

*Supplementary Material*

**Supplementary Table 1. Inclusion and exclusion criteria of the study.**

|                    | Healthy young adults                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Healthy elderly                                                           | Elderly with CKD                                                                              |
|--------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|
| Inclusion criteria |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                                           |                                                                                               |
|                    | Thai male or female, aged 20 to 40 years (inclusive)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Thai male or female aged ≥60 years                                        |                                                                                               |
|                    | Body mass index 18-25 kg/m <sup>2</sup> (inclusive)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | Body mass index 18-30 kg/m <sup>2</sup> (inclusive)                       |                                                                                               |
|                    | Healthy by medical history, physical examination, and vital signs                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                                                                           | Diagnosed with CKD according to the Kidney Disease Improving Global Outcomes 2012 criteria    |
|                    | Laboratory values at screening visit <sup>a</sup> are within the normal range or showing no clinically significant abnormalities as confirmed by the clinical investigator                                                                                                                                                                                                                                                                                                                                                                    |                                                                           |                                                                                               |
|                    | eGFR <sup>b</sup> ≥90 mL/min/1.73 m <sup>2</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | eGFR <sup>b</sup> >60 mL/min/1.73 m <sup>2</sup>                          | eGFR <sup>b</sup> 15-60 mL/min/1.73 m <sup>2</sup>                                            |
| Exclusion criteria |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                                           |                                                                                               |
|                    | History of drug allergy to midazolam, dabigatran etexilate, pitavastatin, atorvastatin, rosuvastatin, and rifampicin                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                           |                                                                                               |
|                    | History of any illness that, in the opinion of the clinical investigator, might confound the result of the study or pose an additional risk in administrating the study drug to the subjects. This may include but is not limited to: a history of relevant drug or food allergies; history or cardiovascular disease, gastrointestinal, central nervous system disease, renal and hepatic impairment; history or presence of clinically significant illness; or history of mental illness that may affect compliance with study requirements | History of hypertension, diabetes mellitus, other major systemic diseases | History of renal transplantation, liver transplantation, hemodialysis and peritoneal dialysis |
|                    | Receive any medical prescription within 14 days before the first administration of the microdose cocktail, especially drug related to the study drug metabolizing enzyme                                                                                                                                                                                                                                                                                                                                                                      |                                                                           | -                                                                                             |
|                    | History of heavy smoking (> 10 cigarettes per day) or moderate smoking (< 10 cigarettes per day) and cannot omit smoking a least one day before the study and until the completion of the study phase                                                                                                                                                                                                                                                                                                                                         |                                                                           |                                                                                               |
|                    | History of alcoholic (more than 2 years) or moderate drinker (more than 3 drinks per day) or history of any drug abuse                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                                           |                                                                                               |
|                    | Pregnant or breastfeeding (female)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | -                                                                         | -                                                                                             |

<sup>a</sup>Laboratory tests include complete blood count, fasting blood sugar, blood urea nitrogen, serum creatinine, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, total bilirubin, direct bilirubin, total protein, albumin, total cholesterol, triglyceride, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, electrolytes, parathyroid hormone, and phosphate.

<sup>b</sup>Modification of Diet in Renal Disease estimated glomerular filtration rate for Thai formula (Praditpornsilpa K, *et al.* The need for robust validation for MDRD-based glomerular filtration rate estimation in various CKD populations. *Nephrol Dial Transplant.* 2011 Sep;26(9):2780-5)

CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate.

**Supplementary Table 2.** Geometric means (95% confidence interval) of pharmacokinetic parameters between midazolam powder formulation and midazolam solution formulation in elderly with chronic kidney disease.

| <b>PK parameters</b>               | <b>Powder formulation (n = 13)</b> | <b>Solution formulation (n = 4)</b> |
|------------------------------------|------------------------------------|-------------------------------------|
| AUC <sub>0-last</sub> (pg/mL.hr)   | 441.13 (323.81, 600.95)            | 437.37 (193.88, 986.69)             |
| C <sub>max</sub> (pg/mL)           | 150.06 (111.23-202.46)             | 152.84 (118.73, 196.75)             |
| T <sub>max</sub> (hr) <sup>a</sup> | 0.67 (0.67-0.67)                   | 1.00 (0.33-1.00)                    |
| T <sub>1/2</sub> (hr) <sup>a</sup> | 5.50 (3.38-5.99)                   | 6.46 (6.21-10.93)                   |

<sup>a</sup>Data are presented in the median (interquartile range).

AUC<sub>0-last</sub>: area under the concentration-time curve of time zero to the last time point; C<sub>max</sub>: maximum plasma concentration; T<sub>max</sub>: time to maximum plasma concentration; T<sub>1/2</sub>: half-life.

**Supplementary Table 3. Genotype frequency of *CYP3A5*\*3 (rs776746, 6986A > G) in three groups of participants**

| Genotype frequency (n)            | Healthy young adults (n=20) | Healthy elderly (n=16) | Elderly patients with chronic kidney disease (n=17) | Total (n=53) |
|-----------------------------------|-----------------------------|------------------------|-----------------------------------------------------|--------------|
| rs776746 (6986A > G) <sup>a</sup> |                             |                        |                                                     |              |
| 6986AA (wild-type)                | 3                           | 0                      | 5                                                   | 8            |
| 6986AG                            | 9                           | 8                      | 5                                                   | 22           |
| 6986GG                            | 8                           | 8                      | 7                                                   | 23           |

<sup>a</sup>Venous blood sample (3 mL) was collected from each participant into an EDTA tube. Genomic DNA was extracted by PureLink® Genomic DNA kit (ThermoFisher Scientific). *CYP3A5*\*3 c.6986A>G (rs776746) was detected by TaqMan® Real-time Polymerase Chain Reaction (PCR) using TaqMan Drug Metabolism Genotyping Assays (Applied Biosystem, ThermoFisher Scientific). PCR was performed with the quantitative real-time PCR (q-PCR) system (StepOnePlus™ Real-Time PCR System, ThermoFisher Scientific).

**Supplementary Table 4.** List of concomitant medication in elderly patients with chronic kidney disease

| Concomitant drugs                  | Number of patients |
|------------------------------------|--------------------|
| Alfuzosin                          | 2                  |
| Allopurinol                        | 7                  |
| Amlodipine                         | 5                  |
| Aspirin                            | 5                  |
| Atenolol                           | 2                  |
| Benzbromarone                      | 1                  |
| Betahistine                        | 1                  |
| Calciferol                         | 6                  |
| Calcium carbonate                  | 3                  |
| Candesartan                        | 1                  |
| Carvedilol                         | 2                  |
| Cetirizine                         | 1                  |
| Clopidogrel                        | 1                  |
| Colchicine                         | 3                  |
| Diacerein                          | 1                  |
| Doxazosin                          | 3                  |
| Dutasteride                        | 1                  |
| Enalapril                          | 5                  |
| Eprex                              | 1                  |
| Ezetimibe                          | 1                  |
| Ferrous sulfate                    | 4                  |
| Folic acid                         | 7                  |
| Furosemide                         | 4                  |
| Gabapentin                         | 1                  |
| Glipizide                          | 3                  |
| Hemax injection                    | 2                  |
| Hydralazine                        | 1                  |
| Insulin mixtard HM30               | 1                  |
| Insulin novo mix penfill injection | 1                  |
| Irbesartan                         | 2                  |
| Lercanidipine                      | 1                  |
| Linagliptin                        | 3                  |
| Lorazepam                          | 1                  |
| Losartan                           | 4                  |
| Manidipine                         | 6                  |
| Melatonin                          | 1                  |
| Metoprolol                         | 1                  |
| Nicergoline                        | 1                  |
| Omeprazole                         | 2                  |
| Perindopril                        | 1                  |
| Pioglitazone                       | 2                  |
| Pocitrin (K, Na)                   | 1                  |
| Pregabalin                         | 1                  |
| Propranolol                        | 1                  |
| Sennoside b                        | 1                  |
| Silodosin                          | 1                  |
| Simethicone                        | 1                  |
| Simvastatin                        | 6                  |
| Sodamint                           | 7                  |
| Sodium chloride                    | 1                  |
| Vitamin B6                         | 1                  |

**Supplementary Table 5.** Multivariable analysis of rosuvastatin with genetic variation.

| Multivariate model                     | Rosuvastatin <sup>a</sup>      |
|----------------------------------------|--------------------------------|
| <b>n</b>                               | 52                             |
| <b>AUC<sub>0-last</sub> (pg/mL.hr)</b> |                                |
| Healthy young adults                   | 1.00                           |
| Healthy elderly                        | 1.43 (0.95-2.16) <sup>ns</sup> |
| Elderly with CKD                       | 1.83 (1.25-2.69)*              |
| Elderly with CKD vs Healthy elderly    | 1.28 (0.85-1.93) <sup>ns</sup> |
| <i>ABCG2</i> wild-type                 | 1.00                           |
| <i>ABCG2</i> 421AA                     | 3.06 (1.51-6.18)*              |
| <i>ABCG2</i> 421CA                     | 1.67 (1.18-2.35)*              |
| <b>C<sub>max</sub> (pg/mL)</b>         |                                |
| Healthy young adults                   | 1.00                           |
| Healthy elderly                        | 1.12 (0.77-1.64) <sup>ns</sup> |
| Elderly with CKD                       | 1.59 (1.12-2.26)*              |
| Elderly with CKD vs Healthy elderly    | 1.42 (0.97-2.07) <sup>ns</sup> |
| <i>SLCO1B1</i> wild-type               | -                              |
| <i>SLCO1B1</i> *15/*17                 | -                              |
| <i>ABCG2</i> wild-type                 | 1.00                           |
| <i>ABCG2</i> 421AA                     | 2.18 (1.14-4.16)*              |
| <i>ABCG2</i> 421CA                     | 1.43 (1.04-1.96)*              |

Data are presented in the geometric mean ratio (95% confidence interval).

Variables with p-value <0.1 in the univariate analysis were included in the multivariable analysis.

<sup>a</sup>24 subjects with *ABCG2* variants (rs2231142, 421AA, and 421CA) were included in the model.

\*p <0.05

AUC<sub>0-last</sub>: area under the concentration-time curve of time zero to the last time point; C<sub>max</sub>: maximum plasma concentration; ns: non-significant.

**Supplementary Table 6.** Pharmacokinetic parameters of midazolam in wild-type *CYP3A5* participants

| Drugs                              | Healthy young adults (n=3) |                        |                   | Healthy elderly (n=0) |              | Elderly patients with chronic kidney disease (n=5) |                   |
|------------------------------------|----------------------------|------------------------|-------------------|-----------------------|--------------|----------------------------------------------------|-------------------|
|                                    | Microdose                  | Microdose + Rifampicin |                   | Microdose             |              | Microdose                                          |                   |
|                                    | GM (95% CI)                | GM (95% CI)            | GMR (95% CI)      | GM (95% CI)           | GMR (95% CI) | GM (95% CI)                                        | GMR (95% CI)      |
| <b>Midazolam</b>                   |                            |                        |                   |                       |              |                                                    |                   |
| AUC <sub>0-last</sub> (pg/mL.hr)   | 244 (140-424)              | 344 (183-646)          | 1.41 (0.82-2.42)  | -                     | -            | 619 (411-931)                                      | 2.53 (1.49-4.31)* |
| AUC <sub>0-inf</sub> (pg/mL.hr)    | 254 (136-473)              | 360 (195-665)          | 1.42 (0.81-2.49)  | -                     | -            | 653 (435-980)                                      | 2.57 (1.49-4.42)* |
| C <sub>max</sub> (pg/mL)           | 95 (89-102)                | 119 (111-128)          | 1.26 (1.18-1.34)* | -                     | -            | 174 (144-210)                                      | 1.83 (1.46-2.28)* |
| T <sub>max</sub> (hr) <sup>a</sup> | 0.7 (0.7-1.0)              | 0.7 (0.7-1.5)          | -                 | -                     | -            | 0.7 (0.7-1.0)                                      | -                 |
| T <sub>1/2</sub> (hr)              | 2.9 (0.9-8.7)              | 4.7 (0.5-42.2)         | 1.65 (0.34-8.06)  | -                     | -            | 8.4 (5.2-13.7)                                     | 2.96 (1.42-6.13)* |

<sup>a</sup>Data are presented in the median (interquartile range).

\*p-value <0.05, healthy young adult as a reference group.

<sup>†</sup>p-value <0.05, healthy elderly as a reference group.

AUC<sub>0-inf</sub>: area under the concentration-time curve of time zero to infinity; AUC<sub>0-last</sub>: area under the concentration-time curve of time zero to the last time point; CI: confidence interval; C<sub>max</sub>: maximum plasma concentration; GM: geometric mean; GMR: geometric mean ratio; T<sub>max</sub>: time to maximum plasma concentration; T<sub>1/2</sub>: half-life.

**Supplementary Table 7.** Pharmacokinetic parameters of midazolam in healthy young adults (n=20) categorized by *CYP3A5* genotypes.

| Drugs                              | 6986AA (wild-type, n=3) | 6986AG (n=9)  |                  | 6986GG (n=8)  |                  |
|------------------------------------|-------------------------|---------------|------------------|---------------|------------------|
|                                    | GM (95% CI)             | GM (95% CI)   | GMR (95% CI)     | GM (95% CI)   | GMR (95% CI)     |
| <b>Midazolam</b>                   |                         |               |                  |               |                  |
| AUC <sub>0-last</sub> (pg/mL.hr)   | 244 (140-424)           | 177 (111-280) | 0.72 (0.38-1.40) | 192 (147-252) | 0.78 (0.40-1.53) |
| AUC <sub>0-inf</sub> (pg/mL.hr)    | 254 (136-473)           | 184 (117-289) | 0.72 (0.38-1.38) | 200 (153-261) | 0.79 (0.41-1.52) |
| C <sub>max</sub> (pg/mL)           | 95 (89-102)             | 77 (51-118)   | 0.82 (0.46-1.45) | 78 (63-97)    | 0.82 (0.46-1.47) |
| T <sub>max</sub> (hr) <sup>a</sup> | 0.7 (0.7-1.0)           | 0.7 (0.7-0.7) | -                | 0.7 (0.7-0.7) | -                |
| T <sub>1/2</sub> (hr)              | 2.9 (0.9-8.7)           | 2.3 (1.5-3.4) | 0.79 (0.43-1.44) | 2.9 (2.4-3.6) | 1.02 (0.55-1.88) |

<sup>a</sup>Data are presented in the median (interquartile range).

\*p-value <0.05, 6986AA wild-type as a reference group.

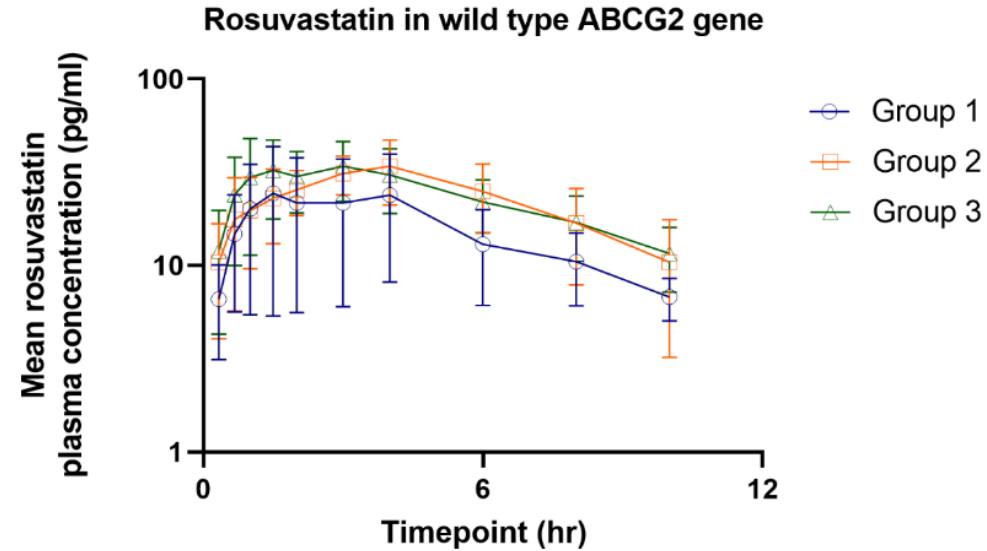
AUC<sub>0-inf</sub>: area under the concentration-time curve of time zero to infinity; AUC<sub>0-last</sub>: area under the concentration-time curve of time zero to the last time point; CI: confidence interval; C<sub>max</sub>: maximum plasma concentration; GM: geometric mean; GMR: geometric mean ratio; T<sub>max</sub>: time to maximum plasma concentration; T<sub>1/2</sub>: half-life.



**Supplementary Table 8.** Safety laboratory parameters on first and last visits.

| Parameters                       | Healthy young adults |               | Healthy elderly |               | Elderly patients with chronic kidney disease |               |
|----------------------------------|----------------------|---------------|-----------------|---------------|----------------------------------------------|---------------|
|                                  | First visit          | Last visit    | First visit     | Last visit    | First visit                                  | Last visit    |
| Blood urea nitrogen (mg/dL)      | 10 (10-12)           | 11 (9-13)     | 11 (10-14)      | 12 (11-16)    | 26 (21-37)                                   | 28 (20-42)    |
| Serum creatinine (g/dL)          | 0.7 (0.7-0.9)        | 0.7 (0.7-0.9) | 0.7 (0.6-0.8)   | 0.6 (0.6-0.7) | 2.2 (1.8-2.9)                                | 2.0 (1.5-2.8) |
| eGFR corrected by BSA (mL/min)   | 112 (106-118)        | 116 (106-118) | 95 (86-110)     | 110 (100-123) | 33 (24-40)                                   | 38 (25-49)    |
| Parathyroid hormone (pg/mL)      | 55 (45-60)           | 52 (46-59)    | 63 (49-76)      | 42 (35-66)    | 113 (76-168)                                 | 135 (101-166) |
| Fasting plasma glucose (mg/dL)   | 85 (81-93)           | 85 (80-88)    | 95 (90-102)     | 93 (89-102)   | 98 (88-105)                                  | 98 (83-118)   |
| Total protein (g/dL)             | 7.8 (7.5-7.9)        | 7.5 (7.0-8.0) | 7.7 (7.3-7.8)   | 7.4 (7.0-8.1) | 7.6 (7.1-7.7)                                | 7.6 (7.2-7.6) |
| Albumin (g/dL)                   | 4.5 (4.3-4.6)        | 4.5 (4.3-4.8) | 4.3 (4.3-4.5)   | 4.4 (4.0-4.5) | 4.3 (4.2-4.4)                                | 4.2 (4.0-4.3) |
| Total bilirubin (mg/dL)          | 0.6 (0.5-0.8)        | 0.4 (0.3-0.8) | 0.6 (0.6-0.9)   | 0.6 (0.5-0.7) | 0.6 (0.5-0.7)                                | 0.6 (0.5-0.6) |
| Direct bilirubin (mg/dL)         | 0.2 (0.2-0.4)        | 0.2 (0.2-0.3) | 0.3 (0.2-0.3)   | 0.2 (0.2-0.3) | 0.3 (0.2-0.3)                                | 0.2 (0.2-0.3) |
| Aspartate aminotransferase (U/L) | 16 (15-20)           | 15 (14-17)    | 22 (19-24)      | 20 (16-21)    | 18 (16-26)                                   | 17 (14-28)    |
| Alanine aminotransferase (U/L)   | 15 (13-20)           | 13 (10-18)    | 19 (16-22)      | 17 (14-20)    | 19 (16-25)                                   | 17 (13-27)    |
| Alkaline phosphatase (U/L)       | 54 (47-66)           | 54 (46-64)    | 67 (56-75)      | 65 (60-77)    | 64 (53-73)                                   | 63 (53-73)    |
| Total cholesterol (mg/dL)        | 203 (176-225)        | 196 (172-214) | 242 (209-270)   | 228 (198-256) | 167 (152-177)                                | 174 (142-210) |
| Triglyceride (mg/dL)             | 87 (61-107)          | 94 (78-136)   | 130 (94-191)    | 113 (78-189)  | 91 (87-141)                                  | 103 (73-124)  |

Data are presented in the median (interquartile range). BSA: body surface area; eGFR: estimated glomerular filtration rate.



**Supplementary Figure 1.** Plasma concentration-time curve of rosuvastatin of wild-type *ABCG2* gene in 3 groups of participants.

Group 1: healthy young subjects

Group 2: healthy elderly subjects

Group 3: elderly patients with chronic kidney disease