorthalidone combination groups, an increase in water intake was observed in males in all groups and an increase in urine output was observed in males in all groups and females in some groups ( 100/300, 1000,100 and 1000 and 3000 mg/kg/day). Urine chemistry: In the single test article group receiving chlorthalidone alone, potassium (creatinine ratio) was increased in males in all the groups. In the TAK-491/chlorthalidone combination groups, potassium/creatinine ratio was increased in males in some groups) (100.300, 1000/100 and 1000, 300 mg/kg/day). In the TAK-536 MII groups, potassium (creatinine ratio) was increased in males and chloride/creatinine ratio was increased in females in one group (2000/300 mg/kg/day).

## Gross Pathology

Sponsor asserts that a careful examination of all organs/tissues in the cephalic, thoracic and abdominal cavities was made at necropsy. No treatment-related abnormalities were observed in any group.

## Organ Weights

The following organs were weighed: brain, pituitary, thyroids, adrenals, thymus, spleen, heart, lungs (inc. bronchi), salivary glands, liver, kidneys, testes, prostate, seminal vesicles, ovaries and uterus. In the TAK-491-only group, the heart weight was decreased in both sexes and the thymus weight was decreased in males. In the 2 chlorthalidone-only groups, the kidney weight was increased and the thymus weight decreased in males. In the 4 TAK-491/chlorthalidone groups, heart weight was decreased in both sexes and thymus weight was decreased in males. The kidney weight was increased in some groups (100/300 mg/kg/day for males and 1000/100 and 1000/300 mg/kg/day for both sexes) and the ovarian weight was decreased (100/300 and 1000/300 mg/kg/day). In the 2 TAK-536 MII/chlorthalidone groups, the thymus weight was decreased in males and the kidney weight was increased in males in 1 group (2000/300 mg/kg/day).

## Histopathology

### Adequate Battery

Tissues from animals in the control group, the chlorthalidone only group (300 mg/kg/day group), the TAK-491 plus chlorthalidone group (1000/300 mg/kg/day group) and the TAK-536 MII/chlorthalidone group (2000/300 mg/kg/day group) were examined microscopically for any histopathology. In the other groups, only the heart, adrenals, liver and stomach were examined for histopathology. The organs listed above were analyzed histologically along with cerebrum, cerebellum, spinal cord (cervical, thoracic and lumbar), sciatic nerves, eyeballs, optic nerves, Harderian glands, pituitary, thyroids, parathyroids, submandibular lymph node, mesenteric lymph node, thoracic aorta, trachea, tongue, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, submandibular and sublingual glands, parotid glands, pancreas, urinary bladder, epididymides, vagina, mammary glands, sternum, femurs, femoral skeletal muscles, skin (inguinal region, both sides) and gross lesions. Lastly, oviducts, extraorbital lacrimal glands, Zymbal's glands, larynx, nasal cavity and site of animal identification (ear auricle) were preserved.

### Peer Review

Yes.

### Histological Findings

In the TAK-491 only group, hypertrophy of the juxtaglomerular cells in the kidney was observed in all animals and atrophy of the zona glomerulosa in the adrenal glands was observed in 3 males and 3 females. In the chlorthalidone only groups, mild tubular basophilia in the kidney was observed in 1 male in the low and 3 males in the high dose groups, and mild mineralization of the cortico-medullary junction in the kidney was observed in 2 females in the 300 mg/kg/day group. In the TAK-491/chlorthalidone groups, hypertrophy of the juxtaglomerular cells in the kidney (100/100 mg/kg/day: 2 males and all females; 100/300 mg/kg/day: 1 male and all females; 1000/100 mg/kg/day: all animals; 1000/300 mg/kg/day: all animals) and atrophy of the zona glomerulosa in the adrenal glands (100/100 mg/kg/day: 2 males and all females; 100/300 mg/kg/day: 2 males and all females; 1000/100 mg/kg/day: 3 males and all females; 1000/300 mg/kg/day: all animals) were observed in both sexes in all the groups and the frequency and severity in these combination groups were equivalent to those in the TAK-491 only group. Mild tubular

basophilia and mild mineralization of the cortico-medullary junction in the kidney were not observed in any combination group.

## Special Evaluation

None

## Toxicokinetics

Plasma concentrations ( $T_{max}$ ,  $C_{max}$  and  $AUC_{0-24h}$ ) of TAK-491, TAK-536, TAK-536 MI, TAK-536 MII and chlorthalidone were measured in the satellite groups before dosing (except for the first dose) and at 1, 2, 4, 8 and 24 hr on Days 1 and 16.

Results: TAK-491 treated groups: TAK-491F concentrations were less than the lower limit of quantification at all time points in all groups. The  $T_{max}$  values for TAK-536 and TAK-536 MII ranged from 1-2 hr. Those for TAK-536 MI ranged from 1 to 8 hr. The  $C_{max}$  and  $AUC_{0-24h}$  values for TAK-536 and TAK-536 MII increased with the increase in dosage levels of TAK-491, but those for TAK-536 MI did not. The  $C_{max}$  and  $AUC_{0-24h}$  values for TAK-536, TAK-536 MI and TAK-536 MII in the TAK-491/chlorthalidone-combination groups were almost equivalent to those in the TAK-491 only group except for those for TAK-536 MI in females in the 1000/300 group and for TAK-536 MII in both sexes in the 1000/100 and 1000/300 mg/kg/day groups. TAK-536 MII levels increased with repeated dosing.

TAK-536 MII treated groups: The  $T_{max}$  values for TAK-536 MII ranged from 1 to 4 hr. The  $C_{max}$  and  $AUC_{0-24h}$  values for TAK-536 MII in the TAK-536 MII/chlorthalidone combination groups were almost equivalent to those in the TAK-536 MII-only group. The  $C_{max}$  and  $AUC_{0-24h}$  values for TAK-536 MII decreased with repeated dosing in both the TAK-536 MII-only group and the combination groups.

Chlorthalidone treated groups: The  $T_{max}$  values for chlorthalidone in plasma ranged from 1 to 4 hr. The  $C_{max}$  and the  $AUC_{0-24h}$  increased with the increase in dosage levels. No sex differences in these values were noted. There were no apparent effects with repeated dosing on the  $C_{max}$  or  $AUC_{0-24h}$  values for chlorthalidone in plasma except for those in the TAK-536 MII/chlorthalidone combination groups, which decreased with repeated dosing. The  $C_{max}$  and  $AUC_{0-24h}$  values for chlorthalidone in the TAK-491/chlorthalidone combination groups were higher than those in the chlorthalidone-only groups at both the first and the sixteenth doses.

## Stability and Homogeneity

The stability was confirmed as follows:

Parameters	Analyte	Concentration	Period or conditions	Study number
Long-term stock solution	Chlorthalidone	1 mg/mL	30 days from 2°C to 8°C	JCL072061 (Final report amendment-1)
Long-term standard solution	Chlorthalidone	5 ng/mL	30 days from 2°C to 8°C	
Long-term stock solution of concomitant drug	TAK-491F	100 µg/mL	90 days at 5±4°C	JCL041441 (Final report amendment-1)
	TAK-536			
	TAK-536 M-I TAK-536 M-II	100 µg/mL	212 days from 2°C to 8°C	JCL041441 (Final report amendment-2)
Long-term concomitant drug solution (Mixed solution of TAK-491F, TAK-536, TAK-536 M-I, and TAK-536 M-II)	TAK-491F TAK-536 TAK-536 M-I TAK-536 M-II	4 ng/mL 10 µg/mL	92 days from 2°C to 8°C	JCL042032
Short-term stock solution of concomitant drug	TAK-491F TAK-536 TAK-536 M-I TAK-536 M-II	100 µg/mL	6 hours at room temperature (approx. 25°C)	JCL042031
Short-term concomitant drug solution (Mixed solution of TAK-491F, TAK-536, TAK-536 M-I, and TAK-536 M-II)	TAK-491F TAK-536 TAK-536 M-I TAK-536 M-II	4 ng/mL 10 µg/mL		

Summary Table

Animals		F344/Jel Rats, 6 weeks of age										
Test article	Control <sup>1)</sup>	TAK-491	TAK-536 M-II	CLD		TAK-491/CLD		TAK-491/CLD		TAK-536 M-II/CLD		
Dosage level (mg/kg/day)	0	1000 <sup>2)</sup>	2000	100	300	100 <sup>2)</sup> /100	100 <sup>2)</sup> /300	1000 <sup>2)</sup> /100	1000 <sup>2)</sup> /300	2000 /100	2000 /300	
Dosage volume (mL/kg/day)	10	10	10	10	10	10	10	10	10	10	10	
Number of animals (M:F)	4:4	4:4 <sup>3)</sup>	4:4 <sup>3)</sup>	4:4 <sup>3)</sup>	4:4 <sup>3)4)</sup>	4:4 <sup>3)</sup>	4:4 <sup>3)</sup>	4:4 <sup>3)</sup>	4:4 <sup>3)</sup>	4:4 <sup>3)</sup>	4:4 <sup>3)</sup>	
Mortality (M:F)	0:0	0:0	0:0	0:0	0:0	0:0	0:0	0:0	0:0	0:0	0:0	
Clinical signs	-	-	-	-	-	-	-	-	-	-	-	
Body weights	-	-	-	-	-	-	-	-	-	-	-	
Food consumption	-	↓	-	↓(Week1)		↓		↓		↓(Week1)		
Water intake	-	-	-	-	-	↑(M)		↑(M)		-	-	
Urine output	-	-	-	-	-	↑(M)	↑	↑		-	-	
Urinalysis (urinary test strip)	-	-	-	-	-	-	-	-	-	-	-	
Urine chemistry	K <sup>5)</sup>	-	-	↑(M)		-	↑(M)	↑(M)		-	↑(M)	
	Cl <sup>5)</sup>	-	-	-	-	-	-	-	-	-	↑(F)	
Hematology	RBC·Ht·Hb	-	↓(F)	-	-	↓		↓		-	-	
	WBC·Lymph	-	↑(F)	-	-	↑(F)		↑(F)		-	-	
Blood chemistry	UN	-	↑(M)	-	↑(M)	↑	↑↑	↑↑		-	↑(M)	
	Na	-	-	-	-	-	↓	↓		-	-	
	Cl	-	↑(F)	-	↓	↓(M)		↓(M)		↓	↓	
	K	-	-	-	↓(M)	↓	-	-	-	↓(M)	↓	
	TP·Alb	-	-	-	-	-	-	↑(F)	-	↑(F)	-	-
	TCho	-	-	-	-	-	↑(M)		↑(M)		-	-
	AST	-	↓(M)	-	-	-	↓(M)		↓(M)		-	-
	ALT	-	↓	-	-	-	↓(F)	↓	↓		-	-
CK	-	↓	-	-	-	↓		↓		-	-	

M: Male, F: Female, -: No abnormalities, ↑: Increase, ↑↑: Additive increase (enhancement by combination dosing)

↓: Decrease, CLD: Chlorthalidone

1) 0.5 w/v% methylcellulose solution containing 0.47 w/v% citric acid, 2) As TAK-491F (TAK-491 free acid)

3) Additional 4 animals/sex/group were used for toxicokinetics (plasma levels).

4) Additional 4 animals/sex were used for determination of blood CLD levels., 5) Creatinine ratio

Animals		F344/Jcl Rats, 6 weeks of age									
Test article	Control <sup>1)</sup>	TAK-491	TAK-536 M-II	CLD		TAK-491/CLD		TAK-491/CLD		TAK-536 M-II /CLD	
Dosage level (mg/kg/day)	0	1000 <sup>2)</sup>	2000	100	300	100 <sup>2)</sup> /100	100 <sup>2)</sup> /300	1000 <sup>2)</sup> /100	1000 <sup>2)</sup> /300	2000 /100	2000 /300
Dosage volume (mL/kg/day)	10	10	10	10	10	10	10	10	10	10	10
Number of animals (M:F)	4:4	4:4 <sup>3)</sup>	4:4 <sup>3)</sup>	4:4 <sup>3)</sup>	4:4 <sup>3)4)</sup>	4:4 <sup>3)</sup>	4:4 <sup>3)</sup>	4:4 <sup>3)</sup>	4:4 <sup>3)</sup>	4:4 <sup>3)</sup>	4:4 <sup>3)</sup>
Mortality (M:F)	0:0	0:0	0:0	0:0	0:0	0:0	0:0	0:0	0:0	0:0	0:0
Necropsy	-	-	-	-	-	-	-	-	-	-	-
Organ weights	Heart	-	↓	-	-	-	↓	-	↓	-	-
	Kidneys	-	-	-	↑(M)	-	↑(M)	↑	-	↑(M)	-
	Thymus	-	↓(M)	-	↓(M)	↓(M)	↓(M)	↓(M)	↓(M)	↓(M)	-
	Ovaries	-	-	-	-	-	↓	-	↓	-	-
Histopathology											
Kidney (M:F)	-	Hyper-trophy of JG cells <sup>5)</sup> (4:4)	-	Tubular basophilia		Hypertrophy of JG cells <sup>5)</sup>		Hypertrophy of JG cells <sup>5)</sup>		-	-
				(1:0)	(3:0)	(2:4)	(1:4)	(4:4)	(4:4)		
Adrenal gland (M:F)	-	Atrophy of zona glomerulosa (3:3)	-	-	-	Atrophy of zona glomerulosa		Atrophy of zona glomerulosa		-	-
						(2:4)	(2:4)	(3:4)	(4:4)		

M: Male, F: Female, -: No abnormalities, ↑: Increase, ↓: Decrease, CLD: Chlorthalidone  
 1) 0.5 w/v% methylcellulose solution containing 0.47 w/v% citric acid, 2) As TAK-491F (TAK-491 free acid)  
 3) Additional 4 animals/sex/group were used for toxicokinetics (plasma levels).  
 4) Additional 4 animals/sex were used for determination of blood CLD levels.  
 5) JG cells: Juxtaglomerular cells

Animals	F344/Jcl Rats, 6 weeks of age										
Test article	Control <sup>1)</sup>	TAK-491	TAK-536 M-II	CLD		TAK-491/CLD		TAK-491/CLD		TAK-536M-II /CLD	
Dosage level (mg/kg/day)	0	1000 <sup>2)</sup>	2000	100	300	100 <sup>2)</sup> /100	100 <sup>2)</sup> /300	1000 <sup>2)</sup> /100	1000 <sup>2)</sup> /300	2000/100	2000/300
Dosage volume (mL/kg/day)	10	10	10	10	10	10	10	10	10	10	10
Number of animals (M:F)	4:4	4:4 <sup>3)</sup>	4:4 <sup>3)</sup>	4:4 <sup>3)</sup>	4:4 <sup>3)4)</sup>	4:4 <sup>3)</sup>	4:4 <sup>3)</sup>	4:4 <sup>3)</sup>	4:4 <sup>3)</sup>	4:4 <sup>3)</sup>	4:4 <sup>3)</sup>
Toxicokinetics (Mean, n=2 for each sampling point)											
TAK-491F											
Tmax (h)	1st	NC	NA	NA	NA	NC	NC	NC	NC	NA	NA
	16th	NC	NA	NA	NA	NC	NC	NC	NC	NA	NA
Cmax (µg/mL)	1st	0:0	NA	NA	NA	0:0	0:0	0:0	0:0	NA	NA
	16th	0:0	NA	NA	NA	0:0	0:0	0:0	0:0	NA	NA
AUC <sub>0-24h</sub> (µg·h/mL)	1st	0:0	NA	NA	NA	0:0	0:0	0:0	0:0	NA	NA
	16th	0:0	NA	NA	NA	0:0	0:0	0:0	0:0	NA	NA
TAK-536											
Tmax (h)	1st	1.0:1.0	NA	NA	NA	1.0:1.0	1.0:1.0	2.0:1.0	2.0:1.0	NA	NA
	16th	2.0:1.0	NA	NA	NA	1.0:1.0	1.0:1.0	1.0:1.0	1.0:1.0	NA	NA
Cmax (µg/mL)	1st	316:325	NA	NA	NA	143:150	135: 94	246:289	279:319	NA	NA
	16th	354:322	NA	NA	NA	172:171	183:138	405:388	458:423	NA	NA
AUC <sub>0-24h</sub> (µg·h/mL)	1st	1840 :1541	NA	NA	NA	782 :701	754 :614	1549 :1486	1838 :1562	NA	NA
	16th	2291 :1664	NA	NA	NA	711 :686	969 :791	1683 :1985	1978 :2455	NA	NA
TAK-536 M-I											
Tmax (h)	1st	1.0:1.0	NA	NA	NA	1.0:2.0	1.0:2.0	2.0:2.0	8.0:1.0	NA	NA
	16th	2.0:4.0	NA	NA	NA	2.0:4.0	1.0:1.0	2.0:2.0	4.0:4.0	NA	NA
Cmax (µg/mL)	1st	1.44:1.16	NA	NA	NA	0.66:0.85	0.59:2.61	1.52:1.11	0.86:1.12	NA	NA
	16th	1.08:0.90	NA	NA	NA	0.98:0.93	0.39:1.15	0.90:1.37	0.83:3.90	NA	NA
AUC <sub>0-24h</sub> (µg·h/mL)	1st	8.50 :10.69	NA	NA	NA	9.13 :5.89	5.37 :11.92	10.73 :7.41	14.05 :7.12	NA	NA
	16th	13.22 :8.33	NA	NA	NA	5.62 :8.11	3.93 :5.50	6.19 :11.05	9.13 :29.10	NA	NA
TAK-536 M-II											
Tmax (h)	1st	1.0:1.0	2.0:2.0	NA	NA	1.0:1.0	2.0:1.0	1.0:1.0	2.0:1.0	2.0:2.0	4.0:2.0
	16th	1.0:1.0	2.0:1.0	NA	NA	1.0:1.0	1.0:1.0	1.0:1.0	1.0:1.0	2.0:1.0	2.0:2.0
Cmax (µg/mL)	1st	0.48 :0.41	10.17 :9.77	NA	NA	0.20 :0.18	0.15 :0.14	0.36 :0.43	0.48 :0.52	11.63 :10.40	9.77 :11.02
	16th	0.35:0.35	6.66:5.61	NA	NA	0.18:0.21	0.23:0.62	0.84:1.30	0.93:1.39	4.97:5.45	6.06:6.07
AUC <sub>0-24h</sub> (µg·h/mL)	1st	2.1:1.7	41.6:57.0	NA	NA	0.8:0.8	0.8:0.7	1.9:2.2	3.0:2.3	61.1:46.3	61.9:94.9
	16th	2.1:1.2	32.4:23.5	NA	NA	0.8:0.9	1.0:3.0	5.4:6.3	5.6:7.2	26.9:22.2	34.3:34.5
CLD											
Tmax (h)	1st	NA	NA	2.0:2.0	4.0:2.0	2.0:2.0	2.0:2.0	4.0:4.0	2.0:2.0	2.0:2.0	1.0:2.0
	16th	NA	NA	2.0:2.0	2.0:2.0	2.0:1.0	2.0:2.0	2.0:2.0	2.0:1.0	2.0:1.0	2.0:2.0
Cmax (µg/mL)	1st	NA	NA	0.99:1.01	1.65:1.83	2.84:2.29	3.84:3.00	2.24:2.24	3.86:4.37	1.36:1.43	2.13:1.68
	16th	NA	NA	1.09:1.34	1.58:2.08	2.41:2.71	3.62:3.21	2.15:2.77	4.43:4.62	0.62:0.84	0.91:1.18
AUC <sub>0-24h</sub> (µg·h/mL)	1st	NA	NA	5.3:8.0	20.6:17.0	20.6:16.2	26.3:28.9	21.6:25.0	44.1:49.7	7.1:6.5	12.2:22.1
	16th	NA	NA	6.5:7.7	15.1:11.4	22.1:21.3	37.9:32.1	26.9:36.9	51.6:43.1	4.7:3.8	5.4:7.6

M: Male, F: Female, NC: Not calculated, NA: Not applicable, CLD: Chlorthalidone

1) 0.5 w/v% methylcellulose solution containing 0.47 w/v% citric acid, 1) As TAK-491F (TAK-491 free acid), 3) Additional 4 animals/sex/group were used for toxicokinetics., 4) Additional 4 animals/sex were used for determination of blood CLD levels (1st dose; Tmax: 2.0/2.0h, Cmax: 10.74/10.15 µg/mL, AUC<sub>0-24h</sub>: 154.4/163.1 µg·h/mL, 16th dose; Tmax: 2.0/1.0h, Cmax: 8.13/10.68 µg/mL, AUC<sub>0-24h</sub>: 144.0/171.3 µg·h/mL, M/F).

Animals	F344/Jcl Rats, 6 weeks of age										
Test article	Control <sup>1)</sup>	TAK-491	TAK-536 M-II	CLD		TAK-491/CLD		TAK-491/CLD		TAK-536M-II /CLD	
Dosage level (mg/kg/day)	0	1000 <sup>2)</sup>	2000	100	300	100 <sup>2)</sup> /100	100 <sup>2)</sup> /300	1000 <sup>2)</sup> /100	1000 <sup>2)</sup> /300	2000/100	2000/300
Dosage volume (mL/kg/day)	10	10	10	10	10	10	10	10	10	10	10
Number of animals (M:F)	4:4	4:4 <sup>3)</sup>	4:4 <sup>3)</sup>	4:4 <sup>3)</sup>	4:4 <sup>3)4)</sup>	4:4 <sup>3)</sup>	4:4 <sup>3)</sup>	4:4 <sup>3)</sup>	4:4 <sup>3)</sup>	4:4 <sup>3)</sup>	4:4 <sup>3)</sup>
Conclusion	Plasma chlorthalidone levels increased by dosing in combination with TAK-491, and an additive increase in plasma urea nitrogen and increases in water intake, urine output and plasma total cholesterol were observed in the TAK-491/chlorthalidone combination groups but were not accompanied by any toxicologically significant findings. No clear combination effects were observed from dosing the combination of TAK-536 M-II and chlorthalidone.										

M: Male, F: Female, CLD: Chlorthalidone

1) 0.5 w/v% methylcellulose solution containing 0.47 w/v% citric acid, 1) As TAK-491F (TAK-491 free acid), 3) Additional 4 animals/sex/group were used for toxicokinetics., 4) Additional 4 animals/sex were used for determination of blood CLD levels.

### 6.2.1 Repeat-Dose Toxicity- Second Study

Study title: Thirteen-week oral gavage toxicity study of TAK-491 plus TAK-536 M-II plus chlorthalidone (triple combination) in rats

Study no.: B-6429  
 Study report location: electronic  
 Conducting laboratory and location: Developmental Research Center; Takeda Pharmaceutical Company, Ltd., Osaka, Japan  
 Date of study initiation: 04/22/08  
 GLP compliance: yes  
 QA statement: yes  
 Drug, lot #, and % purity: TAK-491, lot # M491-020; 99.9%  
 TAK-536 MII, lot # M117-M1006; 99.8%  
 Chlorthalidone, lot # MA06-005, 99.8%

### Key Study Findings

TAK-491 is the pro-drug for the active drug, TAK-536. In humans, the major metabolite of TAK-536 is TAK-536 MII. The double combination administered in this study contains the prodrug TAK-491 with TAK-536 MII. Two groups of rats received this double combination: One group received 100 mg/kg of TAK-491 with 2000 mg/kg of MII and the second group received 1000 mg/kg TAK-491 with 2000 mg/kg of MII. In this second group, there was a significant decrease in body weight gain and food consumption. This effect was enhanced in all groups receiving the triple combination containing chlorthalidone. Chlorthalidone given by itself at one dose of 300 mg/kg increased BUN and adrenal weight. It also increased the incidence and severity of background renal tubular regeneration. All these effects were enhanced by co-administration with the double combination (TAK-491 and TAK-536 MII). The increase in kidney weight seen with chlorthalidone alone was similar to that seen in the triple combination. APTT

prolongation, decreased potassium and increase in incidence and severity of renal cortical mineralization attributable to chlorthalidone were not observed by co-administration with TAK-491 and TAK-536 MII. Increased plasma creatinine, and neutrophil count, decreased uterine weight, hypertrophy of the adrenal zona fasciculata and dilatation of the renal Henle's ascending tubules with dose-dependency of TAK-491 and chlorthalidone in its incidence were observed only in the triple combination group in which plasma chlorthalidone levels were increased compared to the cohort receiving chlorthalidone *per se*.

#### Methods

Doses: See below  
Frequency of dosing: Once/day for 13 weeks  
Route of administration: Oral gavage  
Dose volume: 10 ml  
Formulation/Vehicle: Agents were dissolved in 0.5 w/v% methylcellulose solution containing 0.5 w/v% citric acid.  
Species/Strain: Rat/ F344/Jcl  
Number/Sex/Group: 10  
Age: 6 weeks  
Weight: 99- 129 g males; 89- 111 g females  
Satellite groups: 10 for TK analysis  
Unique study design: None  
Deviation from study protocol: None

Test group	Dosage level <sup>a, b)</sup> (mg/kg/day)	Concentration <sup>a, b)</sup> (mg/mL)	Dosage volume (mL/kg/day)	Sex	Main group		Satellite group	
					No. of animals	Animal No.	No. of animals	Animal No.
Control*	0/0/0	0/0/0	10	M	10	1001-1010	10	1201-1210
				F	10	1101-1110	10	1301-1310
TAK-491 /TAK-536 M-II	100/2000/0	10/200/0	10	M	10	2001-2010	10	2201-2210
	1000/2000/0	100/200/0	10	F	10	2101-2110	10	2301-2310
CLD	0/0/300	0/0/30	10	M	10	3001-3010	10	3201-3210
				F	10	3101-3110	10	3301-3310
TAK-491 /TAK-536 M-II /CLD <sup>c)</sup>	100/2000/100	10/200/10	10	M	10	4001-4010	10	4201-4210
	100/2000/300	10/200/30	10	F	10	4101-4110	10	4301-4310
	1000/2000/300	100/200/30	10	M	10	5001-5010	10	5201-5210
				F	10	5101-5110	10	5301-5310
	1000/2000/300	100/200/30	10	M	10	6001-6010	10	6201-6210
				F	10	6101-6110	10	6301-6310
	1000/2000/300	100/200/30	10	M	10	7001-7010	10	7201-7210
				F	10	7101-7110	10	7301-7310

\*: 0.5 w/v% MC solution containing 0.5 w/v% citric acid

CLD: Chlorthalidone, M: Male, F: Female

a): Dosage or concentration of TAK-491 indicated as TAK-491F, TAK-491 free acid

b): TAK-491/TAK-536 M-II/Chlorthalidone

c): Triple combination

## Observations and Results

### Mortality

Mortality was monitored 3X a day (before dosing, immediately after and 2 hr after dosing). No animals died in any treatment group.

### Clinical Signs

Clinical signs were monitored 3X a day (before dosing, immediately after and 2 hr after dosing). No abnormal clinical signs were noted in any treatment group.

### Body Weights/ Feed Consumption

Body weight was measured 3X in week 1 and thereafter 2X/ week. Food consumption was measured twice: on days 1 and 7 and then at 7 day intervals.

Body weight gain and food consumption were suppressed in both sexes in the double combination group. These effects were considered to be a result of the pharmacological effects of the drugs. There was no suppression in body weight gain or food consumption in the chlorthalidone group. In the triple combination groups, there were also decreases in body weight gain and food consumption. In males at the 1000/2000/300 dose, these effects were accompanied by increased adrenal weight and hypertrophy of the zona fasciculata suggesting stress. These changes at this dose in males were judged to be toxic.

## Ophthalmoscopy

Ophthalmological examinations were performed before and at month 3 of dosing. There were no treatment-related effects in either sex.

## ECG

Not analyzed.

## Hematology

Hematological analysis was tested at necropsy the day after the last dosing. Results: In the double combination groups, there were decreases in red blood cell count, hemoglobin concentration, hematocrit values, and reticulocyte ratio; and there were increases in MCV and MCH values in one or both sexes. The effects on the erythrocyte parameters are due to the effect of the TAK-536 on the hematopoietic system. The increase in leukocyte parameters and white blood cells was due most likely to stomach erosion that was seen in the histopathology. In the chlorthalidone group, increases in hemoglobin concentration and MCHC values were seen. Decreases in hematocrit and MCV values were observed in one or both sexes. A prolongation of APTT was observed in males. In the triple combination group, decreases in red blood cell count, hemoglobin concentrations, hematocrit, and reticulocytes and decreases in MCV, MCH and MCHC values were exacerbated by co-administration with chlorthalidone but were not judged to be toxicologically significant. Increases in white blood cell counts, lymphocytes and neutrophil counts were seen in the triple combination groups in both sexes. An increase in platelet count was enhanced by co-administration with chlorthalidone but was not judged to be of toxicological significance. Shortening of PT and APTT without toxicological significance were observed in one or both sexes.

## Clinical Chemistry

Blood was collected at necropsy by abdominal aortic puncture the day after the last dosing.

Results: In the double combination groups, there was an increase in BUN in both sexes. This was believed to result from the pharmacological effects of the drugs. There was a decrease in plasma sodium concentration and an increase in plasma potassium concentration. These effects were not judged to be toxicologically significant. In the chlorthalidone group, an increase in BUN and decreases in potassium, sodium, and chloride values were seen in one or both sexes. Only the effect on BUN was believed to be toxic. In the triple combination groups, chlorthalidone enhanced the increase in BUN seen in the double combination groups. Also, an increase in creatinine was seen in both sexes receiving all three drugs. These effects were toxic because there was an increase in the incidence and severity of histopathological renal tubular regeneration observed in the triple combination groups.

## Urinalysis and Water Intake

Urinalysis (including water intake) was performed in month 3.

Results: In the double combination group, decreases in osmolality and output of sodium, chloride and potassium were observed in one or both sexes. These effects on urinalysis were not judged to be toxic. In the chlorthalidone group, no adverse effects were observed in the urinalysis. In the triple combination groups, effects on urine osmolality and electrolyte excretion were seen like in the double combination groups, but were enhanced by chlorthalidone. With chlorthalidone, water intake increased, urine volume increased and urinary pH increased. These effects were not judged to be toxic, but just related to the pharmacological action of the drugs.

## Gross Pathology

Sponsor asserts that a careful examination of all organs/tissues in the cephalic, thoracic and abdominal cavities was made at necropsy.

Results: In the double combination groups, there was an increased incidence of dark red foci in the glandular stomach which was enhanced by chlorthalidone in the triple combination groups. Chlorthalidone alone did not demonstrate any changes at necropsy in either sex.

## Organ Weights

The brain, pituitary, thyroids, adrenals, thymus, spleen, heart, lungs (inc. bronchi), salivary glands, liver, kidneys, testes, prostate, seminal vesicles, ovaries and uterus were weighed.

Results: In the double combination groups, increased adrenal weight in males and decreased heart weight in both sexes was observed. The effect on the adrenals was judged to be toxic. In the chlorthalidone group, increased kidney weight was seen in both sexes. This effect was considered toxic. In the triple combination group, increased adrenal weight and decreased heart weight were observed as seen in the double combination group, and increased kidney and adrenal weights were observed as seen in the chlorthalidone group. The increased adrenal weight was enhanced by co-administration with TAK-491/TAK-536 MII and judged to be toxic since histopathological examination revealed hypertrophy of the zona fasciculata.

## Histopathology

### Adequate Battery

Tissues were examined microscopically for any histopathology. The organs listed were analyzed histologically along with cerebrum, cerebellum, spinal cord (cervical, thoracic and lumbar), sciatic nerves, eyeballs, optic nerves, Harderian glands, pituitary, thyroids, parathyroids, submandibular lymph node, mesenteric lymph node, thoracic aorta, trachea, tongue, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, submandibular

and sublingual glands, parotid glands, pancreas, urinary bladder, epididymides, vagina, mammary glands, sternum, femurs, femoral skeletal muscles, skin (inguinal region, both sides) and gross lesions. Lastly, oviducts, extraorbital lacrimal glands, Zymbal's glands, larynx, nasal cavity and site of animal identification (ear auricle) were preserved.

#### Peer Review

Yes.

#### Histological Findings

In the double combination groups, treatment-related changes were observed in the stomach, kidney and adrenals. There was an increased incidence and/or severity of erosion in the glandular stomach in both sexes. Hypertrophy of the juxtaglomerular cells and medial hypertrophy of the arterial wall in the kidney with dose-dependency of TAK-491 in their severity were observed in both sexes, and were not considered to be toxic. Atrophy of the zona glomerulosa of the adrenal was not considered to be a toxic effect. In the chlorthalidone group, treatment was associated with an increased incidence and severity of background tubular regeneration and induced corticomedullary mineralization *de novo*. These, along with hypertrophy of the glomerulosa cells in the adrenal, were considered to be related to drug effect and not toxic manifestations. In the triple combination groups, increased incidence and/or severity of erosion in the glandular stomach, hypertrophy of the juxtaglomerular cells and medial hypertrophy of the arterial wall in the kidney, and atrophy of the zona glomerulosa in the adrenal were observed in both sexes in all triple combination groups. Lesion severity depended on the dose of TAK-491, as seen in the double combination groups. Increased incidence and severity of renal tubular regeneration was observed as seen in the chlorthalidone group, but the finding in the 1000/2000/300 mg/kg group was more severe than that in any other triple combination group and chlorthalidone group. Hypertrophy of the zona fasciculata in the adrenal was also observed in males. Dilatation of the renal Henle's ascending tubules was observed in some animals and is most likely related to the pharmacological action of chlorthalidone. The reason that this effect was only seen in the triple combination groups is most likely due to the fact that plasma chlorthalidone levels are increased when combined with the TAK compounds. These effects were not associated with any regressive inflammatory changes and deemed not to be toxic.

#### Special Evaluation

None

## Toxicokinetics

Plasma concentrations ( $T_{max}$ ,  $C_{max}$  and  $AUC_{0-24h}$ ) of the compound were measured in the satellite groups after the first dose at 0.5, 1, 2, 4, 8 and 24 hr. Measurements were also made on day 90 of week 13 before, and 0.5, 1, 2, 4, 8 and 24 hr after dosing.

Results: The  $T_{max}$  values for TAK-536, TAK-536 MI and TAK-536 MII ranged from 0.5 to 2.0 hr. The  $C_{max}$  and  $AUC_{0-24h}$  values for TAK-536 and MI increased with increment of TAK-491 dosage levels but not of MII dosage levels. The  $C_{max}$  and  $AUC_{0-24h}$  values for TAK-536, MI and MII in each triple combination group were comparable with those in the double combination group at the same TAK-491 level. The  $C_{max}$  and  $AUC_{0-24h}$  values for TAK-536, MI and MII were not affected by repeated doses.

The  $T_{max}$  values for chlorthalidone ranged from 1-4 hr. The  $C_{max}$  and  $AUC_{0-24h}$  values for chlorthalidone increased when co-administered with TAK-491/TAK-536 MII, i.e., the values for chlorthalidone in the triple combination groups including the 100/2000/100 mg/kg group were higher than those in the 0/0/300 mg/kg group. The  $C_{max}$  and  $AUC_{0-24h}$  values in the triple combinations groups increased with increment of chlorthalidone. There were no apparent changes in the  $C_{max}$  or  $AUC_{0-24h}$  values for chlorthalidone during repeated administration.

## Stability and Homogeneity

### Stability and homogeneity of TAK-491/TAK-536 M-II/CLD in the vehicle

It was confirmed by (b) (4) that TAK-491/TAK-536 M-II/CLD (Lot No. M491-020/M117-M1006/MA06-005) 10/200/0, 100/200/0, 0/0/30, 10/200/10, 10/200/30 and 100/200/30 mg/mL suspensions (vehicle: 0.5 w/v% MC solution containing 0.05 or 0.5 w/v% citric acid) were stable and homogeneous in a brown glass bottle for 24 hours at room temperature following 4 or 8 days in a refrigerator

**Thirteen-week oral gavage toxicity study of TAK-491 plus TAK-536 M-II plus chlorthalidone (triple combination) in rats (B-6429) (1/5)**

Animal	F344/Jcl rats, 6 weeks of age						
Test article	Control <sup>a)</sup>	TAK-491 /TAK-536 M-II		Chlor- thalidone	TAK-491/TAK-536 M-II /Chlorthalidone		
Dosage level <sup>b, c)</sup> (mg/kg/day)	0/0/0	100/2000 /0	1000/2000 /0	0/0/300	100/2000 /100	100/2000 /300	1000/2000 /300
Dosage volume (mL/kg/day)	10	10	10	10	10	10	10
No. of animals (M:F)	10:10 <sup>d)</sup>	10:10	10:10	10:10	10:10	10:10	10:10
Mortality (M:F)	0:0	0:0	0:0	0:0	0:0	0:0	0:0
Clinical signs	-	-	-	-	-	-	-
Body weight	-	-	↓	-	↓ (M)	↓	↓ (M*#, F)
Food consumption	-	-	↓	-	↓	↓	↓ (M*#, F)
Ophthalmology	-	-	-	-	-	-	-
Urinalysis (including water intake)							
pH	-	-	-	-	↑ (F)	↑ (F)	↑ (F)
Urine volume	-	-	-	-	-	↑ (M)	↑ (M)
Water intake	-	-	-	-	↑ (M)	↑ (M)	↑ (M)
Osmolality	-	↓ (M)	↓ (M)	-	↓ (M*)	↓ (M*, F)	↓ (M*, F)
Na	-	↓	↓	-	↓	↓	↓ (M, F*)
K	-	-	↓ (M)	-	↓ (F)	↓ (F)	↓
Cl	-	↓ (M)	↓	-	↓	↓	↓ (M, F*)
Hematology							
RBC	-	↓	↓	-	↓	↓	↓
HGB	-	↓	↓	↑ (M)	↓	↓	↓
HCT	-	↓	↓	↓ (F)	↓	↓ (M, F**)	↓ (M, F**)
MCV	-	↑	↑	↓ (F)	↑ (M*)	↑ (M*)	↑ (M*, F)
MCH	-	↑ (M)	↑	-	↑ (M*, F)	↑ (M*, F)	↑*
MCHC	-	-	-	↑	↑ (F)	↑ (F)	↑ (F)
Reticul	-	-	↓ (F)	-	↓ (M)	↓	↓ (M, F*)
WBC	-	-	↑#	-	↑ (F)#	↑ (F)#	↑#
Lymph	-	-	↑#	-	↑ (F)#	↑ (F)#	↑ (F)#
Neut	-	-	-	-	-	-	↑#
PLT	-	↑	↑	-	↑ (F)	↑ (F)	↑ (M*, F)
PT	-	-	-	-	↓ (M)	↓ (M)	↓
APTT	-	-	-	↑ (M)#	↓	↓	↓

a): 0.5 w/v% methylcellulose solution containing 0.5 w/v% citric acid, b): Dosage of TAK-491 indicated as TAK-491F (TAK-491 free acid, conversion factor: 1.067), c): TAK-491/TAK-536 M-II/Chlorthalidone,

d): Additional 10 animals/sex/group were used as satellite groups for toxicokinetics including the control group.

M: Male, F: Female, -: No treatment-related effects, ↑: Increase, ↓: Decrease, #: Toxicologically significant change

\*: Enhancement of effect attributed to TAK-491/TAK-536 M-II by co-administration with chlorthalidone

\*\* : Enhancement of effect attributed to chlorthalidone by co-administration with TAK-491/TAK-536 M-II

**Thirteen-week oral gavage toxicity study of TAK-491 plus TAK-536 M-II plus chlorthalidone (triple combination) in rats (B-6429) (2/5)**

Animal	F344/Jcl rats, 6 weeks of age						
Test article	Control <sup>a)</sup>	TAK-491 /TAK-536 M-II		Chlor- thalidone	TAK-491/TAK-536 M-II /Chlorthalidone		
Dosage level <sup>b, c)</sup> (mg/kg/day)	0/0/0	100/2000 /0	1000/2000 /0	0/0/300	100/2000 /100	100/2000 /300	1000/2000 /300
Dosage volume (mL/kg/day)	10	10	10	10	10	10	10
No. of animals (M:F)	10:10 <sup>d)</sup>	10:10	10:10	10:10	10:10	10:10	10:10
Mortality (M:F)	0:0	0:0	0:0	0:0	0:0	0:0	0:0
<b>Blood chemistry</b>							
AST	-	↓	↓	-	-	↓	↓ (M, F*)
ALT	-	↓	↓	-	↓	↓ (M*, F)	↓*
LDH	-	-	↓	-	↓ (M)	↓ (M)	↓
CK	-	-	↑ (F)	↓ (F)	-	↓ (F)	↓ (F)
AIP	-	-	-	-	↑ (M)	↑	↑
T-CHO	-	-	↓ (F)	-	-	↑ (M)	↑ (M)
TG	-	↑ (F)	↑ (F)	-	↓ (M)	↓ (M)	↓ (M)
GLU	-	-	-	-	-	↑ (F)	↑ (F)
BUN	-	↑	↑	↑#	↑**	↑***#	↑***#
CRNN	-	-	-	-	↑#	↑#	↑#
Na	-	↓ (M)	↓	↓ (M)	↓	↓ (M**, F)	↓ (M**, F*)
K	-	↑ (M)	↑	↓#	-	↑ (F)	↑
Cl	-	-	-	↓	↓	↓	↓
Ca	-	-	↓ (M)	↑ (F)	↓ (M)	↓ (M)	↓ (M)
P	-	-	-	-	-	-	↑ (F)
TP	-	↓	↓	-	↓ (M)	↓ (M)	↓
ALB	-	↓	↓	↑ (F)	-	-	-
A/G	-	-	↑	-	↑ (M)	↑ (M)	↑ (M*, F)
<b>Organ weights</b>							
Thyroid	-	-	-	↑ (M)	-	-	-
Thymus	-	-	↓ (M)	↑ (F)	-	-	↓ (M)
Heart	-	↓ (F)	↓	-	↓	↓	↓
Spleen	-	-	↓ (M)	-	-	-	-
Liver	-	-	-	↑ (F)	-	-	-
Kidney	-	-	-	↑#	↑ (F)#	↑ (F)#	↑ (F)#
Adrenal	-	-	↑ (M)#	↑ (M)	-	-	↑ (M**)#
Uterus	-	-	-	-	-	↓ (F)#	↓ (F)#

a): 0.5 w/v% methylcellulose solution containing 0.5 w/v% citric acid, b): Dosage of TAK-491 indicated as TAK-491F (TAK-491 free acid, conversion factor: 1.067), c): TAK-491/TAK-536 M-II/Chlorthalidone,

d): Additional 10 animals/sex/group were used as satellite groups for toxicokinetics including the control group.

M: Male, F: Female, -: No treatment-related effects, ↑: Increase, ↓: Decrease, #: Toxicologically significant change

\*: Enhancement of effect attributed to TAK-491/TAK-536 M-II by co-administration with chlorthalidone

\*\* : Enhancement of effect attributed to chlorthalidone by co-administration with TAK-491/TAK-536 M-II

\*\*\*: Concomitant enhancement by co-administration of TAK-491/TAK-536 M-II and chlorthalidone

**Thirteen-week oral gavage toxicity study of TAK-491 plus TAK-536 M-II plus chlorthalidone (triple combination) in rats (B-6429) (3/5)**

Animal		F344/Jcl rats, 6 weeks of age						
Test article		Cont- rol <sup>a)</sup>	TAK-491 /TAK-536 M-II		Chlor- thalidone	TAK-491/TAK-536 M-II /Chlorthalidone		
Dosage level <sup>b, c)</sup> (mg/kg/day)		0/0/0	100/2000 /0	1000/2000 /0	0/0/300	100/2000 /100	100/2000 /300	1000/2000 /300
Dosage volume (mL/kg/day)		10	10	10	10	10	10	10
No. of animals (M:F)		10:10 <sup>d)</sup>	10:10	10:10	10:10	10:10	10:10	10:10
Mortality (M:F)		0:0	0:0	0:0	0:0	0:0	0:0	0:0
Gross pathology								
Stomach	Dark red focus in glandular stomach	1:1	1:1	8#:4#	0:0	1:1	1:0	6#:2#
Histopathology								
Stomach	Erosion in glandular stomach (±)	2:1	1:0	4#:4#	0:0	2:1	1:0	2#:1#
	(+)	0:0	0:0	3#:0	0:0	0:0	0:0	3#:1#
Adrenal	Atrophy of zona glomerulosa (±)	0:0	8:10	3:8	0:0	7:10	9:10	3:8
	(+)	0:0	2:0	7:2	0:0	3:0	1:0	7:2
	Hypertrophy of glomerulosa cell (±)	0:0	0:0	0:0	6:7	0:0	0:0	0:0
	Hypertrophy of zona fasciculata (±)	0:0	0:0	0:0	0:0	0:0	0:0	5#:0
Kidney	Tubular regeneration (±)	7:1	6:1	9:1	8:5#	8:5#	7:6#	4:8#
	(+)	0:0	0:0	0:0	2#:0	2#:0	2#:0	5#:0
	Corticomedullary mineralization (±)	0:10	1:7	1:6	8#:2	0:1	0:1	0:0
	(+)	0:0	0:0	0:0	0:8#	0:0	0:0	0:0
	Hypertrophy of juxtglomerular cell (±)	0:0	8:4	9:7	0:0	9:4	8:5	7:8
	(+)	0:0	0:0	1:0	0:0	0:0	1:0	3:0
	Medial hypertrophy of arterial wall (±)	0:0	10:5	4:10	0:0	10:9	10:9	3:8
(+)	0:0	0:0	6:0	0:0	0:0	0:1	7:2	
Dilatation of Henle's ascending tubule (±)	0:0	0:0	0:0	0:0	0:1#	1#:3#	1#:7#	

a): 0.5 w/v% methylcellulose solution containing 0.5 w/v% citric acid, b): Dosage of TAK-491 indicated as TAK-491F (TAK-491 free acid, conversion factor: 1.067), c): TAK-491/TAK-536 M-II/Chlorthalidone,

d): Additional 10 animals/sex/group were used as satellite groups for toxicokinetics including the control group.

M: Male, F: Female, #: Toxicologically significant change, ±: Minimal, +: Mild

**Thirteen-week oral gavage toxicity study of TAK-491 plus TAK-536 M-II plus chlorthalidone (triple combination) in rats (B-6429) (4/5)**

Animal	F344/Jcl rats, 6 weeks of age						
Test article	Control <sup>a)</sup>	TAK-491 /TAK-536 M-II		Chlor- thalidone	TAK-491/TAK-536 M-II /Chlorthalidone		
Dosage level <sup>b, c)</sup> (mg/kg/day)	0/0/0	100/2000 /0	1000/2000 /0	0/0/300	100/2000 /100	100/2000 /300	1000/2000 /300
Dosage volume (mL/kg/day)	10	10	10	10	10	10	10
No. of animals (M:F)	10:10 <sup>d)</sup>	10:10	10:10	10:10	10:10	10:10	10:10
Mortality (M:F)	0:0	0:0	0:0	0:0	0:0	0:0	0:0
<b>Toxicokinetic parameters (n=3, mean, M:F)</b>							
<b>TAK-491F</b>							
$T_{max}$ (h)	Day 1	NC:NC	NC:NC	NE	NC:NC	NC:NC	NC:NC
	Week 13	NC:NC	NC:NC	NE	NC:NC	NC:NC	NC:NC
$C_{max}$ (ng/mL)	Day 1	0:0	0:0	NE	0:0	0:0	0:0
	Week 13	0:0	0:0	NE	0:0	0:0	0:0
$AUC_{0-24h}$ (ng-h/mL)	Day 1	0:0	0:0	NE	0:0	0:0	0:0
	Week 13	0:0	0:0	NE	0:0	0:0	0:0
<b>TAK-536</b>							
$T_{max}$ (h)	Day 1	1.0:1.0	1.0:0.5	NE	0.5:1.0	1.0:1.0	1.0:1.0
	Week 13	0.5:0.5	1.0:1.0	NE	1.0:0.5	0.5:1.0	1.0:1.0
$C_{max}$ (ng/mL)	Day 1	179916 :174770	341195 :260352	NE	174827 :187357	175193 :202567	305395 :320443
	Week 13	215833 :209458	384894 :394192	NE	211760 :210450	168112 :216434	319147 :407104
$AUC_{0-24h}$ (ng-h/mL)	Day 1	1020017 :915171	2053248 :1530491	NE	1124931 :1051464	1191813 :1047078	2367111 :1839304
	Week 13	1196001 :964239	2035731 :1778745	NE	1154941 :1036028	1273600 :1046577	1933390 :1878036
<b>TAK-536 M-I</b>							
$T_{max}$ (h)	Day 1	1.0:1.0	1.0:1.0	NE	2.0:2.0	1.0:2.0	1.0:2.0
	Week 13	1.0:0.5	1.0:1.0	NE	2.0:0.5	0.5:1.0	2.0:1.0
$C_{max}$ (ng/mL)	Day 1	1262 :12057	2229 :23551	NE	1235 :1443	1049 :1990	2052 :3560
	Week 13	2083 :2081	3113 :3756	NE	1476 :1221	1692 :1831	7432 :2088
$AUC_{0-24h}$ (ng-h/mL)	Day 1	8906 :17256	15658 :30631	NE	11120 :12272	10570 :13900	18213 :20922
	Week 13	17521 :11216	24540 :19970	NE	13749 :12229	14522 :13892	30109 :20409

a): 0.5 w/v% methylcellulose solution containing 0.5 w/v% citric acid, b): Dosage of TAK-491 indicated as TAK-491F (TAK-491 free acid, conversion factor: 1.067), c): TAK-491/TAK-536 M-II/Chlorthalidone, d): Additional 10 animals/sex/group were used as satellite groups for toxicokinetics including the control group. M: Male, F: Female, NC: Not calculated, NE: Not examined, Determination of plasma drug concentrations was also conducted on the control group (2 hours after dosing on day 1 and in week 13), and the concentrations of all analytes were less than the quantification limit (5 ng/mL for TAK-491F, TAK-536, TAK-536 M-I and TAK-536 M-II, 0.05 µg/mL for chlorthalidone).

**Thirteen-week oral gavage toxicity study of TAK-491 plus TAK-536 M-II plus chlorthalidone (triple combination) in rats (B-6429) (5/5)**

Animal	F344/Jcl rats, 6 weeks of age						
Test article	Control <sup>a)</sup>	TAK-491 /TAK-536 M-II		Chlor- thalidone	TAK-491/TAK-536 M-II /Chlorthalidone		
Dosage level <sup>b, c)</sup> (mg/kg/day)	0/0/0	100/2000 /0	1000/2000 /0	0/0/300	100/2000 /100	100/2000 /300	1000/2000 /300
Dosage volume (mL/kg/day)	10	10	10	10	10	10	10
No. of animals (M:F)	10:10 <sup>d)</sup>	10:10	10:10	10:10	10:10	10:10	10:10
Mortality (M:F)	0:0	0:0	0:0	0:0	0:0	0:0	0:0
Toxicokinetic parameters (n=3, mean, M:F)							
<b>TAK-536 M-II</b>							
T <sub>max</sub> (h)	Day 1	1.0:1.0	1.0:1.0	NE	1.0:1.0	2.0:1.0	2.0:1.0
	Week 13	2.0:1.0	2.0:1.0	NE	1.0:1.0	2.0:1.0	2.0:1.0
C <sub>max</sub> (ng/mL)	Day 1	13216 :12058	16795 :12561	NE	14001 :12178	14164 :13209	15344 :20662
	Week 13	16242 :12823	17706 :16461	NE	12396 :14558	13914 :14503	15433 :17086
AUC <sub>0-24h</sub> (ng·h/mL)	Day 1	81977 :53524	129762 :81667	NE	86976 :74268	115109 :74716	138607 :115503
	Week 13	123576 :55469	122790 :77770	NE	125382 :62365	167165 :84297	129455 :100825
<b>Chlorthalidone</b>							
T <sub>max</sub> (h)	Day 1	NE	NE	2.0:2.0	2.0:1.0	2.0:2.0	4.0:4.0
	Week 13	NE	NE	4.0:2.0	2.0:1.0	2.0:2.0	4.0:2.0
C <sub>max</sub> (µg/mL)	Day 1	NE	NE	1.07:1.92	3.71:3.22	3.57:3.91	3.28:2.83
	Week 13	NE	NE	1.00:1.54	2.40:3.63	3.61:4.28	2.52:3.80
AUC <sub>0-24h</sub> (µg·h/mL)	Day 1	NE	NE	8.4:13.1	18.9:17.1	29.6:27.7	45.0:41.9
	Week 13	NE	NE	9.7:7.2	19.3:19.2	36.0:42.6	36.2:37.6
Conclusion	Toxicologically significant changes were observed in the TAK-491/TAK-536 M-II group and chlorthalidone group. Suppression of body weight gain and decreased food consumption attributable to TAK-491/TAK-536 M-II were enhanced by co-administration with chlorthalidone. Increased adrenal weight and increased incidence and severity of renal tubular regeneration attributable to chlorthalidone were enhanced by co-administration with TAK-491/TAK-536 M-II, and an APTT prolongation, decreased plasma potassium and increased incidence and severity of renal corticomedullary mineralization attributable to chlorthalidone were not observed by co-administration with TAK-491/TAK-536 M-II. Increased plasma BUN was enhanced by co-administration with TAK-491/TAK-536 M-II and chlorthalidone. Increased plasma creatinine and neutrophil count, decreased uterine weight, hypertrophy of the adrenal zona fasciculata and dilatation of the renal Henle's ascending tubules with dose-dependency of TAK-491 and chlorthalidone in its incidence were observed only in the triple combination group in which plasma chlorthalidone levels increased by the triple administration.						

a): 0.5 w/v% methylcellulose solution containing 0.5 w/v% citric acid, b): Dosage of TAK-491 indicated as TAK-491F (TAK-491 free acid, conversion factor: 1.067), c): TAK-491/TAK-536 M-II/Chlorthalidone, d): Additional 10 animals/sex/group were used as satellite groups for toxicokinetics including the control group. M: Male, F: Female, NE: Not examined, Determination of plasma drug concentrations was also conducted on the control group (2 hours after dosing on day 1 and in week 13), and the concentrations of all analytes were less than the quantification limit (5 ng/mL for TAK-491F, TAK-536, TAK-536 M-I and TAK-536 M-II, 0.05 µg/mL for chlorthalidone).

## 9 Reproductive and Developmental Toxicology

### 9.1 Embryonic Fetal Development

Study title: Effects of TAK-491 plus TAK-536 MII plus Chlorthalidone (triple combination) on Embryo-fetal Development in Rats

Study no: SBL010-128

Study report location: electronic

Conducting laboratory and location:

(b) (4)

Date of study initiation: 07/24/08

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: TAK-491, lot # M491-015, 100.2%  
TAK-536 MII, lot # M117-M1005, 99.7%  
Chlorthalidone, lot # MA06-002, 99.3%

### Key Study Findings

TAK-491 is the pro-drug for the active drug, TAK-536. In humans, the major metabolite of TAK-536 is TAK-536 MII. The double combination administered in this study contains the prodrug TAK-491 with the human metabolite TAK-536 MII. The triple combination group was administered TAK-491, TAK-536 MII and chlorthalidone together. The final group received chlorthalidone alone. In reference to embryo-fetal development in rats, severity of general toxicity in dams was increased, fetal growth was retarded and indices of some visceral variations and wavy ribs were increased by the triple combination. Despite maternal toxicity, neither increased fetal mortality nor teratogenicity was observed in the triple combination group in this study.

## Methods

Doses: See below  
 Frequency of dosing: Once per day from day 6 to Day 17 of gestation  
 Dose volume: 10 ml  
 Route of administration: Oral gavage  
 Formulation/Vehicle: Agents were dissolved in 0.5 w/v% methylcellulose solution containing 0.5 w/v% citric acid  
 Species/Strain: Rat/ Sprague-Dawley  
 Number/Sex/Group: 20-27 /group  
 Satellite groups: none  
 Study design: See below  
 Deviation from study protocol: None

Toxicity groups: 1 control group and 3 test article groups

Group	Test or control article	Dosage level <sup>1,2)</sup> (mg/kg/day)	Dosage volume (mL/kg/day)	Concentration <sup>2)</sup> (w/v%)	Number of animals confirmed to have copulated (Animal No.)*
1	Control article <sup>3)</sup>	-	10	-	20 (10001-10020)
2	TAK-491/ TAK-536 M-II	1000/2000/0	10	10/20/0	20 (10021-10040)
3	CLD	0/0/300	10	0/0/3	20 (10041-10060)
4	TAK-491/ TAK-536 M-II/ CLD	1000/2000/300	10	10/20/3	27 (10061-10087) <sup>4)</sup>

1) Dosage level (conversion factor: 1.067) as TAK-491 free acid (TAK-491F) for TAK-491

2) Expressed as TAK-491/TAK-536 M-II/CLD (chlorthalidone)

3) 0.5 w/v% MC solution containing 0.5 w/v% citric acid

4) In Group 4, 7 spare animals were set in case of an occurrence of death. However, no dams died in this group, and a sufficient number of alive dams was confirmed without these animals. Therefore, these animals (Nos. 10081 to 10087) were excluded from the study before necropsy.

\*: Non-pregnant animals: Nos. 10003 and 10042

## Observations and Results

### Mortality

**Mortality was monitored 2X a day (before dosing and 1-2 hr after dosing). No dams died in any group.**

### Clinical Signs

**Clinical signs were monitored 2X a day (before dosing and 1-2 hr after dosing). In the double and triple treatment groups, white stool was observed and was due to unabsorbed test articles.**

### Body Weight and Feed Consumption

Body weight was measured in the dams on Days 0, 6, 8, 10, 12, 14, 16, 18 and 20 (the day of gross pathology examination) in the toxicity groups and on the same days (except for Day 20) in the satellite group. Food consumption was measured on Days 6, 7, 9, 11, 13, 15, 17, 19 and 20 of gestation. Body weight gain and food consumption were suppressed in the double combination group during the dosing period. In the chlorthalidone alone group, there was a suppression of body weight gain and a decrease in food consumption during the early phase of dosing which returned to normal by gestation Day 20. In the triple combination groups, there were also decreases in body weight gain and food consumption. These effects were greater than those effects seen in the double combination group and the chlorthalidone alone group.

### Toxicokinetics

Plasma concentrations ( $T_{max}$ ,  $C_{max}$  and  $AUC_{0-24h}$ ) of the compound were measured in the satellite groups on 2 days: on Day 6 of gestation at 0.5, 1, 2, 4, 8 and 24 hr. Measurements were also made on Day 17 of gestation before, and 0.5, 1, 2, 4, 8 and 24 hr after dosing.

Results: The  $T_{max}$  values for chlorthalidone, TAK-536, TAK-536 MI and TAK-536 MII in the triple combination group increased when compared with the double combination or the chlorthalidone alone groups. The  $C_{max}$  and  $AUC_{0-24h}$  values for chlorthalidone in the triple combination group were higher than those in the chlorthalidone alone group, but those for TAK-536, TAK-536 MI and TAK-536 MII in the triple combination group were almost comparable with those in the double combination group, except for the  $C_{max}$

values for TAK-536 MI which was higher than that in the triple combination group. The concentration of TAK-491, the prodrug, was below the lower limit of quantification or very low in the double and triple combination groups.

### **Stability and Homogeneity**

Data are submitted for the stability and homogeneity of the test articles. These are found in Attachment A, pages 84-92.

### **Necropsy**

On Day 20 of gestation, dams were euthanized and necropsied. External appearance and internal organs and tissues were observed macroscopically. There were no treatment-related effects in gross pathological findings.

### **Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)**

The numbers of corpora lutea and implantations were recorded, and the preimplantation loss rate  $[(\# \text{ of corpora lutea} - \# \text{ of implantation}) / \text{number of corpora lutea} \times 100]$  was calculated. Thoraco-abdominal organs (including uterus and ovaries) were collected and stored in formalin. Results: There were no treatment effects on the number of corpora lutea, implantations, live fetuses or embryonic/fetal deaths; on fetal viability rate, postimplantation loss rate, and sex ratio; or on placental appearance or weight.

### **Offspring (Malformations, Variations, etc.)**

All live fetuses were removed at necropsy on Day 20 of gestation, and observed, and the following parameters were recorded: Number of live fetuses, number of embryonic/fetal deaths, and their condition (classified as implantation site, placental remnant or dead fetus). The fetal viability rate  $[(\# \text{ of live fetuses} / \# \text{ of implantations}) \times 100]$  and the postimplantation loss rate  $[(\# \text{ of embryo-fetal deaths} / \# \text{ of implantations}) \times 100]$  for each dam was calculated. The placentae and external features (including oral cavity) of each live fetus were examined. The sex of each live fetus was determined, and the sex ratio  $(\# \text{ of live male fetuses} / \# \text{ of live fetuses})$  for each litter was calculated. Half of the live fetuses were fixed in formalin for subsequent visceral examination and the other half fixed in ethanol for subsequent skeletal examination. Visceral examinations of cephalic, thoracic and abdominal organs and the other half fixed in ethanol and stained with Alizarin Red S for subsequent skeletal examination. Visceral examinations were made. The number of ossified sacral and caudal vertebrae was examined as an ossification index.

Results: In the 1000/2000/300 mg/kg dose, fetal body weights were low. Incidence of visceral variations and thymic remnant in the neck, dilated renal pelvis, dilated ureter and convoluted ureter as visceral variations were increased in the triple combination

group. Also observed in this group were wavy ribs as a skeletal variation and delayed ossification considered to be related to growth retardation.

(No. 1)

Effects of TAK-491 plus TAK-536 M-II plus Chlorthalidone (Triple Combination) on Embryo-Fetal Development in Rats

(Study No. SBL010-128)

Animal	Rat, CrI:CD(SD), 12 to 15-week-old males and 11 to 14-week-old females (at mating)			
Treatment	Orally by gavage Dosing period: from Day 6 to Day 17 of gestation			
Test article	Control article <sup>a)</sup>	TAK-491/ TAK-536 M-II	Chlorthalidone	TAK-491/ TAK-536 M-II/ Chlorthalidone
Dosage level (mg/kg/day) <sup>b, c)</sup>	0/0/0	1000/2000/0	0/0/300	1000/2000/300
Dosage volume (mL/kg/day)	10	10	10	10
No. of dams	19	20	19	20
<b>Dams</b>				
No. of deaths	0	0	0	0
Clinical signs	-	White stool	-	White stool
Body weight	-	↓GD20 <sup>S1</sup> #	-	↓GD 8 <sup>S1, @@2</sup> # ↓GD10 <sup>SS1, @@2, S3</sup> # ↓GD12 to 18 <sup>SS123</sup> # ↓GD20 <sup>@@123</sup> #
Body weight gain	-	↓GD 6-12 <sup>SS1</sup> # ↓GD12-18 <sup>@@1</sup> # ↓GD 6-18 <sup>@@1</sup> # ↓GD 0-20 <sup>SS1</sup> #	↓GD 6-12 <sup>SS1</sup> # ↓GD 6-18 <sup>SS1</sup> # ↑GD18-20 <sup>SS1</sup>	↓GD 6-12 <sup>SS123</sup> # ↓GD12-18 <sup>@@13, SS2</sup> # ↓GD 6-18 <sup>@@13, SS2</sup> # ↑GD18-20 <sup>@1, @@3</sup> # ↓GD 0-20 <sup>@@123</sup> #
Food consumption	-	↓GD14-15 <sup>SS1</sup> # ↓GD16-17 <sup>@@1</sup> # ↓GD18-19 <sup>SS1</sup> # ↓GD19-20 <sup>SS1</sup> #	↓GD 6- 7 <sup>S1</sup> # ↑GD18-19 <sup>SS1</sup> ↑GD19-20 <sup>SS1</sup>	↓GD 6-7 <sup>@@123</sup> # ↓GD 8-9 <sup>@@13, SS2</sup> # ↑GD10-11 <sup>@@123</sup> # ↓GD12-13 <sup>@@1, SS23</sup> # ↓GD14-15 <sup>@@13, @2</sup> # ↓GD16-17 <sup>@@13, S2</sup> # ↓GD18-19 <sup>@@13, @2</sup> # ↓GD19-20 <sup>@@13, @2</sup> #
Gross pathological findings	-	-	-	-
No. of corpora lutea <sup>d)</sup>	15.2 ± 1.1	15.6 ± 1.7	15.6 ± 1.6	14.7 ± 1.6
No. of implantations <sup>d)</sup>	14.3 ± 1.1	14.6 ± 1.6	14.6 ± 1.5	14.5 ± 1.5
Preimplantation loss rate (%) <sup>d)</sup>	5.4 ± 6.1	6.1 ± 8.7	6.6 ± 6.2	1.7 ± 2.9 <sup>*1, **3</sup>

a): 0.5 w/v% Methylcellulose solution containing 0.5 w/v% citric acid

b): TAK-491 is as TAK-491F (TAK-491 free acid)

c): Dose: TAK-491/TAK-536 M-II/Chlorthalidone, d): Mean ± SD, #: Adverse effects

-: No treatment-related effects, ↓: Decreased/Suppressed, ↑: Increased, GD: Gestation day

\$ P<0.05, \$\$ P<0.01: Significantly different by the Student t-test from control (1), 1000/2000/0 (2) and 0/0/300 (3)

@ P<0.05, @@ P<0.01: Significantly different by the Aspin-Welch t-test from (1), (2) and (3)

\* P<0.05, \*\* P<0.01: Significantly different by the Wilcoxon test from (1) and (3)

(No. 2)

## Effects of TAK-491 plus TAK-536 M-II plus Chlorthalidone (Triple Combination) on Embryo-Fetal Development in Rats

(Study No. SBL010-128)

Test article	Control article <sup>a)</sup>	TAK-491/ TAK-536 M-II	Chlorthalidone	TAK-491/ TAK-536 M-II/ Chlorthalidone
Dosage level (mg/kg/day) <sup>b, c)</sup>	0/0/0	1000/2000/0	0/0/300	1000/2000/300
<b>Dams</b>				
<b>Placentae</b>				
Placental weight (g): males <sup>d)</sup>	0.48 ± 0.07	0.46 ± 0.03	0.48 ± 0.04	0.46 ± 0.11
Placental weight (g): females <sup>d)</sup>	0.46 ± 0.04	0.45 ± 0.04	0.46 ± 0.07	0.45 ± 0.12
Placental abnormalities (%) <sup>d)</sup>	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
<b>Fetuses</b>				
Postimplantation loss rate (%) <sup>d)</sup>	4.4 ± 5.8	4.4 ± 5.3	5.6 ± 6.6	3.9 ± 7.2
No. of live fetuses <sup>d)</sup>	13.7 ± 1.4	14.0 ± 1.6	13.8 ± 1.8	13.9 ± 1.8
Sex ratio (male/total) <sup>d)</sup>	0.514 ± 0.156	0.499 ± 0.145	0.528 ± 0.173	0.484 ± 0.119
Body weight (g): males <sup>d)</sup>	3.63 ± 0.24	3.54 ± 0.24	3.69 ± 0.23	3.04 ± 0.33 <sup>@@123#</sup>
Body weight (g): females <sup>d)</sup>	3.46 ± 0.23	3.32 ± 0.22	3.52 ± 0.27	2.91 ± 0.26 <sup>\$\$123#</sup>
<b>External findings (%)</b>				
Malformations (%) <sup>d)</sup>	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Visceral abnormalities (%) <sup>d)</sup>	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Visceral variations (%) <sup>d)</sup>	5.46 ± 8.51	3.72 ± 8.58	6.77 ± 12.94	27.09 ± 26.83 <sup>**123#</sup>
<b>Main type (%)</b>				
Thymic remnant in neck	2.19	0.72	2.56	6.38#
Dilated renal pelvis	1.63	1.34	2.59	12.27 <sup>*12#</sup>
Dilated ureter	1.63	0.00	0.66	9.29 <sup>*23#</sup>
Convolutated ureter	0.88	2.38	2.51	8.22#
Skeletal abnormalities (%) <sup>d)</sup>	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.63 ± 2.80
<b>Main type (%)</b>				
Misshapen scapula	0.00	0.00	0.00	0.63
Skeletal variations (%) <sup>d)</sup>	20.46 ± 17.65	13.73 ± 16.55	23.86 ± 22.44	22.47 ± 22.24
<b>Main type (%)</b>				
Cervical rib	0.00	0.00	0.00	1.43
Dumbbell-shaped thoracic centrum	1.76	3.73	3.70	0.00 <sup>*23</sup>
Splitting of thoracic centrum	0.00	0.72	1.41	0.00
Full supernumerary rib	0.00	0.84	0.75	0.63
Short supernumerary rib	16.29	8.79	19.63	11.66
Wavy rib	1.75	1.34	0.00	9.38 <sup>*3#</sup>
Supernumerary lumber vertebra	0.00	0.84	0.00	0.00
No. of ossified sacral and caudal vertebrae <sup>d)</sup>	8.16 ± 0.46	7.72 ± 0.39 <sup>\$\$1</sup>	8.11 ± 0.59	6.40 ± 1.12 <sup>@@123#</sup>

a): 0.5 w/v% Methylcellulose solution containing 0.5 w/v% citric acid

b): TAK-491 is as TAK-491F (TAK-491 free acid)

c): Dose: TAK-491/TAK-536 M-II/Chlorthalidone, d): Mean ± SD, #: Adverse effects

\$ P&lt;0.05, \$\$ P&lt;0.01: Significantly different by the Student t-test from control (1), 1000/2000/0 (2) and 0/0/300 (3)

@ P&lt;0.05, @@ P&lt;0.01: Significantly different by the Aspin-Welch t-test from (1), (2) and (3)

\* P&lt;0.05, \*\* P&lt;0.01: Significantly different by the Wilcoxon test from (1), (2) and (3)

(No. 3)

Effects of TAK-491 plus TAK-536 M-II plus Chlorthalidone (Triple Combination) on Embryo-Fetal Development in Rats

(Study No. SBL010-128)

Test article	Control article <sup>a)</sup>	TAK-491/ TAK-536 M-II	Chlorthalidone	TAK-491/ TAK-536 M-II/ Chlorthalidone
Dosage level (mg/kg/day) <sup>b, c)</sup>	0/0/0	1000/2000/0	0/0/300	1000/2000/300
Toxicokinetic parameters <sup>d)</sup> (Day 6 of gestation / Day 17 of gestation)				
Chlorthalidone	T <sub>max</sub> (h)	ND	-	3.3 / 2.3
	C <sub>max</sub> (µg/mL)	ND	-	0.52 / 0.54
	AUC <sub>0-24h</sub> (µg·h/mL)	ND	-	5.1 / 4.0
TAK-491F	T <sub>max</sub> (h)	ND	24.0 <sup>e)</sup> / ND	8.0 <sup>e)</sup> / ND
	C <sub>max</sub> (ng/mL)	ND	2 / 0	3 / 0
	AUC <sub>0-24h</sub> (ng·h/mL)	ND	0 / 0	27 / 0
TAK-536	T <sub>max</sub> (h)	ND	1.2 / 2.0	1.2 / 2.3
	C <sub>max</sub> (ng/mL)	ND	238737 / 295143	219861 / 249809
	AUC <sub>0-24h</sub> (ng·h/mL)	ND	2055927 / 2424633	2090431 / 2934630
TAK-536 M-I	T <sub>max</sub> (h)	ND	2.0 / 1.2	1.7 / 5.7
	C <sub>max</sub> (ng/mL)	ND	4839 / 4118	2185 / 1755
	AUC <sub>0-24h</sub> (ng·h/mL)	ND	24930 / 20249	17071 / 23517
TAK-536 M-II	T <sub>max</sub> (h)	ND	1.7 / 1.7	2.0 / 6.0
	C <sub>max</sub> (ng/mL)	ND	28449 / 22695	34299 / 23524
	AUC <sub>0-24h</sub> (ng·h/mL)	ND	234802 / 217552	276189 / 346084
Conclusion: combined administration of TAK-491, TAK-536 M-II and chlorthalidone	Dams	Severity of general toxicity was increased but reproductive toxicity was not noted.		
	Embryo-fetal development	Fetal growth retardation was induced and incidences of some visceral variations and wavy ribs were increased but fetal mortality or teratogenicity was not induced.		

a): 0.5 w/v% Methylcellulose solution containing 0.5 w/v% citric acid

b): TAK-491 is as TAK-491F (TAK-491 free acid)

c): Dose: TAK-491/TAK-536 M-II/Chlorthalidone, d): Mean, e): n=1

ND: Not determined or all the values determined were below the quantification limit, -: No data

## 10 Special Toxicology Studies

None

## 11 Integrated Summary and Safety Evaluation

TAK-491 is a pro-drug of TAK-536 (azilsartan), a highly selective and potent antagonist at All type 1 (AT1) receptors. TAK-536 M-II (O-dealkylated metabolite of TAK-536) is present in human plasma at concentrations higher than those observed in animal toxicity studies conducted with TAK-491 and TAK-536. Therefore, the nonclinical safety of TAK-536 M-II also was evaluated. Chlorthalidone is a thiazide-like diuretic. The diuretic effect of chlorthalidone is due to inhibition of sodium reabsorption in the kidney, which leads to increased water excretion. The

initial blood pressure-lowering effect of chlorthalidone is likely due to decreases in plasma volume and cardiac output related to diuresis; however, with continued treatment, plasma volume returns to pre-drug levels, but arterial blood pressure decreases persist, possibly due to its activity to vasodilate arterioles.

The toxicity of combination treatment with TAK-491 and chlorthalidone was evaluated in single- and repeat-dose toxicokinetic and toxicity studies in rats for up to 13 weeks in duration, and embryo-fetal development studies in rats.

In the first pivotal rat toxicity study, rats were administered once/day for 2 weeks by oral gavage either vehicle, TAK-491 alone (1000 mg/kg/day), TAK-536 M-II alone (2000 mg/kg/day), Chlorthalidone alone (100 and 300 mg/kg/day), TAK-491 with chlorthalidone (100 mg/kg/day of both; 100 TAK-491 and 300 CLD; 1000 TAK-491 and 100 CLD and 1000 TAK-491 and 300 CLD); finally TAK-536 M-II with chlorthalidone (2000 TAK-536 M-II and 100 CLD; 2000 TAK-536 M-II and 300 CLD). Plasma chlorthalidone levels were increased by dosing in combination with TAK-491, and an additive increase in plasma urea nitrogen and increases in water intake, urine output and plasma total cholesterol were also observed in the TAK-491/chlorthalidone combination groups. No clear combination effects were observed from dosing the combination of TAK-536 MII and chlorthalidone.

In the second pivotal rat toxicity study, rats were administered once/day for 13 weeks by oral gavage either vehicle, TAK-491 and TAK-536 M-II (100 TAK-491 and 2000 TAK-536 M-II; 1000 TAK-491 and 2000 TAK-536 M-II), chlorthalidone alone (300 CLD), or the triple combination (100 TAK-491, 2000 TAK-536 M-II and 100 CLD; 100 TAK-491, 2000 TAK-536 M-II and 300 CLD; finally, 1000 TAK-491, 2000 TAK-536 M-II and 300 CLD). TAK-491 is the pro-drug for the active drug, TAK-536. In humans, the major metabolite of TAK-536 is TAK-536 MII. The double combination administered in this study contains the prodrug TAK-491 with TAK-536 MII. Two groups of rats received this double combination: One group received 100 mg/kg of TAK-491 with 2000 mg/kg of MII and the second group received 1000 mg/kg TAK-491 with 2000 mg/kg of MII. In this second group, there was a significant decrease in body weight gain and food consumption. This effect was enhanced in all groups receiving the triple combination containing chlorthalidone. Chlorthalidone given by itself at one dose of 300 mg/kg increased BUN and adrenal weight. It also increased the incidence and severity of background renal tubular regeneration. All these effects were enhanced by co-administration with the double combination (TAK-491 and TAK-536 MII). The increase in kidney weight seen with chlorthalidone alone was similar to that seen in the triple combination. APTT prolongation, decreased potassium and increase in incidence and severity of renal cortical mineralization attributable to chlorthalidone were not observed by co-administration with TAK-491 and TAK-536 MII. Increased plasma creatinine, and neutrophil count, decreased uterine weight, hypertrophy of the adrenal zona fasciculata and dilatation of the renal Henle's ascending tubules with dose-dependency of TAK-491 and chlorthalidone in its incidence were observed only in the triple combination group in which plasma chlorthalidone levels were increased compared to the cohort receiving chlorthalidone *per se*.

In the last pivotal study, there was no evidence of teratogenicity in rat embryo/fetal development studies conducted with TAK-491/TAK-536 M-II in combination with chlorthalidone at maternally toxic doses. No new toxicities were observed in this study, although there were increases in the severity or incidence of toxicologically significant findings in the groups receiving the triple combination. These included fetal growth retardation, and increased incidence of visceral variations in the fetuses such as wavy ribs.

In a clinical study with TAK-491, patients with moderate to severe essential hypertension were treated daily for 6 weeks with either 25 mg chlorthalidone and placebo (monotherapy), with 40 mg TAK-491 and 25 mg chlorthalidone, or with 80 mg TAK-491 and 25 mg chlorthalidone. Patients receiving the coadministration treatment demonstrated statistically significant greater increases in plasma creatinine levels at final visit compared to those patients receiving either drug alone.

One can interpret the increase in serum creatinine levels as a pharmacologic response to renin-angiotensin aldosterone system blockade in the setting of potent diuresis and extensive reductions in blood pressure and intraglomerular pressure, rather than a toxicologic effect. These creatinine elevations are associated with reductions in blood pressure and are reversible once drug is discontinued. Therefore this effect appears to be associated with pharmacological effects of the drug and not necessarily nephrotoxicity. Indeed, the use of combination drug therapy for the treatment of hypertension has been frequently associated with increases in creatinine. ARBs or ACE inhibitors, either alone or in combination with diuretics may lead to an acute increase in serum creatinine within the first 2 to 3 months of treatment. In patients with chronic kidney disease treated with ACE inhibitors, acute increases of creatinine up to 30% are associated with improved long-term preservation of renal function without increased risk of hyperkalemia.

## 12. Appendix/Attachments

None

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**This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.**  
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/s/  
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PHILIP J GATTI  
07/18/2011

ALBERT F DEFELICE  
07/18/2011