Report on the Deliberation Results

March 15, 2013
Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau
Ministry of Health, Labour and Welfare

[Brand name]	Stribild Combination Tab.
[Non-proprietary name]	Elvitegravir/Cobicistat/Emtricitabine/Tenofovir Disoproxil Fumarate (JAN*)
[Applicant]	Japan Tobacco Inc.
[Date of application]	December 6, 2012

[Results of deliberation]
In the meeting held on March 13, 2013, the Second Committee on New Drugs concluded that the product may be approved and that this result should be presented to the Pharmaceutical Affairs Department of the Pharmaceutical Affairs and Food Sanitation Council.

The product is not classified as a biological product or a specified biological product. The re-examination period is 10 years. Elvitegravir and cobicistat among the drug substances, as well as the drug product, are classified as powerful drugs.

[Conditions for approval]
The applicant is required to:

1. Request physicians to obtain patients' informed consent to the use of the product after having thoroughly informed the patients that the collection of additional data on the efficacy and safety of the product is still ongoing, in light of the fact that a pharmacokinetic study on the product is planned to be conducted in Japan.

2. Submit periodical reports on the progress status of the pharmacokinetic study to be conducted in Japan as well as the results and analysis of the study promptly after the study completion. The results and analyses of ongoing or planned foreign clinical studies should also be submitted promptly after the study completion.

3. Conduct the post-marketing surveillance which must, as a general rule, cover all patients treated with the product in Japan, until the expiration of the re-examination period, and collect information on the use status of the product (patient characteristics, efficacy and safety [including the efficacy and safety of the product in concomitant use with other drugs], data on drug-drug interactions), thereby submitteing periodical reports. Also, the final results of the surveillance should be submitted in support of the application for re-examination.

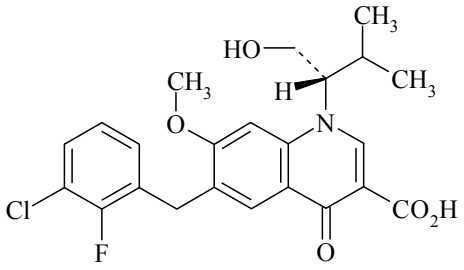
**Japanese Accepted Name (modified INN)*

Review Report

February 19, 2013
Pharmaceuticals and Medical Devices Agency

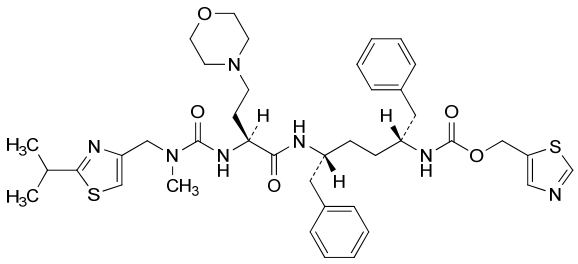
The results of a regulatory review conducted by the Pharmaceuticals and Medical Devices Agency on the following pharmaceutical product submitted for registration are as follows.

[Brand name]	Stribild Combination Tab.
[Non-proprietary name]	Elvitegravir/Cobicistat/Emtricitabine/Tenofovir Disoproxil Fumarate
[Applicant]	Japan Tobacco Inc.
[Date of application]	December 6, 2012
[Dosage form/Strength]	Each tablet contains150 mg of elvitegravir, 150 mg of cobicistat, 200 mg of emtricitabine, and 300 mg of tenofovir disoproxil fumarate (245 mg as tenofovir disoproxil)
[Application classification]	Prescription drug (1) Drug with new active ingredients and (2) new combination drug
[Chemical structure]	Elvitegravir



Molecular formula: $C_{23}H_{23}ClFNO_5$
Molecular weight: 447.88
Chemical name:
6-[(3-Chloro-2-fluorophenyl)methyl]-1-[(2*S*)-1-hydroxy-3-methylbutan-2-yl]-7-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid

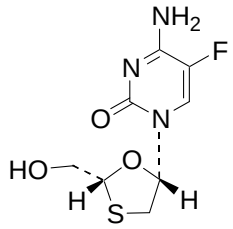
Cobicistat



Molecular formula: $C_{40}H_{53}N_7O_5S_2$
Molecular weight: 776.0

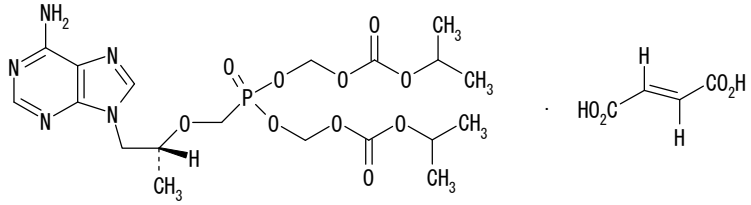
Chemical name:
1,3-Thiazol-5-ylmethyl{(2*R*,5*R*)-5-[(2*S*)-2-(3-methyl-3-{[2-(1-methylethyl)-1,3-thiazol-4-yl]methyl}ureido)-4-(morpholin-4-yl)butanamido]-1,6-diphenylhexan-2-yl}carbamate

Emtricitabine



Molecular formula: $C_8H_{10}FN_3O_3S$
Molecular weight: 247.25
Chemical name:
4-Amino-5-fluoro-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2(1*H*)-one

Tenofovir disoproxil fumarate



Molecular formula: $C_{19}H_{30}N_5O_{10}P \cdot C_4H_4O_4$
Molecular weight: 635.51
Chemical name:
Bis(isopropoxycarbonyloxymethyl){[(1*R*)-2-(6-amino-9*H*-purin-9-yl)-1-methylethoxy]methyl}phosphonate monofumarate

[Items warranting special mention]

- The product is eligible for prior assessment based on the PMSB/ELD Notification No. 1015 dated November 12, 1998.

- Orphan drug (Notification No. 1114-1 from the Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, MHLW, dated November 14, 2012 [elvitegravir, cobicistat]; PFSB/ELD Notification No. 1013001 dated October 13, 2004 [emtricitabine]; PFSB/ELD Notification No. 1212001 dated December 12, 2003 [tenofovir disoproxil fumarate])

[Reviewing office] Office of New Drug IV

Review Results

February 19, 2013

[Brand name]	Stribild Combination Tab.
[Non-proprietary name]	Elvitegravir/Cobicistat/Emtricitabine/Tenofovir Disoproxil Fumarate
[Applicant]	Japan Tobacco Inc.
[Date of application]	December 6, 2012

[Results of review]
Based on the submitted data, it is concluded that the efficacy of the product in treatment-naïve patients with HIV-1 infection has been demonstrated and its safety is acceptable in view of its observed benefits. The safety information of the product in Japanese patients with HIV-1 infection should be carefully collected after the market launch. Also, a post-marketing clinical study should be conducted to investigate the pharmacokinetics of the product in the Japanese population, and information obtained should be appropriately evaluated and provided to be made available in clinical practice.

As a result of its regulatory review, the Pharmaceuticals and Medical Devices Agency has concluded that the product may be approved for the following indication and dosage and administration with the following conditions for approval.

[Indication]	HIV-1 infection
[Dosage and administration]	The usual adult dosage is one tablet (containing 150 mg of elvitegravir, 150 mg of cobicistat, 200 mg of emtricitabine, and 300 mg of tenofovir disoproxil fumarate) administered orally once daily during or immediately after a meal.

[Conditions for approval]
The applicant is required to:

1. Request physicians to obtain patients' informed consent to the use of the product after having thoroughly informed them that the collection of additional data on the efficacy and safety of the product is still ongoing, in light of the fact that a pharmacokinetic study on the product is planned to be conducted in Japan.

2. Submit periodical reports on the progress status of the pharmacokinetic study to be conducted in Japan, as well as the results and analysis of the study promptly after the study completion. The results and analyses of ongoing or planned foreign clinical studies should also be submitted promptly after the study completion.

3. Conduct the post-marketing surveillance which must, as a general rule, cover all patients treated with the product in Japan, until the expiration of the re-examination period, and collect information on the use status of the product (patient characteristics, efficacy and safety [including the efficacy and safety of the product in concomitant use with other drugs], data on drug-drug interactions), thereby submitting periodical reports. Also, the final results of the surveillance should be submitted in support of the application for re-examination.

Prior Assessment Report (1)

December 4, 2012

I. Product Submitted for Prior Assessment

[Intended brand name] Stribild Combination Tab.
[Prior assessment requestor] Japan Tobacco Inc.
[Non-proprietary name] Elvitegravir/Cobicistat/Emtricitabine/Tenofovir Disoproxil Fumarate
[Dosage form/Strength] Each tablet contains150 mg of elvitegravir, 150 mg of cobicistat, 200 mg of emtricitabine, and 300 mg of tenofovir disoproxil fumarate (245 mg as tenofovir disoproxil)
[Intended indication] HIV-1 infection
[Intended dosage and administration]
The usual adult dosage is one tablet (containing 150 mg of elvitegravir, 150 of mg cobicistat, 200 mg of emtricitabine, and 300 mg of tenofovir disoproxil fumarate) administered orally once daily during or immediately after a meal.
[Date of preparatory meeting for prior assessment] July 26, 2012
[Items warranting special mention]
Orphan drug (PFSB/ELD Notification No. 1114-1 dated November 14, 2012 [elvitegravir and cobicistat]; PFSB/ELD Notification No. 1013001 dated October 13, 2004 [emtricitabine]; PFSB/ELD Notification No. 1212001 dated December 12, 2003 [tenofovir disoproxil fumarate])
The product is eligible for prior assessment based on the PMSB/ELD Notification No. 1015 dated November 12, 1998.
Date of approval in the United States: August 27, 2012
This prior assessment is based on the application dossier submitted in the U.S.

II. Comments from the Pharmaceuticals and Medical Devices Agency (PMDA) Given to the Prior Assessment Requestor at the Preparatory Meeting for Prior Assessment and Its Evaluation Results

1. Origin or history of discovery and usage conditions in foreign countries etc.

Stribild Combination Tab. was developed by Gilead Sciences, Inc. in the U.S. (Gilead) as a combination drug comprising elvitegravir (EVG), which is an integrase strand transfer inhibitor (INSTI) for human immunodeficiency virus type 1 (HIV-1), cobicistat (COBI), which inhibits cytochrome P450 (CYP) 3A, and emtricitabine (FTC) and tenofovir disoproxil fumarate (TDF), both of which have been approved as nucleoside or nucleotide reverse transcriptase inhibitors (N(t)RTIs). (Stribild Combination Tab. is hereinafter referred to as the EVG/COBI/FTC/TDF combination.)

EVG was discovered by Japan Tobacco Inc. It inhibits HIV-1 integrase (IN), thereby suppressing the incorporation of HIV-DNA into the host DNA, resulting in the inhibition of HIV-1 provirus formation and viral replication. COBI, discovered by Gilead, does not inhibit HIV-1 protease although COBI is a structural analog of ritonavir (RTV); however, it inhibits CYP3A activity. COBI is included in the EVG/COBI/FTC/TDF combination to increase the exposure level of EVG. FTC (brand name, Emtriva Capsules 200 mg) and TDF (brand name, Viread Tab. 300 mg),

both discovered by Gilead, were approved in Japan, on March 23, 2005 and on March 25, 2004, respectively, for the indication for HIV-1 infection. A combination drug of FTC and TDF (brand name, Truvada Combination Tab.[1)]) was also approved on March 23, 2005 for the same indication.

Anti-HIV drugs approved in Japan are classified into 5 categories: N(t)RTIs, non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), INSTIs, and CC chemokine receptor 5 (CCR5) inhibitors. The *Guideline for anti-HIV treatment*[2)] recommends, as the initial antiretroviral (ARV) treatment, one of the following combinations: (i) 2 N(t)RTIs and 1 NNRTI; (ii) 2 N(t)RTIs and 1 PI (combination with low-dose RTV); or (iii) 2 N(t)RTIs and 1 INSTI. Once the treatment has started, the treatment goal is to suppress HIV RNA below the detection limit, and adherence to a treatment regimen is considered most important. For this purpose, an easy-to-adhere regimen with fewer pill counts and less dosing frequency as well as ARV therapy with fewer adverse reactions is sought after to ensure a high level of adherence.

During the early stage of development of the EVG/COBI/FTC/TDF combination, the development of EVG as a single drug preceded the combination product, and the once-daily dose of EVG in combination with a low-dose RTV was considered to be an optimal regimen [see "4.(ii).B.(5) Dosage and administration"]. However, since RTV inhibits HIV protease, concomitant use of EVG with a low-dose RTV posed a concern of inducing PI-resistant virus. Therefore, COBI was developed as a CYP3A inhibitor without anti-HIV activity. Also, to meet the needs in the clinical settings for a once-daily combination tablet, it was decided to develop the EVG/COBI/FTC/TDF combination in preference to EVG as a single drug.

Under the circumstances, results of the foreign clinical phase III studies (Studies GS-US-236-0102 and GS-US-236-0103) demonstrated the non-inferiority of the EVG/COBI/FTC/TDF combination to the comparators (efavirenz [EFV]/FTC/TDF and atazanavir sulfate [ATV]/RTV + FTC/TDF, respectively) in the virological effect (achievement ratio of HIV-1 RNA levels <50 copies/mL) at Week 48, together with good tolerability. Therefore, the EVG/COBI/FTC/TDF combination could serve as a treatment option for patients with HIV-1 infection. In the U.S., an application for the EVG/COBI/FTC/TDF combination was submitted in October 2011 ahead of EVG and COBI,[3)] and the combination product was approved on August 27, 2012. In Europe, an application was submitted in November 2011 and is currently under review. The EVG/COBI/FTC/TDF combination is approved in 2 foreign countries (the U.S., Canada) as of November 2012.

In Japan, Japan Tobacco Inc. has requested the prior assessment of the EVG/COBI/FTC/TDF combination.

2. Physicochemical properties and specifications

2.A. Summary of the submitted data

2.A.(1) Drug substance

- The drug substance is comprised of EVG, a mixture of COBI and silicon dioxide, FTC, and TDF.

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1) The product was approved on March 23, 2005 under the brand name "Truvada Tab." and, according to the "Handling of Labels and Brand Names of Drugs for the Prevention of Medication Accident (PMSB Notification No. 935, dated September 19, 2000)," a new application for replacement of license to change brand name of drug was submitted as a means to prevent medical accidents. As a result, the product was approved on December 15, 2008 under the name of "Truvada Combination Tab."

2) *Guideline for anti-HIV treatment*, March 2012, by the Research Group for Conquering HIV Infection and Complications, The Research Project on HIV/AIDS funded by the FY 2011 Health and Labour Sciences Research Grants

3) During the development process of EVG and COBI, each as a single drug, and the EVG/COBI/FTC/TDF combination, regulatory submission of COBI and EVG was delayed due to the developmental strategy. Therefore, under the agreement with the U.S. FDA, an application for the EVG/COBI/FTC/TDF combination was submitted ahead of the single drugs. An application for EVG was submitted in May 2012 in the U.S. and in June 2012 in the EU. An application for COBI was submitted in April 2012 in the U.S. and in June 2012 in EU. Both are currently under review.

- FTC and TDF are identical to the drug substances used in the approved products (FTC [Emtriva Capsules 200 mg], TDF [Viread Tab. 300 mg], FTC and TDF [Truvada Combination Tab.]).

2.A.(1).1) EVG

- EVG is white to pale yellow powder. Its tertiary structure, melting point, acid dissociation constant, distribution coefficient, hygroscopicity, and solubility have been determined. EVG is known to exist in 3 crystalline forms ([redacted]), but it is confirmed that only the crystalline form [redacted] is produced in the manufacturing process under the production scale and is stable in room temperature conditions.

- The chemical structure of EVG has been elucidated by nuclear magnetic resonance spectroscopy (^{1}H-NMR, ^{13}C-NMR), mass spectrometry (MS), infrared spectrophotometry (IR), ultraviolet-visible spectrophotometry (UV), elemental analysis, and single-crystal X-ray crystallography.

- [redacted].

- [redacted].

- [redacted].

- The following table shows the results of the stability studies of EVG. Photostability studies demonstrated the stability of the drug substance against light.

Table. Stability studies of EVG

Test	Primary batches	Temperature	Humidity	Storage container	Storage period
Long-term test	4 batches	25°C	60% RH	Polyethylene bag (double-layered) + high-density polyethylene drum	60 months (3 batches) 24 months (1 batch)
Accelerated test	4 batches	40°C	75% RH		6 months

- Based on the above results, a retest period of 60 months has been proposed for the drug substance when stored at ≤30°C in a double-layered polyethylene bag placed in a high-density polyethylene drum.

2.A.(1).2) Mixture of COBI and silicon dioxide

- The mixture of COBI and silicon dioxide is white to pale yellow powder. Its tertiary structure, melting point, acid dissociation constant, partition coefficient, hygroscopicity, and solubility have been determined.

- [redacted].
- [redacted].
- The proposed specifications for the mixture of COBI and silicon dioxide include content, description, identification (UV, IR, silicic acid), purity (heavy metals, related substances [HPLC], residual solvents [GC], optical isomers [HPLC]), water content, bulk density, and assay (HPLC).
- The following table shows the results of the stability studies of the mixture of COBI and silicon dioxide. Photostability studies demonstrated the stability of the drug substance against light.

Table. Stability studies of mixture of COBI and silicon dioxide

Test	Primary batches	Temperature	Humidity	Storage container	Storage period
Long-term test	4 batches	5°C	-	Polyethylene bag (double-layered) + high-density polyethylene drum	24 months (2 batches) 18 months (2 batches)
Accelerated test	4 batches	25°C	60% RH		6 months

- Based on the above results, the retest period of 24 months has been proposed for the mixture of COBI and silicon dioxide when stored at 2°C to 8°C in a double-layered polyethylene bag placed in a high-density polyethylene drum according to the "Guideline for the Evaluation of Stability Data" (PFSB/ELD Notification No. 0603004 dated June 3, 2003, ICH Q1E Guideline). The long-term testing is to be continued up to [redacted] months.

2.A.(2) Drug product

2.A.(2).1) Description and composition of the drug product and formulation development

- The drug product contains 150 mg of EVG, 150 mg of COBI, 200 mg of FTC, and 300 mg of TDF in each tablet. [redacted].
- [redacted].

- The drug product is manufactured by a process comprising granulation/drying, sizing, blending, compressing/sizing, blending, tableting, coating, and packaging. [redacted].

- The proposed specifications for the drug product include content, description, identification (HPLC), purity (degradation products [HPLC]), water content, uniformity of dosage units (HPLC), dissolution (HPLC), and assay (HPLC).

- The following table shows the results of the stability studies of the drug product. Photostability studies demonstrated the stability of the drug product against light.

Table. Stability studies of drug product

Test	Primary batches	Temperature	Humidity	Storage container	Storage period
Long-term test	4 batches	25°C	60% RH	High-density polyethylene bottle, silica gel (desiccant)	24 months (1 batch) 12 months (3 batches)
Intermediate test	4 batches	30°C	65% RH		
Accelerated test	4 batches	40°C	75% RH		6 months

- Based on the above results, a shelf life of 24 months has been proposed for the drug product when stored at room temperature in a high-density polyethylene bottle together with silica gel desiccant, according to the ICH Q1E Guideline. The long-term testing is to be continued up to [redacted] months.

2.B. Outline of the prior assessment by PMDA

- Based on the submitted data and on the results of the following review, PMDA considers that the quality of the drug substance and the drug product is controlled appropriately.

- PMDA asked the prior assessment requestor to explain the reason for having considered it appropriate to control COBI as the mixture with silicon dioxide.

  The prior assessment requestor explained as follows:

  [redacted].

  PMDA accepted the above explanation of the prior assessment requestor.

- [redacted].

  The prior assessment requestor explained as follows:

[redacted].

PMDA accepted the above explanation of the prior assessment requestor.

3. Non-clinical data

3.(i) Summary of Pharmacology studies

3.(i).A. Summary of the submitted data

3.(i).A.(1) Primary pharmacodynamics

- EVG was investigated for the following properties: inhibition of HIV-1 IN; association and dissociation kinetics of EVG with HIV-1 IN; the *in vitro* antiviral activities of EVG against laboratory strains of HIV-1 and human immunodeficiency virus type 2 (HIV-2) and clinical isolates of HIV-1; the effect of serum protein on *in vitro* antiviral activity; and emergence of resistance. COBI was investigated for CYP3A inhibitory and enzyme inactivating actions. Also, EVG, COBI, FTC, and TDF were investigated for the interactions in the following cases: when 2 to 4 of them were concomitantly administered; and when they were concomitantly administered with other anti-HIV-1 drugs. Data in this section are expressed in mean values.

3.(i).A.(1).1) EVG

- Inhibitory activities of EVG against all integration activities of HIV-1 IN (assembly, 3'-processing, strand transfer) and against strand transfer activity were investigated by enzyme assay. The 50% inhibitory concentration (IC_{50}) values against the respective activities above were 5.3 nM and 8.8 nM. IC_{50} values of EVG and its major metabolites M1 and M4 against the strand transfer activity of HIV-1 IN were 7.4, 38.8, and 281.2 nM, respectively.

- The association and dissociation rates for EVG or raltegravir (RAL), an INSTI, binding to purified HIV-1 IN/DNA complex were investigated by scintillation proximity assay. The association rate constants (K_{on}) of EVG and RAL were 1.1 and 0.7 $\mu M^{-1}s^{-1}$, respectively, and their dissociation rate constants (K_{off}) were 0.17 and 0.18 s^{-1}, respectively. The elimination half-lives ($t_{1/2}$) of EVG and RAL bound to purified HIV-1 IN/DNA complex were 11.1 and 11.0 hours, respectively.

- The inhibitory action against HIV-1 DNA integration into the host DNA was investigated in HIV-1-infected MT-4 or SupT1 cells using Alu-PCR assay. As a result, HIV-1 DNA integration into the MT-4 cell DNA was inhibited by EVG (10 nM); EFV (10 nM), an NNRTI; and nelfinavir (NFV, 100 nM), a PI; by 94.6%, 96.5%, and 21.5%, respectively. The amounts of viral DNA synthesized by reverse transcriptase (RT) in the presence of EVG (10 nM), EFV (10 nM), and NFV (100 nM) were 0.9, 0.4, and 0.9 times that in the presence of the vehicle (0.2% dimethyl sulfoxide [DMSO]), respectively. IC_{50} values of EVG, EFV, and amprenavir (APV), a PI, against HIV-1 DNA integration into the SupT1 cell DNA were 0.3, 0.5, and >1000 nM, respectively.

- The antiviral activity of EVG was investigated using laboratory strain (HIV-1/NL4-3, HIV-1/IIIB)-infected MT-4 cells and laboratory strain (HIV-1/IIIB)-infected MT-2 cells, and EC_{50} values of EVG were 0.38, 0.17, and 0.6 nM, respectively. In HIV-1/Ba-L-infected monocytes/macrophages, EC_{50} values of EVG, zidovudine (ZDV), and EFV were 0.67,

0.20, and 2.12 nM, respectively. In HIV-1/IIIB infected MT-2 cells, EC_{50} values of EVG, M1, and M4 were 0.6, 5.6, and 4 nM, respectively. In HIV-1 clinical isolate (8 subtypes: group M [A, B, C, D, E, F, G] and group O)-infected human peripheral blood mononuclear cells (PBMCs), EC_{50} values of EVG, ZDV, EFV, and NFV were 0.10 to 1.26, 0.60 to 25.3, 0.30 to 47.0, and <0.10 to 70.5 nM, respectively. In PBMCs infected with a clinical isolate of HIV-2, EC_{50} values of EVG, ZDV, EFV, and NFV were 0.53, 1.14, >1000, and 16.1 nM, respectively.

- Using HIV-1/III-infected MT-2 cells, the relationship between the time required for the antiviral action of the test drugs to become effective and the time required for the viral DNA to be integrated into the host chromosomes and the intracellular antiviral persistence of the test drugs were investigated. Results showed that EVG (250 nM) and RAL (1.25 μM) exhibited antiviral effect (assessed by the decrease in p24 level compared with the level in the absence of the drugs) when added until the time of viral DNA intergration into the host DNA (12-15 hours after infection). EVG, RAL, lopinavir (LPV), and tenofovir (TFV) were added at high concentrations[4)] to MT-2 cells at 8 hours before HIV-1/IIIB infection, and p24 level was measured at 48 hours after infection. As a result, when the drugs were removed before infection, the antiviral activity of EVG, RAL, and LPV decreased to ≤1/20 times the level observed when the drugs were not removed, whereas the antiviral activity of TFV remained unchanged even after the removal.

- The effect of serum protein on the antiviral action of EVG was investigated using HIV-1/IIIB infected human PBMCs. The antiviral activity (95% inhibitory concentration [EC_{95}]) in the absence of human serum (HS) was 1.25 nM (0.61 ng/mL), and combined protein-adjusted EC_{95}[5)], calculated from the results of an experiment conducted in the presence of human serum albumin (HSA) and α1-acid glycoprotein (AAG), was 100 nM (44.8 ng/mL).

- When HIV-1/IIIB-infected MT-2 cells were serially passaged with increasing EVG concentrations, selected IN amino acid substitutions had the following combinations: H51Y, T66I/K, E92Q, F121Y, S147G, S153Y, E157Q, and/or R263K.[6)] EC_{50} value of EVG against HIV-1/IIIB (containing the combination of T66I, F121Y, and/or S153Y mutations) after passages 6 to 12 (a passage counted up by 1 each time EVG concentration doubled, starting from passage 1 when the subcultivation started) increased 41.9 to 585 times that before passaging. However, no cross-resistance was observed with RAL or TFV. The EC_{50} value of EVG against HIV-1/IIIB (containing the combination of H51Y, E92Q, S147G, and/or E157Q mutations) in MT-4 cells increased 14.9- to 96.8-fold after 30 to 80 passages, compared with that before passaging. However, no cross-resistance was observed with ZDV, EFV, or NFV. Similarly, when HIV-1/IIIB-infected MT-2 cells were serially passaged with increasing concentrations of M1 or M4, selected IN amino acid substitutions had the following combinations: H51Y, T66I and/or S147G, and T66A, E92G, and/or S153F. EC_{50} values of EVG and M1 against HIV-1/IIIB at the passage 11 using M1 were 54 and 245 times those observed before passaging, respectively. Also, EC_{50} values of EVG and M4 against HIV-1/IIIB at the passage 9 using M4 were 93.1 and 115 times those observed before passaging, respectively.

- SupT1 cells infected with HIV-1/HXB2 at the multiplicity of infection (MOI) of 0.1 were serially passaged with EVG or RAL at a constant concentration to investigate whether or not EVG resistance was induced. Amino acid mutations in IN selected in the presence of EVG were T66I/T and Q148R, and those in the presence of RAL were N155H and Q148K.

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4) Concentrations of EVG, RAL, and LPV were 500 times the EC_{50}, and concentration of TFV was 100 times the EC_{50}.
5) Estimated EC_{95} in the presence of 100% HS.
6) Amino acid substitutions are represented with 2 single-letter codes and amino acid sequence numbers, with original amino acid codes first and the mutated amino acid codes last.

- Using purified IN[7] (derived from HIV-1/NL4-3) with single or multiple amino acid mutations introduced, the effect of IN's resistance to EVG acquired from each amino acid mutation was investigated by strand transfer assay. As a result, all mutant IN showed weaker strand transfer activity than the wild-type IN. Of these, IC_{50} values of EVG against strand transfer activity of mutant IN with E92Q or E92Q + S147G increased 4.3 and 7.6 times that against wild-type IN, respectively.

- The antiviral activities (FC[8]) of EVG, RAL, TFV, FTC, ZDV, EFV, and LPV against HIV-1/NL4-3 or HIV-1/HXB2 with amino acid mutations introduced into IN were as shown in the following table.

Table. Antiviral activity (FC) of EVG and other anti-HIV drugs against HIV-1/NL4-3 or HIV-1/HXB2 with mutations introduced into IN

IN genotype [a)]	Resistance (FC)						
	EVG	RAL	TFV	FTC	ZDV	EFV	LPV
H51Y	1.53-3.6	0.83-1.2	1.04-1.5	—	1.32	0.75-1.01	1.1-1.81
T66I	14.5-30.8	1.3-2.3	1.0-3.8	0.9	—	—	—
T66K	39.9-40.8	19.1-19.4	1.2	—	—	—	—
E92Q	32.7-79.2	5.26-11.2	0.9-3.3	1.03-1.1	0.94-1.70	0.84-1.02	1.0-1.58
F121Y	11.8-12.1	7.2-7.3	1.0	—	—	—	—
S147G	2.08-9.7	1.01-1.3	0.84-1.6	—	1.30	0.82-0.99	0.75-1.4
Q148R	107.6-109	34.0-37.6	0.7	—	—	—	0.9
Q148K	49.6	26.1	0.7	—	—	—	0.7
S153Y	4.9-5.0	1.7	1.0-1.1	—	—	—	—
N155H	35.2-67.3	21.0-25.9	1.1-2.8	—	—	—	0.9
E157Q	0.55-2.33	1.64	1.01	—	1.09	0.40-0.98	0.98-1.04
R263K	6.3-6.4	1.0-1.4	0.5-1.2	1.2	—	—	1.6
H51Y + S147G	33	2.1	2.5	—	—	—	1.4
T66I + F121Y	37.2-38.0	10.2-10.4	0.6	—	—	—	—
T66I + S153Y	41.3-42.2	0.9	0.6	—	—	—	—
T66I + R263K	105-107.5	1.0	0.5	0.8	—	—	0.9
E92Q + S147G	69.5-358	6.5-8.44	0.68-0.8	1.0-1.05	0.6-1.48	0.99-1.18	0.76-0.9
E92Q + S147G + H51Y	143-405	6.8-7.0	0.78-0.9	0.9-0.91	0.69-1.33	0.75-1.14	0.77-1.0
E92Q + S147G + H51Y + E157Q	154-437	6.1-6.24	0.9-0.97	0.95-1.1	0.54-0.75	0.29-1.31	0.28-1.0

a) Amino acid mutations obtained in resistance selection experiments with EVG were tested. IN mutants with multiple amino acid mutations simultaneously observed in each test were also tested.

Furthermore, antiviral activities of EVG and other anti-HIV drugs against HIV-1/NL4-3 and HIV-1/HXB2 with other mutations introduced were investigated. EC_{50} values of EVG against mutant IN with T66A/I/K, E92G/Q, F121Y, P145S, Q146I/L, Q148K/R, and N155H/S increased ≥10 fold compared with the EC_{50} value against wild-type IN, and EC_{50} values of RAL against mutant IN with T66K, E92Q, Y143R, Q148H/K/R, and N155H/S increased ≥10 fold compared with the EC_{50} value against wild-type IN. Except H51Y mutation, the sensitivity of mutant IN to M1 and M4 was similar to that to EVG.

- Antiviral activity (EC_{50}) of EVG against HIV-1 with mutations in RT or protease and against HIV-1 with genes resistant to NRTI, NNRTI or PI was investigated. None of the mutant strains showed cross-resistance to EVG.

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7) The following 7 mutations were introduced: E92Q, H51Y, S147G, E157Q, E92Q + S147G, H51Y + E92Q + S147G, and H51Y + E92Q + S147G + E157Q.

8) Fold-change in EC_{50}, (EC_{50} against mutant strain)/(EC_{50} against wild-type strain)

- In the EVG/COBI/FTC/TDF combination groups in the phase III studies (Studies GS-US-236-0102 and GS-US-236-0103) and in the phase II study (Study GS-US-236-0104),[9] T66I, E92Q, Q148R, and N155H mutations were found in IN.[10] In 11 of 23 patients in whom results of analysis of resistance to EVG were available, the sensitivity to EVG decreased 67 fold on average. Also, the sensitivity to RAL decreased 7.9 fold.

3.(i).A.(1).2) COBI

- CYP3A inhibitory and inactivating actions were compared between COBI and RTV. IC_{50} values of COBI and RTV against human liver microsomal CYP3A activity[11] ranged from 0.03 to 0.29 μM and from 0.02 to 0.28 μM, respectively. Parameters of COBI for CYP3A inactivation based on mechanism-based inhibition (MBI) (k_{inact} [theoretical maximum inactivation rate constant] = 0.47 min^{-1}, K_I [inactivation dissociation constant] = 1.1 μM) were similar to those of RTV (k_{inact} = 0.23 min^{-1}, K_I = 0.26 μM).

3.(i).A.(1).3) Concomitant use with 2 to 4 drugs from among EVG, COBI, FTC, and TDF

- Anti-HIV-1 activity of 2–drug combinations, EVG and FTC, EVG and TFV, and FTC and TFV, was investigated based on the inhibition rate of HIV-1 infection when 2-fold serial dilutions of each drug were applied alone or in combination as stated above. Results were as shown in the following table, which demonstrated a synergistic effect of the concomitant use of 2 drugs.

Table. Anti-HIV 1 activity following administrationof of EVG in combination with other anti-HIV drugs

Combination of drugs	Cells	Volume[a] (μM²%)	
		Synergistic effect	Antagonistic effect
EVG + FTC	MT-2	167.5	-7.84
EVG + TFV	MT-2	133.2	-4.0
TFV + FTC	MT-2	114.9	-0.5

a) Calculated by MacSynergy II software analysis and assessed as follows: <25 μM²%, slight synergistic (additive) effect; ≥25 and ≤50 μM²%, weak synergistic/antagonistic effect; ≥50 and <100 μM²%, moderate synergistic/antagonistic effect; ≥100 μM²%, strong synergistic/antagonistic effect

In a similar manner, the effect of concomitant use of EVG with other approved anti-HIV-1 drugs (NRTIs, NNRTIs, PIs), a fusion inhibitor (enfuvirtide), a CCR5 inhibitor (maraviroc [MVC]), and RAL was investigated. All drugs studied exhibited an additive or synergistic effects with EVG.

- Anti-HIV-1 activity following administration of 3- or 4-drug combinations including EVG, TFV, and/or FTC, was investigated based on the EC_{50} and the inhibitory rate against HIV-1 infection when 1.5-fold serial dilutions of each drug were applied alone or in combination including the above drugs. Results were as shown in the following table.

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9) HIV-1 strains isolated from the plasma samples collected from subjects with virological failure and subjects with HIV-1 RNA level exceeding 400 copies/mL at Week 48 of administration (or Week 60 in phase II study [Study GS-US-236-0104]) or at treatment discontinuation were subjected to analysis for resistance, and 27 of 749 subjects (3.6%) in the proposed drug product group were subjected to analysis for the emergence of resistance.

10) These mutations were explained as major resistant mutations against EVG. Also, H51Y, L68V, G140C, S153A, and E157Q mutations in IN were observed in 1 subject each together with the major resistant mutations, and they were explained as secondary resistant mutations.

11) Midazolam (MDZ)1'-hydroxylation reaction, testosterone 6β-hydroxylation reaction, terfenadine t-butylhydroxylation reaction, EVG hydroxylation reaction, ATV oxidation, and telaprevir oxidation reaction.

Table. Anti-HIV-1 activities following administration of3- or 4-drug combinations including EVG and other anti-HIV drugs

Combination of drugs	Cells	Combination Index (CI)[a)]± SD	Interaction
EVG + TFV + FTC	MT-2	0.45 ± 0.10	Synergistic
EVG + TFV + FTC (25 μM COBI)	MT-2	0.45 ± 0.06	Synergistic
FTC + FTC + FTC	MT-2	0.92 ± 0.06	Additive
EFV + TFV + FTC	MT-2	0.56 ± 0.08	Synergistic
d4T + ZDV + RBV	MT-2	>5.9	Antagonistic

d4T: Stavudine

a) Calculated by CalcuSyn software analysis and assessed as follows: Combination Index (CI) >1.1, antagonistic effect; $0.9 \leq CI \leq 1.1$, additive effect; CI <0.9, synergistic effect.

3.(i).A.(2) Secondary pharmacodynamics

- EVG was investigated for an inhibitory effect on RT and protease, and an antiviral activity against viruses other than HIV-1, *in vitro* cytotoxicity, mitochondrial toxicity, etc. COBI was investigated for antiviral activity against HIV-1, HBV, and hepatitis C virus (HCV), the effect on the antiviral activity of anti-HIV drugs, *in vitro* cytotoxicity, metabolic toxicity, etc. All data in this section are expressed in mean values.

3.(i).A.(2).1) EVG

- The inhibitory activity of EVG against RT and protease of HIV-1 was investigated. No inhibitory effect was found (IC_{50} >50 μM for both activities).

- The *in vitro* antiviral activity of EVG against HBV and HCV was investigated. EVG had no antiviral effects on these viral species (EC_{50} values of >6.25 and 22.9 μM, respectively).

- *In vitro* cytotoxicity of EVG was investigated. The concentration of EVG necessary to decrease the cell viability by 50% (50% cytotoxicity concentration, CC_{50}) was >100 μM against primary cultured human PBMCs, 40 μM against primary cultured human T lymphocytes, >500 μM against primary cultured human monocytes/macrophages, and 25.6 μM against primary cultured human macrophages.

- EVG up to 10 μM showed no binding inhibitory or stimulatory effects on 22 types of receptors,[12)] 7 types of enzymes,[13)] or 3 types of cell-based assay systems.[14)] The inhibitory effect (IC_{50}) values against human topoisomerase I and II were >50 μM and >150 μM, respectively.

- The effect of EVG on mitochondrial DNA synthesis in HepG2 hepatocytes was investigated. The amount of mitochondrial DNA remained unchanged even after treatment with EVG (10 μM) for 14 days.

3.(i).A.(2).2) COBI

- The inhibitory effect of COBI on HIV-1 protease was investigated. COBI had no inhibitory effect up to 30 μM, whereas IC_{50} value of RTV was 0.6 nM. In a test of cytopathic effect of HIV-1 in MT-2 cells, COBI (30 μM) did not inhibit HIV-1 replication in the absence of HS, and COBI (90 μM) did not inhibit HIV-1 replication even in the presence of 40% HS, HSA at physiological concentration (35 mg/mL), or AAG (1.0 mg/mL). In the human

12) DPCPX, prazosin, RX 821002, (-)CGP 12177 (β_1, β_2), WIN 55212-2, SCH 23390, GABA, CGP 39653, pyrilamine, APT, (R)α-Me-histamine, QNB, cytisine, α-bungarotoxin, naloxone, serotonin, ketanserin, BRL 43694, DTG, (+)PN 200-11, and glibenclamide receptors

13) Phospholipase A_2, COX_1, constitutive NOS (endothelial), phosphodiesterase IV, HIV-1 protease, protein kinase C, acetylcholinesterase, and MAO-A

14) Include cell adhesion (ICAM-1/VCAM-1-mediated) and immune cell functions of IL-2 secretion and mixed lymphocyte reaction (splenic lymphocytes).

PBMC infection system, COBI had no antiviral activity against 17 types of HIV-1 strains belonging to group M (subtypes A, B, C, D, E, F, G), N, or O or against 2 types of HIV-2 strains (EC_{50}, >11 μM and >6 μM, respectively). In a test of cytopathic effect of HIV-1 in MT-2 cells, neither COBI nor its metabolites (M21, M26, M31) showed antiviral activity (EC_{50} >30 μM).

- Antiviral activity of COBI against HBV and against HCV was investigated using WT-42 cells[15] and Huh-Luc cells[16], respectively. COBI did not have antiviral activity against either of the viruses (EC_{50}, >12.5 μM and >30 μM, respectively).

- In a test of cytopathic effect of HIV-1 in MT-2 cells, drug-drug interactions between COBI and ARV drugs[17] were investigated. Activities (EC_{50} values) of all ARV drugs tested in the presence of COBI (5 μM) were within the range of approximately 0.7 to 1.9 times the activities seen in the absence of COBI.

- IC_{50} values of COBI against HIV-1 protease and against cathepsin D, a host protease, were both >30 μM, whereas IC_{50} values of RTV against these proteases were 0.6 nM and 0.87 μM, respectively. IC_{50} values of COBI and RTV against host proteasome were 12.8 μM and 7.9 μM, respectively.

- The effect of COBI on adipocyte functions was investigated by measuring lipid accumulation in cultured human adipocytes and insulin-induced glucose uptake in differentiated murine adipocytes. As a result, COBI (30 μM) had no effect either on lipid accumulation (EC_{50} of RTV, 16 μM) or on glucose uptake (9.5% inhibition by COBI [10 μM], 55% inhibition by RTV [10 μM]), which suggested that the metabolism-related toxicity of COBI was lower than that of RTV.

- Potential molecular targets of COBI were investigated by radioligand binding tests using 67 mammalian ion channels and receptors. COBI (10 μM) inhibited human ERG (hERG) potassium ion channel by 54%. IC_{50} values against rat L-type calcium ion channel (benzothiazepine binding site) and sodium ion channel (site 2) were 6.45 and 0.137 μM, respectively.

- *In vitro* cytotoxicity of COBI was investigated. CC_{50} values for MT-2 cells and HepG2 cells were 88.6 μM and 44 μM, respectively.

3.(i).A.(3) Safety pharmacology

- The effects of EVG on major organ systems (central nervous system [CNS], cardiovascular system, respiratory system), digestive system, and renal/urinary system was investigated. The effects of COBI on the major organ systems were investigated.

3.(i).A.(3).1) EVG

- In rats, EVG had no adverse effects on CNS, intestinal charcoal transport, or renal/urinary system (up to 2000 mg/kg, orally [p.o.])

- In dogs, EVG had no adverse effects on the cardiovascular system or respiratory system (up to 100 mg/kg).

- In an *in vitro* system, EVG had no effects on hERG current at 0.1 or 1 μM, while at 10 μM,

15) HepG2 cells stably producing wild-type HBV
16) Huh-7 cells stably replicating HCV replicon, a subgenome of genotype 1b
17) Lamivudine (3TC), abacavir (ABC), FTC, TFV, ZDV, EFV, nevirapine (NVP), ATV, darunavir (DRV), EVG, and RAL

it suppressed the hERG current by 24.3%. Also, EVG up to 3.0 μM had no effect on the action potential of isolated papillary muscles of guinea pigs.

3.(i).A.(3).2) COBI

- In rats, COBI had no adverse effects on CNS up to 50 mg/kg, however, at ≥150 mg/kg, COBI caused salivation and hypoactivity, and decreased exploratory behavior, locomotor activity and body temperature.

- In rats, COBI had no adverse effects on the respiratory system (up to 500 mg/kg).

- In a patchclamp test, COBI inhibited hERG potassium current (IC_{50} = 1.8 μM) and $hCa_v1.2$ L-type calcium channel (IC_{50} = 6 μM), and weakly inhibited $hNa_v1.5$ sodium channel (IC_{50} = 86.5 μM). In rabbit Purkinje fibers (in the absence of protein), COBI decreased the active potential duration (APD) at ≥1 μM, but did not cause triangulation, electric instability, or alternating variation effect of the action potential.

- In a Langendorff assay with isolated rabbit heart (in the absence of protein), COBI, alone at ≥1 μM, exhibited a negative inotropic effect and decreased the monophasic action potential duration (MAPD). In a separate study as well, COBI at ≥1.5 μM exhibited a negative inotropic effect (reduction of left ventricular [LV] function) and prolonged PR intervals. Effects of concomitant use of COBI and ATV on PR intervals and the LV function were similar to those noted with COBI alone.

- In conscious dogs equipped with a telemetric monitoring device, COBI up to the maximum dose of 45 mg/kg[18)] did not show any adverse effects either on hemodynamics or on parameters of electrocardiogram (ECG).[19)]

3.(i).B. Outline of prior assessment by PMDA

- Based on the submitted primary pharmacodynamic data, PMDA considers that both EVG alone and concomitant use of 4 drugs, EVG + COBI + FTC + TFV, are effective against HIV-1. The clinical efficacy of the proposed drug product will be discussed in "4.(ii).B.(2) Efficacy evaluation."

- Since EVG has a quinolone moiety, PMDA asked the prior assessment requestor to explain whether or not EVG has antimicrobial activity and whether or not EVG induces the emergence of resistant bacteria.

  The prior assessment requestor responded as follows:
  In a bacterial reverse mutation test, EVG showed antimicrobial activity, but the activity was extremely weaker than quinolone antimicrobial drugs (46.8-1500 times less potent[20)]). Also, in a 26-week repeated dose toxicity study in rats, administration of 2000 mg/kg of EVG caused only approximately a 2-fold increase in cecum weight[21)] compared with the control group, and did not show any gastrointestinal symptoms such as diarrhea. The results suggest that the antimicrobial effect of EVG is weaker than quinolone antimicrobial drugs

18) Plasma COBI concentration at 1 hour after dosing (45 mg/kg) was 2530 to 8950 ng/mL (3.3-11.5 μM or 2.3-8 times the C_{max} following administration of 150 mg to humans).

19) A slight increase in PR intervals (maximum, 12.2 msec) compared with the vehicle control group occurred mainly at 1 to 6 hours after dosing, but PR intervals (mean, 91.8-99.6 msec) did not exceed the upper limit of normal (130 msec) in dogs at any time point.

20) Comparison of the dose of EVG and the dose of norfloxacin that showed antimicrobial activity in the reverse mutation test using 4 *Salmonella typhimurium* species and 1 *Escherichia coli* species (among quinolones tested for comparison [garenoxacin, lomefloxacin, norfloxacin, balofloxacin], norfloxacin showed antimicrobial activity against 3 bacterial species at the highest dose.)

21) The prior assessment requestor explained that antimicrobial drugs are apt to induce cecal dilatation and diarrhea due to change in the intestinal bacterial flora in rats.

and is extremely unlikely to induce the emergence of resistant bacteria.

Given that EVG has antimicrobial activity and that whether or not EVG induces the emergence of resistant bacteria has not been investigated, PMDA considers that the risk of emerging resistant bacteria cannot be ruled out. Therefore, information should be continuously collected on whether EVG induces the emergence of resistant bacteria and, in addition, on whether EVG shows cross-resistance with quinolone antimicrobial drugs. This information should be provided to healthcare providers in clinical settings in an appropriate manner.

- PMDA asked the prior assessment requestor about the information on the resistance to FTC and TDF (genotypic resistance, phenotypic resistance) in and out of Japan.

  The prior assessment requestor responded as follows:
  It is reported that M184V/I mutation in RT resulted in ≥100-fold increase in FC[8)] for FTC[22)] and that K65R mutation in RT resulted in 2 to 4-fold increase in FC[8)] for TDF.[23)] It is also reported, based on the annual change in the frequency of emergence of resistance from 2003 to 2010 in the U.S., that the frequency of M184V/I mutation decreased from 44.0% to 17.9%, and the frequency of K65R mutation from 4.3% to 2.1%.[24)] In Japan, the frequency of M184V/I mutation was 0.4% (14 of 3688 patients) and the frequency of K65R mutation was 0.1% (2 of 3688 patients), according to the cumulative survey from 2003 to 2010 of drug-resistant HIV in patients with newly diagnosed HIV/AIDS.[25)] However, the frequency of drug-resistant HIV observed showed a tendency to increase each fiscal year (5.9% [16 of 273 patients] in FY2003, 12.5% [64 of 524 patients] in FY2010). After the market launch, information on resistance will be collected from literature reports published in and outside of Japan, and information on the emergence of new resistant mutations, if any, will be provided to healthcare providers in clinical settings in an appropriate manner.

  PMDA considers as follows:
  As regards the emergence of FTC- or TDF-resistant HIV strains, there is a tendency of increase in drug-resistant HIV strains in Japan although frequencies of M184V/I and K65R mutations are low. As to EVG-resistant HIV strains, cross-resistance of HIV is observed between EVG and RAL, and the profile of EVG resistance mutation has not been fully elucidated by clinical and non-clinical studies conducted so far. Therefore, information on resistance to FTC, TDF, and EVG should be continuously collected in and out of Japan after the market launch and appropriately provided to healthcare providers in clinical settings.

22) Wainberg MA et al. *Antivir Ther*. 1999;4:87-94
23) White KL et al. *Antimicrob Agent Chemother*. 2002;46:3437-3446, White KL et al. *AIDS*. 2005;19:1751-1760, McColl DJ et al. *Antivir Ther*. 2008;13:189-197
24) Miller MD et al. *Antivir Ther*. 2012;17:993-999
25) 2010 Annual report on "Studies on the Trend in HIV Genotypes and Drug-resistant Strains Prevalent in Japan and on the Establishment of Treatment Methods," The Research Project on HIV/AIDS, Health and Labour Sciences Research Grants

3.(ii) Summary of Pharmacokinetics studies

3.(ii).A. Summary of the submitted data

- Pharmacokinetics of EVG and COBI was investigated following oral administration of either drug alone to mice, rats, dogs, and monkeys. For FTC and TDF, no new nonclinical pharmacokinetic data were submitted. Pharmacokinetics of each active ingredient in the EVG/COBI/FTC/TDF combination was not investigated because metabolic interactions other than inhibition of EVG metabolism by COBI were considered least likely, based on the pharmacokinetic profile of each active ingredient.[26)] Data in this section are expressed in mean values.

3.(ii).A.(1) Absorption

3.(ii).A.(1).1) EVG

- Transcellular transport of EVG was investigated in an *in vitro* study using porcine kidney-derived cells (LLC-PK1 cells) expressing human MDR1. The transmembrane permeation rate[27)] of EVG in the MDR1-expressing cells (13.6-15.0) was higher than that of the positive control digoxin (9.1-10.3), which suggested that EVG served as a substrate for human MDR1.

- Following a single oral administration of EVG (1, 3, 10 mg/kg) to rats and dogs, plasma EVG concentration reached the maximum level (C_{max}) within 1 hour, with bioavailability (BA) being 30.0% to 34.9% and 26.0% to 33.0%, respectively. Following the administration under fasting conditions, C_{max} of EVG increased approximately 2-fold but the area under the plasma concentration-time curve (AUC) remained unchanged.

- In 7-day repeated oral administration of ^{14}C-labeled EVG (3 mg/kg) to rats, the ratio of the area under plasma concentration-time curve from time 0 to τ, the dosing interval, (AUC_{tau}) on Day 1 to that on Day 7 was 1.16, indicating that EVG was not accumulated after repeated administration. In toxicokinetics (TK) studies, EVG was not accumulated after repeated oral administration for 4 to 104 weeks to mice (100-2000 mg/kg/day), to rats (100-2000 mg/kg/day), or to dogs (10-100 mg/kg/day). In mice and rats, EVG exposure levels were higher in females than in males, probably due to the sexual difference in CYP3A expression levels in rodents.

3.(ii).A.(1).2) COBI

- In an *in vitro* study using human colon cancer-derived cells (Caco-2 cells), the apparent permeation rate of COBI from apical surface to basolateral surface (7.61×10^{-6} cm/s) was similar to that from basolateral surface to apical surface (8.51×10^{-6} cm/s).

- Following a single oral administration of COBI to rats (5 mg/kg), dogs (5 mg/kg), and monkeys (6 mg/kg), BA was 33%, 11%, and 7.3%, respectively. Following a single oral administration of COBI to male and female mice (30, 100, 300 mg/kg), male and female rats (5, 25, 100 mg/kg[28)]), and male dogs (10, 30, 100 mg/kg), more than dose-proportional increases in the exposure level were seen in male and female rats, and male dogs (AUC_{0-t} in male rats at 5, 25, and 100 mg/kg was 594, 13,233, and 65,185 nM·h, respectively; AUC_{0-t} in female rats at 25 and 110 mg/kg was 26,087 and 170,525 nM·h, respectively; and AUC_{0-t} in male dogs at 10, 30, and 100 mg/kg was 355, 34,538, and 102,223 nM·h,

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26) Dogs received capsules filled with EVG only tablets, COBI only tablets, and TDF/FTC combination tablets, or 2 prototypic tablets containing 4 active ingredients (bilayer tablets consisting of EVG + COBI layer and TDF + FTC layer; triple-layer tablets consisting of TDF + FTC layer, EVG + COBI layer, and TDF + FTC layer), and plasma concentrations (t_{max}, C_{max}, AUC_{tau}) were compared. Results confirmed that the exposure level of each active ingredient did not differ significantly between the concomitant use with each ingredient alone and 2 combination tablets.

27) Ratio of the permeation from apical surface to basolateral surface to the permeation vice versa

28) Female rats received only 25 or 110 mg/kg.

respectively). The prior assessment requestor explained that saturation of the first-pass effect contributed to the findings. In mice, no sexual difference was noted in the exposure level, whereas in rats, the exposure level tended to be higher in females (AUC_{0-t} in males at 25 and 100 mg/kg was 13,233 and 65,185 nM·h, respectively; AUC_{0-t} in females at 25 and 110 mg/kg was 26,087 and 170,525 nM·h, respectively).

3.(ii).A.(2) Distribution

3.(ii).A.(2).1) EVG

- In rats, dogs, monkeys, and humans, plasma protein binding rate of EVG (0.1, 1, 10 μg/mL) was as high as 98.8% to 99.9% in all animal species studied. In humans, serum albumin is the major plasma protein that EVG binds (plasma protein binding rate was 99.4% at all EVG concentrations with 5% human serum albumin and 39.1%-40.7% with 0.07% α1-acid glycoprotein). The distribution of EVG (0.1, 1, 10 μg/mL) in blood cells was 2.2% to 3.2% in rats, 25.6% to 32.4% in dogs, 26.1% to 28.6% in monkeys, and 20.8% to 24.0% in humans.

- ^{14}C-labeled EVG (3 mg/kg) was administered orally in a single dose to rats, and tissue distribution was investigated. Radioactivity was distributed in the liver, adrenal, kidneys, heart, lungs, and pancreas at 0.25 hours after dosing. Radioactivity in the plasma and organs, except the epididymis and testis which showed the maximum radioactivity level at 4 hours after dosing, reached the maximum level at 0.5 hours after dosing and decreased below the detection limit at 96 hours after dosing. The tissue/plasma concentration ratio was generally <1 except the liver and the gastrointestinal tract. When RTV (20 mg/kg) was administered to rats at 12 and 2 hours before administration of ^{14}C-labeled EVG (10 mg/kg), tissue radioactivity concentration at 8 hours after EVG administration was 1.5 times that found in rats not pre-treated with RTV, but no change was noted in the tissue/plasma concentration ratio. Distribution of EVG in the central nervous system was not seen regardless of whether or not RTV was administered in advance.

3.(ii).A.(2).2) COBI

- In mice, rats, dogs, monkeys, and humans, plasma protein binding rate of COBI (1, 10, 30 μM) was 90.9% to 97.7%, and the plasma/whole blood concentration ratio was 0.508 to 0.605.

- Following a single oral administration of ^{14}C-labeled COBI (10 mg/kg) to rats, radioactivity concentrations reached the maximum level within 1 hour in all organs except the brain, large intestine, and testis. Even at 24 hours after dosing, radioactivity was detected in tissues other than bone, brain, eyes (lens), and bone marrow. Radioactivity concentrations higher than that in the plasma were found in the liver, heart, large intestine, adrenals, kidneys, lungs, lymph nodes, pancreas, pituitary gland, salivary gland, small intestine, spleen, stomach, thyroid gland, brown fat, white fat, bone marrow, uvea, skeletal muscles, prostate gland, skin, and thymus.

- In pigmented rats, radioactivity was distributed at a higher concentration in the uvea than in plasma (in the uvea and plasma, 5820 and 3060 ngEq/g tissue at 1 hour after dosing, respectively; 6530 and 118 ngEq/g tissue at 24 hours after dosing, respectively). Also, radioactivity concentration was higher in pigmented skin than in non-pigmented skin (in pigmented and non-pigmented skin, 292 and 224 ngEq/g tissue at 0.25 hours after dosing, respectively; 1440 and 1280 ngEq/g tissue at 1 hour after dosing, respectively). These results suggested that COBI has a melanin-binding property. The prior assessment requestor explained that multiple administration of COBI or the proposed drug product to humans was unlikely to pose safety problems related to melanin-containing tissues for the following reasons: (1) Radioactivity in melanin-containing tissues decreased with time, suggesting that

the binding with tissues was reversible; (2) in a 9-month repeated dose toxicity study in dogs, COBI up to 20 mg/kg did not cause any ophthalmological findings or histopathological changes of eyes that were attributable to administration [see "3.(iii).A.(2).2).(g) Nine-month repeated oral dose toxicity study in dogs"], and; (3) in the phase III studies (Studies GS-US-236-0102 and GS-US-236-0103, at Week 48), the incidence of adverse reactions related to eye disorders in the proposed product group (0.7% [5 of 701 patients]) was lower than in the comparator groups (2.0% [7 of 352 patients] in the EFV/FTC/TDF group, 14.4% [51 of 355 patients] in the ATV/RTV + FTC/TDF group), and none of the reported adverse drug reactions (blepharospasm, dry eye, eye pruritus, lacrimation increased, ocular icterus, visual impairment) were related to retinal abnormalities.

3.(ii).A.(3) Metabolism

3.(ii).A.(3).1) EVG

- Metabolic pathways of EVG were investigated in *in vitro* studies using liver microsomes of mice, rats, rabbits, dogs, monkeys, and humans and in *in vivo* studies with rats and dogs. As a result, the metabolic pathways of EVG were postulated as shown in the following figure, which suggested that the major metabolic pathways are CYP3A-mediated hydroxylation (M1) and, in humans, UGT1A1- and UGT1A3-mediated glucuronidation of carbonyl group (M4). Results also suggested hydroxylation of benzylic position (M2), direct glucuronidation (M3), and secondary reactions after hydroxylation (M7, M8).

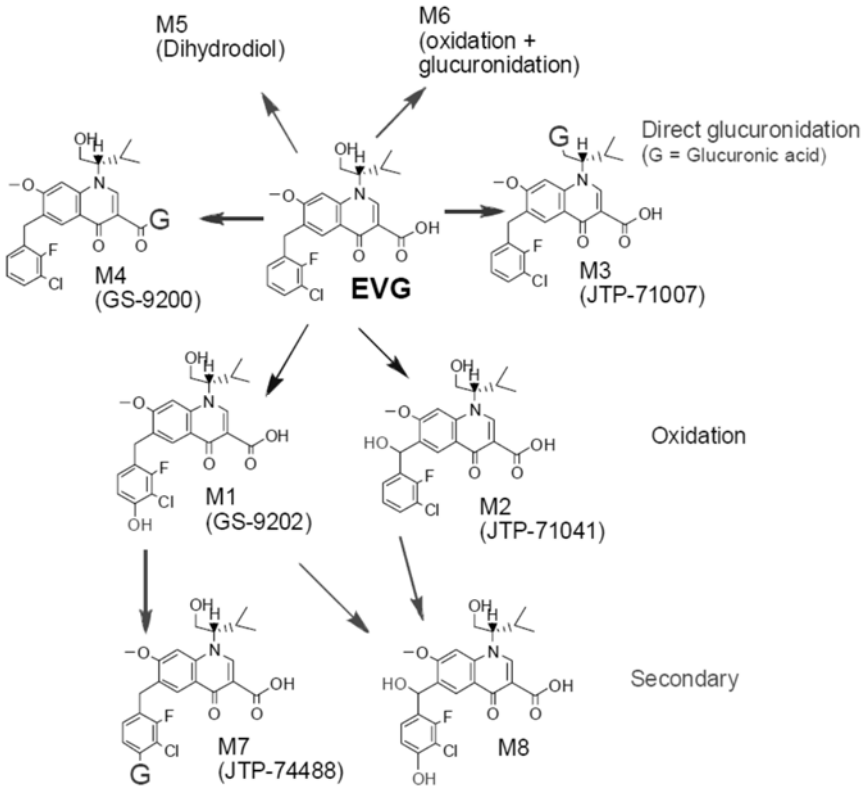

Figure. Postulated metabolic pathways of EVG

3.(ii).A.(3).2) COBI

- Metabolic pathways of COBI were investigated in *in vitro* studies using human liver cells and liver microsomes of mice, rats, dogs, and humans, and in *in vivo* studies with mice, rats, dogs, and humans. As a result, the metabolic pathways of COBI were postulated as

shown in the following figure, which suggested that the major metabolic pathways are oxidation of methine carbon in isopropyl group (M31), breakage of the group adjacent to methylurea (M26), breakage of carbamate ester (M21), and breakage and de-ethylation of morpholine ring (M39). Also, an *in vitro* study using human liver microsomes showed that CYP3A4 and CYP2D6 were chiefly involved in the metabolism of COBI.

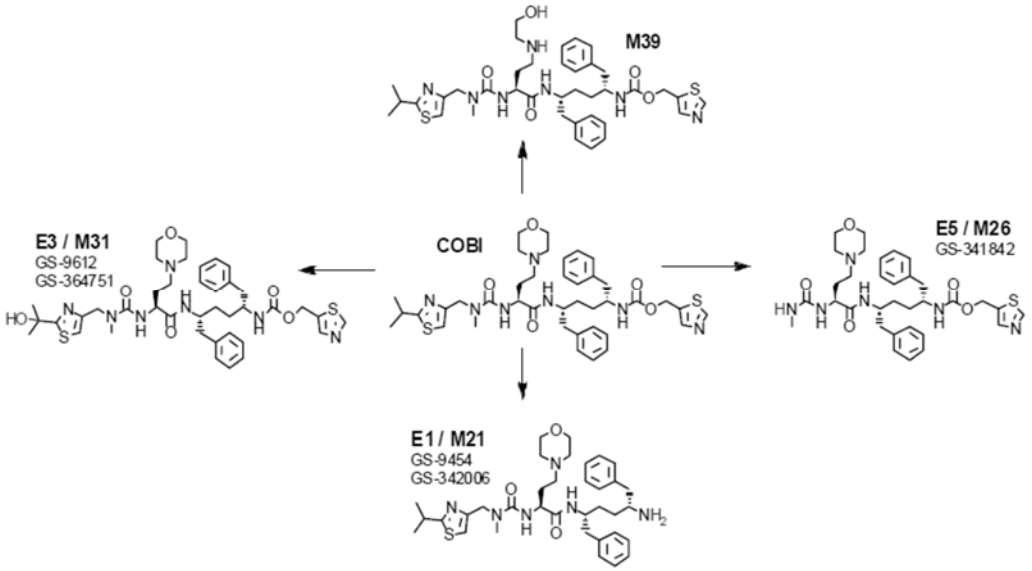

Figure. Postulated metabolic pathways of COBI

3.(ii).A.(4) Excretion

3.(ii).A.(4).1) EVG

- Following the oral administration of ^{14}C-labeled EVG (3 mg/kg) to rats and dogs, 96.7% and 95.5% of the administered radioactivity was recovered up to 48 hours after dosing, respectively. The results were similar to those found after intravenous administration (1 mg/kg). The radioactivity recovered in feces accounted for 96.5% and 95.0% of the administered radioactivity, respectively, while in urine, 0.1% and 0.5% of the administered radioactivity was recovered, respectively. In biliary-cannulated rats, the biliary excretion rate up to 48 hours after dosing was 25.0%. When the recovered bile was intraduodenally administered to rats, the total radioactivity recovered in the bile and urine accounted for 6.0% of the administered radioactivity, which suggested that EVG is unlikely to undergo enterohepatic circulation.

- In the reproductive and developmental toxicity study in rats, EVG (300, 1000, 2000 mg/kg/day) was administered orally from Gestation day 7 to Lactation day 20 or 24. Although EVG was excreted in milk, the milk/plasma concentration ratio at 30 minutes after dosing was approximately 0.1 at any doses.

3.(ii).A.(4).2) COBI

- Following the single oral administration of ^{14}C-labeled COBI (30, 10, 5 mg/kg) to mice, rats, and dogs, the radioactivity recovered up to 168 hours after dosing accounted for 88.7%, 93.5%, and 86.1% of the administered radioactivity, respectively. The radioactivity recovered in feces accounted for 85.9%, 91.4%, and 80.5% of the administered radioactivity, respectively, and the radioactivity recovered in urine accounted for 2.0%, 2.1%, and 2.1% of administered radioactivity, respectively. When ^{14}C-labeled COBI (10 mg/kg) was orally administered to biliary-cannulated rats and dogs, 69.3% of the administered radioactivity was recovered in bile in rats up to 168 hours after dosing and

63.9% in dogsup to 48 hours after dosing.

- In the reproductive and developmental toxicity study in rats, COBI (10, 30, 75 mg/kg/day) was administered orally from Gestation day 6 until day 20 to 22 postpartum. As a result, COBI was excreted in milk, with the milk/plasma concentration ratio of COBI being 1.3 to 1.9 at 2 hours after dosing.

3.(ii).A.(5) Pharmacokinetic drug-drug interactions

3.(ii).A.(5).1) EVG

- The inhibitory action of EVG against CYP450 (CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A) was investigated in an *in vitro* study using human liver microsomes. EVG inhibited CYP3A (IC_{50} = 28.32 μg/mL) but not other CYP450 isoforms tested (IC_{50} >30 μg/mL).

- CYP450 (CYP1A2, CYP2C9, CYP2C19[29)], CYP3A4)-inducing activity of EVG (0.1, 1, 10 μg/mL) was investigated in an *in vitro* study using human liver cells. The activity of EVG to induce CYP1A2, CYP2C9, and CYP3A4 (0.63-1.58, 0.92-2.72, and 1.48-19.1 fold induction[30)], respectively) was weaker than the activity of the positive controls β-naphthoflavone (a CYP1A2 inducer) and rifampicin (a CYP2C9 and CYP2C19 inducer at 20 μM, a CYP3A inducer at 10 μM) (30.6-48.4, 3.14-4.29, and 25.7-34.1 fold induction, respectively). The prior assessment requestor discussed that the CYP3A4-inducing activity of EVG would not have any significant clinical effect because the induced CYP3A4 activity would be inhibited by COBI.

- IC_{50} values of EVG were 0.44 μM against OATP1B3 and >30 μM against MDR1.

- Ketoconazole (a CYP3A4 inhibitor, also a UGT1A1 inhibitor) and ATV (a UGT1A1 selective inhibitor) inhibited M4 formation in human liver microsomes (IC_{50} = 9.6 μM and 0.4 μM, respectively).

3.(ii).A.(5).2) COBI

- In the *in vitro* test using human liver microsomes, the inhibitory effect of COBI against CYP3A (IC_{50} = 0.15 μg/mL, k_{inact} = 0.47 min^{-1}, K_I = 1.1 μM) was comparable to that of RTV (IC_{50} = 0.11 μg/mL, k_{inact} = 0.23 min^{-1}, K_I = 0.26 μM). COBI did not inhibit CYP1A2, CYP2C9, or CYP2C19 (IC_{50} >25 μg/mL), while IC_{50} values against CYP2C8, CYP2D6, and CYP2B6 were 30.1, 9.2, and 2.8 μg/mL, respectively.

- In the *in vitro* test using human liver cells, the activity of COBI (1, 3, 10, 30 μM) to induce CYP1A2, CYP2B6, CYP3A4, UGT1A1, and MDR1 was investigated. The activity of COBI to induce CYP1A2, CYP2B6, CYP3A4, UGT1A1, and MDR1 (1.1-10.1, 0.4-1.7, 4.4-12.6, 1.1-1.7, and 0.8-1.3 fold induction,[31)] respectively) was lower than that of positive controls, 3-methylcholanthrene (a CYP1A2 inducer), phenobarbital (a CYP2B6 inducer), and rifampicin (a CYP3A4, UGT1A1, and MDR1 inducer) (457, 41.3, 45.9, 8.3, and 2.1 fold induction, respectively).

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29) CYP2C19-inducing activity could not be assessed because the metabolite concentration was below the detection limit.

30) Ratio of enzyme activity values (amount of metabolite formed) in the presence of EVG or the positive control to that in the presence of the vehicle. Enzyme activity values (amount of metabolite formed) were calculated using phenacetin (CYP1A2), tolbutamide (CYP2C9), (s)-mephenytoin (CYP2C19), and MDZ (CYP3A).

31) Ratio of the expression level of mRNA in each enzyme in the presence of COBI or the positive control to that in the presence of the vehicle

- The inhibitory action of COBI against various transporters[32] was investigated. IC_{50} values of COBI against OCT2, OCTN1, MATE1, OATP1B1, and OATP1B3 were 8.24, 2.49, 1.87, 3.50, and 1.88 μM, respectively, whereas IC_{50} values against all other transporters (MDR1, MRP1, MRP2, MRP4, BCRP, OAT1, OAT3, MATE2-K) were ≥20.7 μM.

3.(ii).B. Outline of the prior assessment by PMDA

- Taking account of the high protein binding rates of EVG and COBI, PMDA asked the prior assessment requestor to explain the possibility of drug-drug interaction mediated by plasma protein binding of EVG or COBI when concomitantly administered with other drugs.

  The prior assessment requestor explained as follows:
  The binding rates of EVG and COBI to human plasma proteins are high, but not concentration-dependent, suggesting that the binding sites of plasma proteins are not saturated; therefore the binding rate of EVG or COBI is unlikely to change when concomitantly administered with other drugs. Given that both EVG and COBI appear to have relatively large distribution volumes in humans[33] and that hepatic clearance of EVG and COBI decreases because of the inhibition of CYP3A activity by COBI, the concentration of unbound EVG and COBI in the plasma will be least affected. As regards interactions with other concomitant drugs, various clinical studies conducted so far did not suggest any clinically significant drug-drug interactions caused by protein binding. These results suggest that plasma protein binding-mediated drug-drug interaction of EVG or COBI with other concomitant drugs is unlikely to develop.

  PMDA accepted the above explanation of the prior assessment requestor.

3.(iii) Summary of toxicology studies

3.(iii).A. Summary of the submitted data

- The following toxicity studies were conducted for both EVG and COBI as single agents: single and repeated dose toxicity, genotoxicity, carcinogenicity, reproductive and developmental toxicity, local tolerance, phototoxicity, and other toxicities (immunotoxicity, juvenile animal toxicity, antigenicity, study on mechanism of toxicity and study on impurities). Also, repeated dose toxicity studies were conducted on concomitant use of the following combinations: EVG and COBI; EVG and RTV; and COBI and ATV.

3.(iii).A.(1) Single dose toxicity

3.(iii).A.(1).1) EVG

- The approximate lethal dose was determined to be >2000 mg/kg in male and female SD rats and >1000 mg/kg in female beagle dogs. No death occurred in either of the animal species.

3.(iii).A.(1).2) COBI

- The maximum tolerated dose was determined to be 100 mg/kg in male and female Tg-rasH2 wt mice, and the approximate lethal dose was determined to be >500 mg/kg in male and female SD rats.

3.(iii).A.(2) Repeated dose toxicity

- The main studies of EVG conducted were oral dose studies in mice (for 3 months), rats (for 1, 3, 6 months), and dogs (for 1, 9 months), with accompanying studies for reversibility in

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[32] MDR1 (multidrug resistance 1 [P-glyocoprotein]), MRP (multidrug resistance-associated protein) 1, MRP2, MRP4, BCRP (breast cancer resistance protein), OAT (organic anion transporter) 1, OAT3, OCT2 (organic cation transporter 2), OCTN1 (organic cation transporter N1), MATE1 (multidrug and toxin extrusion protein 1 [SLC47A1]), MATE2-K (multidrug and toxin extrusion protein 2-K [SLC47A2]), OATP (organic anion transporting polypeptide) 1B1, OATP1B3

[33] The distribution volumes of EVG and COBI in dogs were 2.6 L/kg and 1.33 L/kg, respectively.

rats and dogs. Also, a study of EVG/RTV combination (for 3 months) was conducted in rats. Of COBI, the main studies conducted were oral dose studies in mice (for 1, 3 months), rats (for 1, 6 months), and dogs (for 1, 9 months), with accompanying studies for reversibility in rats and dogs. Also, an oral dose study of COBI/ATV combination in rats (for 3 months) and an oral dose study of EVG/COBI combination in rats (for 3 months) were conducted, with accompanying studies for reversibility.

- Cecal dilatation was noted in rats receiving EVG. EVG has a quinolone moiety and is shown to have antimicrobial activity in a bacterial reverse mutation test. Therefore, the finding was considered to be caused by the effect on the intestinal bacterial flora unique to rats and not relevant to humans. In rats and dogs receiving EVG, lipid vacuoles containing triglycerides were found in the lamina propria of the upper small intestine. The lipid vacuole formation is associated with lipid intake from food and it takes time until they disappear. However, it was determined that the lipid vacuoles were not any toxicologically significant finding because of the following reasons: the digestive tract did not show any clear toxicity, with no adverse tissue reactions; no changes were found in blood lipid levels or in clinical conditions in the test for the mechanism of lipid vacuole formation; and no marked findings were noted in the upper small intestine in the 2-year carcinogenicity study in rats.

- COBI is an inhibitor of CYP3A, a drug-metabolizing enzyme in human liver, while it induces the enzyme in rodents.[34] Therefore, effects of COBI on the liver noted in mice, rats, and dogs (increased weight, microsomal enzyme induction, hepatocyte hyperplasia, increased hepatic enzyme levels in the blood) and on the thyroid gland noted in rats (increased weight, hyperplasia of thyroid follicular cells, increased level of thyroid stimulating hormone [TSH], and decreased thyroxine [T_4] level) were considered to be adaptive responses and not relevant to humans.

- The no observed adverse effect level (NOAEL) of EVG was 2000 mg/kg/day in mice, 2000 mg/kg/day in rats, and 100 mg/kg/day in dogs. The exposure level[35] at the NOAEL was determined to be approximately 1.9 to 2.6, 20 to 36, and 2.3 to 2.9 times, respectively, compared with the EVG exposure level following multiple administration of the proposed drug product to humans.[36] The NOAEL of COBI was 5 mg/kg/day in male mice, 50 mg/kg/day in female mice, 30 mg/kg/day in rats, and 10 mg/kg/day in dogs. The exposure level in mice, rats, and dogs[37] was determined to be approximately 0.1 to 7.2, 1.2 to 1.6, and 2.1 to 2.4 times, respectively, compared with the exposure level following multiple administration of 150 mg COBI to humans.[38]

3.(iii).A.(2).1) EVG

3.(iii).A.(2).1).(a). Three-month repeated oral dose toxicity study in mice

- EVG (0 [vehicle], 100, 500, 2000 mg/kg/day) was administered orally for 3 months to male and female CD1 mice. Death occurred in 1 of 15 males in the 2000 mg/kg/day group, but the cause of the death was unknown. The treatment-related finding was unkempt coat. Decreased food intake was noted at Week 1 in females of the 500 mg/kg/day group and males and females of the 2000 mg/kg/day group, but the change was transient and not considered as a toxic finding. Based on the above results, the NOAEL was determined to

34) PXR, a drug receptor involved in the expression of CYP3A etc., was little affected by COBI in humans but was activated by COBI in rats in *in vitro* studies. Based on the results, COBI administration may induce CYP3A in rats.

35) 44 to 59 μg·h/mL in mice ($AUC_{0\text{-}t}$), 460 to 836 μg·h/mL in rats ($AUC_{0\text{-}24h}$), and 54 to 66 μg·h/mL in dogs ($AUC_{0\text{-}24h}$).

36) AUC_{tau} based on PPK analysis in the phase III studies (Studies GS-US-236-0102 and GS-US-236-0103) and a phase II study (Study GS-US-236-0104) was 23 μg·h/mL.

37) 0.93 to 60.1 μg·h/mL in mice, 9.9 to 13.3 μg·h/mL in rats, and 16.8 to 19.6 μg·h/mL in dogs (all expressed in $AUC_{0\text{-}t}$).

38) AUC_{tau} based on PPK analysis in phase III studies (Studies GS-US-236-0102 and GS-US-236-0103) and phase II study (Study GS-US-236-0104) was 8.3 μg·h/mL.

be 2000 mg/kg/day.

3.(iii).A.(2).1).(b). One-month repeated oral dose toxicity study in rats

- EVG (0 [vehicle], 100, 300, 1000, 2000 mg/kg/day) was administered orally for 1 month to male and female SD rats. No test drug-related death occurred. Salivation and pale feces were found in the ≥1000 mg/kg/day groups and, in all dose groups, focal retinal atrophy tended to occur increasingly, while this finding was not noted in long-term dosing. A decreasing tendency of urine pH and decreased platelet count were seen in males of the 2000 mg/kg/day group, and occult hematuria was noted infrequently in males of the 2000 mg/kg/day group and in females of the ≥100 mg/kg/day group. But these findings were considered to be of no toxicological significance. Based on the above results, the NOAEL was determined to be 2000 mg/kg/day.

3.(iii).A.(2).1).(c). Three-month repeated oral dose toxicity study in rats

- EVG (0 [vehicle], 100, 300, 1000, 2000 mg/kg/day) was administered orally for 3 months to male and female SD rats. No test drug-related death occurred. A decreasing tendency in urine pH was seen in females of the ≥300 mg/kg/day groups, and increased blood sodium level was found in females of the 100, 300, and 2000 mg/kg/day groups, but these changes were not noted during the recovery period, and were therefore considered not to be toxicologically significant findings. Based on the above, the NOAEL was determined to be 2000 mg/kg/day.

3.(iii).A.(2).1).(d). Six-month repeated oral dose toxicity study in rats

- EVG (0 [vehicle], 100, 300, 2000 mg/kg/day) was administered orally for 6 months to male and female SD rats. No test drug-related death occurred. Salivation was seen in the ≥300 mg/kg/day groups and pale feces in the 2000 mg/kg/day group. Increased red blood cell count was found in the mesenteric lymph node sinus in the 2000 mg/kg/day group and tigroid basophilic cell foci were seen in the liver of females in the ≥300 mg/kg/day groups. However, because of the low frequency and the very low intensity, and also because these findings were noted in the control group, they were considered not to be toxicologically significant findings. Based on the above, the NOAEL was determined to be 2000 mg/kg/day.

3.(iii).A.(2).1).(e). Three-month repeated oral dose toxicity study with concomitant use of RTV in rats

- EVG/RTV (1000/0, 0/10, 100/10, 1000/10 mg/kg/day) was administered orally for 3 months to male and female SD rats. Animals in the control groups received the vehicle of EVG, the vehicle of RTV, or both vehicles in a similar dosing schedule. No test drug-related death occurred, nor was any toxicologically significant finding noted. Based on the above, the NOAEL of EVG/RTV was determined to be 1000/10 mg/kg/day regardless of whether they were administered alone or in combination.

3.(iii).A.(2).1).(f). One-month repeated oral dose toxicity study in dogs

- EVG (0 [vehicle], 10, 30, 100 mg/kg/day) was administered orally for 1 month to male and female beagle dogs. No test drug-related death occurred. Loose stools, diarrhea, and vomiting were noted in all dose groups; however, they were considered to be due to the vehicle because they occurred in the vehicle group as well. Decreased blood potassium was seen in males of the 100 mg/kg/day group. Since these changes were not accompanied by histopathological or electrocardiographic changes, they were considered not to be toxicologically significant findings. Based on the above, the NOAEL was determined to be 100 mg/kg/day.

3.(iii).A.(2).1).(g). Nine-month repeated oral dose toxicity study in dogs

- EVG (0 [water or vehicle], 10, 30, 100 mg/kg/day) was administered orally for 9 months to male and female beagle dogs. No test drug-related death occurred. Except loose stools and diarrhea seen sporadically in the EVG groups, no other test drug-related findings were noted. Based on the above, the NOAEL was determined to be 100 mg/kg/day.

3.(iii).A.(2).2) COBI

3.(iii).A.(2).2).(a). One-month repeated oral dose toxicity study in mice

- COBI (0 [vehicle], 10, 30, 100 mg/kg/day) was administered orally for 1 month to male and female Tg-rasH2 wt mice. No test drug-related death occurred. Decreased renal weight was found in the 100 mg/kg/day group. The change was not accompanied by laboratory or microscopic findings, and the relationship with the test drug was considered unclear. Based on the above, the NOAEL was determined to be 100 mg/kg/day.

3.(iii).A.(2).2).(b). Three-month repeated oral dose toxicity study in mice

- COBI (0 [vehicle], 5, 15, 50 mg/kg/day) was administered orally for 3 months to male and female CD-1 mice. No test drug-related death occurred. The frequency of struggling responses increased in a dose-dependent manner. As regards laboratory test values, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels increased in males of the ≥15 mg/kg/day groups, but did not show apparent dose-dependency in females. Increased liver weight and increased activities of liver metabolic enzymes (CYP2B, CYP3A) were noted in the 50 mg/kg/day group. Based on the above, the NOAEL was determined to be 50 mg/kg/day in females and 5 mg/kg/day in males.

3.(iii).A.(2).2).(c). One-month repeated oral dose toxicity study in rats

- COBI (0 [vehicle], 10, 20, 50, 100 mg/kg/day) was administered orally for 1 month to male and female SD rats. No test drug-related death occurred. In the laboratory test, increased total protein, increased albumin, increased globulin, increased calcium, and decreased AST were seen, but all of them were mild in severity and recovered after withdrawal, which suggested that the findings were of no toxicological significance. Urinalysis results showed increased urine output, increased pH, and decreased urine specific gravity in females of the ≥50 mg/kg/day groups and in males of the 100 mg/kg/day group. The NOAEL was determined to be 50 mg/kg/day.

3.(iii).A.(2).2).(d). Six-month repeated oral dose toxicity study in rats

- COBI (0 [vehicle], 10, 30, 100 mg/kg/day) was administered orally for 6 months to male and female SD rats. Death occurred in 2 of 15 females in the 100 mg/kg/day group, and the causal relationship with the test drug was not ruled out. Decreased food intake, decreased body weight, and reduced body weight gain were seen in males of the 100 mg/kg/day group, but recovered after the 3-month withdrawal period. Hematology tests showed decreased corpuscular volume and decreased corpuscular hemoglobin in the 100 mg/kg/day group and increased platelet count in males of the ≥30 mg/kg/day groups and in females of the 100 mg/kg/day group, but all of these changes were reversible. Blood biochemical tests showed increased cholesterol and increased globulin in females of the ≥30 mg/kg/day groups, increased total protein in females of the ≥30 mg/kg/day groups and in males of the 100 mg/kg/day group, increased albumin in males of the 100 mg/kg/day group, and increased gamma-glutamyltransferase (GGT) and increased calcium in the 100 mg/kg/day group. Except increased cholesterol and increased total protein in females of the 100 mg/kg/day group, these changes were reversible. Thyroid gland-related test results showed a dose-dependent increase in TSH in both males and females and associated T_4 decrease in males of the 100 mg/kg/day group. Decreased T_4 persisted during the administration period but recovered after the withdrawal period. TSH decreased after withdrawal. Urinalysis results showed increased urine output and decreased urine osmolality accompanied by

decreased urine specific gravity in the 100 mg/kg/day group throughout the administration period. Similar but milder findings were noted in the 30 mg/kg/day group as well. Sodium excretion increased mainly in the 100 mg/kg/day group, urinary inorganic phosphorus, calcium, and potassium concentrations decreased mainly in the 100 mg/kg/day group, and urine creatinine level decreased in the ≥30 mg/kg/day group. These changes were reversible after withdrawal.[39] Immunophenotyping results showed COBI-induced increases in lymphocyte count, total T cell count, and B cell count in the 100 mg/kg/day group, and relative changes in some T cell and B cell counts in females of the ≥30 mg/kg/day groups and in males of the 100 mg/kg/day group. After the withdrawal period, the values of these parameters were similar to those found in the control group, from which they were considered not to be toxicologically significant findings. TK study results showed a higher exposure level in females than in males. Based on the results obtained during COBI administration and the recovery period, the NOAEL was determined to be 30 mg/kg/day.

3.(iii).A.(2).2).(e). Three-month repeated oral dose toxicity study with concomitant use of ATV in rats

- COBI (0 [vehicle], 30 mg/kg/day), ATV (20, 50 mg/kg/day), or COBI/ATV (30/20, 30/50 mg/kg/day) was administered orally for 3 months to male and female SD rats. No test drug-related death occurred. Laboratory test showed increased cholesterol in all dose groups except the vehicle group, with particularly marked increase in females. Increased urine output was seen in males of the COBI group and the ATV 50 mg/kg/day group and in males and females of the COBI/ATV 30/20 and 30/50 mg/kg/day groups, but these changes were reversible after withdrawal. TK study showed concomitant use of COBI and ATV caused ATV dose-dependent decrease in COBI exposure level and a marked increase in ATV exposure level.[40] Based on the above, the NOAEL for COBI/ATV was determined to be 30/50 mg/kg/day, regardless of whether they were administered alone or in combination.

3.(iii).A.(2).2).(f). One-month repeated oral dose toxicity study in dogs

- COBI (0 [vehicle], 5, 15, 45 mg/kg/day) was administered orally for 1 month to male and female beagle dogs. In females of the 45 mg/kg/day group, administration was interrupted on Day 11 because of decreased body weight and emaciation, and administration was resumed at a dose of 30 mg/kg/day from Day 14. No test drug-related death occurred. Salivation and vomiting were seen in a dose-dependent manner in the ≥15 mg/kg/day groups and decreased body weight was noted in males of the 45 mg/kg/day group. ECG findings included increased PR intervals in females of the ≥15 mg/kg/day groups on Day 3, increased heart rate in males of the 15 mg/kg/day group on Day 3, and increased RR intervals in males of the 45 mg/kg/day group on Day 22. Since these ECG findings were within the normal range, they were considered not to be physiologically or toxicologically significant changes. No arrhythmia was noted, neither was any change in ECG found after withdrawal. Urinalysis showed decreased urine specific gravity in males of the ≥15 mg/kg/day groups. Although the causal relationship with COBI was unknown, there was no associated histopathological finding, from which the change was considered to be of little toxicological significance. Increased liver weight was seen in males of the ≥15 mg/kg/day groups and in females of the 45 mg/kg/day group, and increased hepatocyte vacuolization was noted in the 45 mg/kg/day group. However, they were reversible and not accompanied by changes such as inflammation or necrosis. Thus, since histopathological findings did not suggest the presence of any drug-induced liver disorder, the changes were considered to be of little toxicological significance. Based on the above, the NOAEL was determined to be 15 mg/kg/day.

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39) Neither vasopressin nor aldosterone level showed test drug-related changes.

40) CYP3A activity after the 1-month withdrawal period in the COBI group, the ATV group, and the COBI/ATV group was similar to that in the vehicle control group, both in males and in females.

3.(iii).A.(2).2).(g). Nine-month repeated oral dose toxicity study in dogs

- COBI (0 [vehicle], 5, 10, 20 mg/kg/day) was administered orally for 9 months to male and female beagle dogs. No test drug-related death occurred. Salivation, vomiting, and changes in fecal conditions were seen in the 20 mg/kg/day group, however, vomiting and changes in fecal conditions in the 5 and 10 mg/kg/day groups were similar to those found in the control group. These changes recovered after the withdrawal period. Decreased body weight was noted in the 20 mg/kg/day group during the administration period, but was reversible after the withdrawal period. Increased platelet count was seen in females of the ≥10 mg/kg/day groups and in males of the 20 mg/kg/day group, and increased ALP (in both males and females) and decreased total protein and albumin (in males) were noted in the 20 mg/kg/day group, but all these changes recovered after the withdrawal period. Urinalysis showed increased urine output, decreased urine osmolality, and decreased urine specific gravity in several females of the 20 mg/kg/day group, resulting in moderate decrease in the concentrations and excretion rates of urine sodium, potassium, chlorine, calcium, and organic phosphorus. Urinalysis also showed frequent bilirubinuria in males of the 20 mg/kg/day group. However, all these changes recovered after the withdrawal period. Based on the above, the NOAEL was determined to be 10 mg/kg/day.

3.(iii).A.(2).3) EVG/COBI

3.(iii).A.(2).3).(a). Three-month repeated oral dose toxicity study in rats

- COBI (0 [vehicle], 30 mg/kg/day), EVG (0, 1000 mg/kg/day), or COBI/EVG (0/0, 30/100, 30/1000 mg/kg/day) was administered orally for 3 months to male and female SD rats. No test drug-related death occurred. Concomitant use of COBI with EVG did not cause any new toxicity or aggravation of the toxicity induced by each drug. TK study results showed a higher COBI exposure level in females than in males but did not show COBI accumulation or any change in COBI exposure level due to the concomitant use of EVG. Concomitant use of COBI caused an increase in EVG exposure level in males, but no EVG accumulation was seen after repeated administration. Concomitant use of EVG did not cause any further increase in the activities of liver metabolic enzymes induced by COBI. The NOAEL of COBI/EVG was determined to be 30/1000 mg/kg/day, regardless of whether they were administered alone or in combination.

3.(iii).A.(3) Genotoxicity

- Bacterial reverse mutation assay (Ames test), chromosomal aberration test with CHL cells, and *in vivo* bone marrow micronucleus tests in rats were conducted, and EVG was negative in all the tests except the chromosomal aberration test. Structural chromosomal aberration was found in 7% to 9% of cells treated for 6 hours at doses of 55, 65, or 75 μg/mL in the absence of S9 mix in the chromosomal aberration test, from which EVG was determined to be pseudo-positive. However, since EVG was negative in all other genotoxicity tests and carcinogenicity studies indicated no carcinogenic potential either, EVG was unlikely to have genotoxicity, based on a comprehensive judgment of the results.

- Genotoxicity of COBI was investigated by bacterial reverse mutation assay (Ames test), TK study with murine lymphoma, and *in vivo* bone marrow micronucleus test in rats. COBI was negative in all the tests.

3.(iii).A.(4) Carcinogenicity

3.(iii).A.(4).1) EVG

3.(iii).A.(4).1).(a). Two-year repeated oral dose carcinogenicity study in mice

- EVG (0 [vehicle], 200, 600, 2000 mg/kg/day), RTV (25 mg/kg/day), or EVG/RTV (0/0 [vehicle], 2000/25 mg/kg/day) was administered orally for 24 months to male and female CD-1 mice. Unexplained death occurred in the EVG/RTV group. Nasal obstruction

due to gastric reflux has not been ruled out as the cause of death, but no related pathological findings were noted. Concomitant use of RTV caused a 4 to 7-fold increase in EVG exposure level, but no difference in incidence of tumor was found between the vehicle control groups and the groups treated with EVG or EVG/RTV, from which EVG had no carcinogenicity in mice regardless of concomitant use of RTV.

3.(iii).A.(4).1).(b). Two-year repeated oral dose carcinogenicity study in rats

- It had been planned to administer orally EVG (0 [vehicle], 100, 300, 2000 mg/kg/day) to male and female SD rats for 24 months. However, the study was terminated after ≥88 weeks in males and after ≥90 weeks in females because the number of surviving animals in the vehicle control group (n = 120) decreased to 20 animals during the early stage of the study. There were no test drug-related changes in body weight, food intake, ocular test values, hematology values, macroscopic findings, or microscopic findings. Lipid vacuoles in the lamina propria of the upper small intestine, noted in the repeated dose toxicity study, were not detected in this study. The incidence of tumor was not different between the vehicle control group and the EVG groups, from which EVG was considered noncarcinogenic in rats.

3.(iii).A.(4).2) COBI

3.(iii).A.(4).2).(a). Two-year repeated oral dose carcinogenicity study in mice

- It had been planned to administer orally COBI (0 [water or vehicle[41)]], 5, 15, 50 mg/kg/day) to male CD-1 mice and COBI (0 [water or vehicle], 10, 30, 100 mg/kg/day) to female CD-1 mice for 24 months. However, because the number of surviving animals decreased, the study was terminated after 95 weeks in males of the 50 mg/kg/day group, after 96 weeks in other male groups, after 87 weeks in females of the 100 mg/kg/day group and after 100 weeks in other female groups. The incidence of tumor did not increase in the COBI groups compared with the vehicle control groups. No COBI-related ophthalmological findings or hematological malignancy were found. Irritation of the upper respiratory tract was seen in an increased frequency in all groups except the water control group, and infiltration of neutrophils into the turbinate, erosion, ulcer, turbinate atrophy, and death related to administration procedure were seen in the COBI 50 mg/kg/day group. Hepatocyte hyperplasia and increase in pigmented Kupffer cell count were found in the COBI 50 mg/kg/day group.[42)] Based on the above, COBI was considered noncarcinogenic in mice.

3.(iii).A.(4).2).(b). Two-year repeated oral dose carcinogenicity study in rats

- It had been planned to administer orally COBI (0 [water or vehicle], 10, 25, 50 mg/kg/day) to male SD rats and COBI (0 [water or vehicle], 5, 15, 30 mg/kg/day) to female SD rats for 24 months. However, the study was terminated after ≥97 weeks in males and after ≥102 weeks in females because the number of surviving animals in the vehicle control group (n = 130) decreased to 20 animals during the early stage of the study. Males in the ≥25 mg/kg/day groups showed decreased body weight and decreased food intake, but these changes did not show a dose-response relationship. There were no COBI-related clinical signs, ophthalmologic findings, hematological malignancy, or macroscopic findings. Males in the ≥25 mg/kg/day groups and females in the 30 mg/kg/day group showed increased incidences of thyroid follicular cell adenoma and thyroid follicular cell carcinoma, suggesting the relationship with hypertrophy of thyroid follicular cells. Non-malignant findings in the liver included centrilobular hepatocyte hypertrophy in males of the ≥10 mg/kg/day groups and in females of the 30 mg/kg/day group and development of hepatocytes containing macronucleus in males of the ≥25 mg/kg/day groups. These malignant and non-malignant findings noted in

41) During the early stage of the study, a number of deaths increased in the group receiving pH-adjusted vehicle. Therefore, non-pH-adjusted vehicle was used from Week 14 onward of the study, but the increase in the number of deaths was not slowed down, whereupon acetate buffer containing 10% propylene glycol was used as the vehicle from Week 26 until the end of the study.

42) Considered to be adaptive responses secondary to liver metabolic enzyme induction.

the thyroid gland and liver were considered as adaptive responses whereby COBI species-specifically activates PXR in rats, causing the induction of liver metabolic enzymes and resultant increase in thyroid hormone clearance, which in turn induces the imbalance of thyroid hormone. Since this mechanism does not work in humans, the above findings were least likely to be relevant to humans.

3.(iii).A.(5) Reproductive and developmental toxicity

- The following studies of EVG were conducted: effects on reproductive function in male and female rats; effects on embryo-fetal development in rats and rabbits; effects on embryo-fetal development in rats with concomitant use of EVG and RTV; effects on pre- and postnatal development in rats; and toxicity studies in juvenile animals. The following studies of COBI were conducted: effects on fertility and early embryonic development in rats; effects on embryo-fetal development in rats and rabbits; effects on pre- and postnatal development in rats; and toxicity studies in juvenile animals.

3.(iii).A.(5).1) EVG

3.(iii).A.(5).1).(a). Studies on fertility and early embryonic development to implantation

i) Studies on fertility and early embryonic development in female rats

- EVG (0 [vehicle], 300, 1000, 2000 mg/kg/day) was administered orally to female SD rats from 2 weeks prior to mating until Gestation day 7. As a result, no effect on fertility or early embryonic development was noted, from which the NOAEL was determined to be ≥2000 mg/kg/day.

ii) Study on fertility in male rats

- EVG (0 [vehicle], 300, 1000, 2000 mg/kg/day) was administered orally to male SD rats from 4 weeks prior to mating for a total of 49 to 52 days, including mating period (maximum 3 weeks). No effect on fertility was noted, from which the NOAEL was determined to be ≥2000 mg/kg/day.

3.(iii).A.(5).1).(b). Studies on embryo-fetal development

i) Study on embryo-fetal development in rats

- EVG (0 [vehicle], 300, 1000, 2000 mg/kg/day) was administered orally to pregnant SD rats from Gestation day 7 to Gestation day 17. No effect was found on food intake, body weight, or macroscopic findings of maternal animals, nor was any teratogenicity, including visceral or skeletal anomalies, noted in fetuses. Based on the above, the NOAEL was determined to be 2000 mg/kg/day for both maternal animals and embryos/fetuses. The exposure level at this dose was 23 times that found in humans receiving multiple doses of EVG.[36)]

ii) Study on embryo-fetal development in rats in concomitant use with RTV

- EVG (0 mg/kg/day [vehicle]), RTV (0 mg/kg/day [vehicle]), or EVG/RTV (0/0 [vehicle], 1000/0, 0/10, 100/10, 1000/10 mg/kg/day) was administered orally to pregnant SD rats from Gestation day 6 to Gestation day 17. No abnormalities were found either in maternal animals or in fetuses, from which the NOAEL was determined to be 1000/10 mg/kg/day for both maternal animals and embryos/fetuses, regardless of whether they were administered alone or in combination.

iii) Study on embryo-fetal development in rabbits

- EVG (0 [vehicle], 50, 150, 450 mg/kg/day) was administered orally to pregnant NZW rabbits from Gestation day 7 to Gestation day 19. Reduced body weight gain and decreased food intake were found in maternal animals of the ≥150 mg/kg/day groups in late pregnancy (after administration period), while no effect was seen in embryos/fetuses. Based on the above, the NOAEL was determined to be 50 mg/kg/day for maternal animals and ≥450 mg/kg/day for fetuses. The exposure level in maternal animals receiving 450 mg/kg/day

was 0.2 times the exposure level in humans receiving multiple doses of EVG 150 mg.[36] The preliminary study[43] showed a decreased number of live fetuses at 600 mg/kg/day and an increased number of resorptions at 300 and 600 mg/kg/day. Effects on embryo-fetal development in rabbits were considered as secondary effects associated with development of maternal toxicity.

3.(iii).A.(5).1).(c). Study of effects on pre- and postnatal development (including direct administration to juvenile animals)

- EVG (0 [vehicle], 300, 1000, 2000 mg/kg/day) was administered orally to pregnant SD rats from Gestation day 7 to day 20 postpartum. No effect was found on maternal animals, on deliveries or on F1 juvenile animals.[44] EVG (0 [vehicle], 300, 1000, 2000 mg/kg/day) was administered orally to F1 juvenile animals from day 22 to day 49 postpartum. No test drug-related effect was noted. Based on the above, the NOAEL was determined to be 2000 mg/kg/day for maternal animals, neonates, and juvenile animals.

3.(iii).A.(5).2) COBI

3.(iii).A.(5).2).(a). Study on fertility and early embryonic development to implantation

- COBI (0 [vehicle], 10, 30, 100 mg/kg/day) was administered orally to male and female SD rats from 4 weeks prior to mating (2 weeks prior to mating in females) until Gestation day 7 (females). Decreased body weight and decreased food intake were seen in the 100 mg/kg/day group. No test drug-related effect was found on fertility or early development in female rats. Male rats in the 100 mg/kg/day group showed a slight decrease in the mean sperm count, and it was due to a marked decrease in the sperm count in only 1 of 22 animals and was considered not to be related to the test drug. Based on the above, the NOAEL was determined to be 30 mg/kg/day regarding general toxicity and ≥100 mg/kg/day regarding fertility and early embryonic development to implantation.

3.(iii).A.(5).2).(b). Studies on embryo-fetal development

i) Study on embryo-fetal development in rats

- COBI (0 [vehicle], 25, 50, 125 mg/kg/day) was administered orally to pregnant SD rats from Gestation day 6 to Gestation day 17. Maternal animals in the 125 mg/kg/day group showed decreased body weight, reduced body weight gain, decreased food intake, decreased activity, loss of fur, etc. Also, decrease in relative uterus weight and increase in the rate of post-implantation loss due to a decreased number of live fetuses were found. In addition, decreased fetal weight associated with the decreased pregnant uterus weight was seen, but this finding was considered to be secondary change due to decreased maternal body weight. No teratogenicity was found in fetuses. In the 125 mg/kg/day group, skeletal anomalies related to abnormal ossification of spine and sternebrae were noted. However, since these changes had no effect on the appearance, survival, or growth of animals, they were considered to be of no toxicological significance. Based on the above, the NOAEL was determined to be 50 mg/kg/day for both maternal animals and embryos/fetuses. The exposure level at this dose was approximately 1.8 times the exposure level in humans receiving multiple doses of COBI 150 mg.[38]

ii) Study on embryo-fetal development in rabbits

- COBI (0 [vehicle], 25, 50, 100 mg/kg/day) was administered orally to pregnant NZW rabbits from Gestation day 7 to Gestation day 20. Maternal animals in the COBI 100 mg/kg/day group showed reduced body weight gain and decreased food intake, but showed no difference in body weight compared with animals of the vehicle control group, from which the changes noted were considered not to be toxic findings. No effect on embryos or

43) Study TX-183-2001

44) For drug concentration in milk, see "3.(ii).A.(4).1) EVG."

fetuses was seen. Based on the above, the NOAEL was determined to be 100 mg/kg/day both for maternal animals and for embryos/fetuses. The exposure level at this dose was approximately 4.3 times the exposure level in humans receiving multiple doses of COBI 150 mg.[38)]

3.(iii).A.(5).2).(c). Study of effects on pre- and postnatal development (including direct administration to juvenile animals)

- COBI (0 [vehicle], 10, 30, 75 mg/kg/day) was administered orally to pregnant SD rats from Gestation day 6 until day 20 to 22 postpartum. No test drug-related death occurred. Maternal animals in the 75 mg/kg/day group showed decreased body weight, reduced body weight gain, and decreased food intake during Gestation days 6 to 11, and low body weight during the lactation period.[45)] No effect was seen in the offspring (F1). COBI (0 [vehicle], 10, 30, 75 mg/kg/day) was administered orally to F1 juvenile animals from day 22 to day 49 postpartum. As a result, decreased body weight, reduced body weight gain, and decreased food intake were noted in the 75 mg/kg/day group. Biochemical tests showed increased globulin in the ≥30 mg/kg/day groups and decreased ALP in males of the ≥30 mg/kg/day groups and in females of the 75 mg/kg/day group. Animals in the ≥10 mg/kg/day groups showed increased TSH, decreased T_4, and decreased tri-iodothyronine (T_3) associated with CYP3A and UGT1A1 induction by rodent PXR activity caused by COBI administration. Females in the ≥10 mg/kg/day groups showed increased thyroid gland weight, females in the ≥30 mg/kg/day groups and males in the 75 mg/kg/day group showed thyroid follicular cell hyperplasia, and animals in the 75 mg/kg/day group showed increased liver weight associated with hepatocyte hyperplasia. Based on the above, the NOAEL was determined to be 30 mg/kg/day for maternal animals and 75 mg/kg/day for pre- and postnatal development and for F1 juvenile animals.

3.(iii).A.(6) Local tolerance

- EVG and COBI were tested for eye irritation and skin irritation, and EVG was also tested for phototoxicity. COBI does not have any absorption spectra in the wavelengths of 290 to 700 nm, the potentially phototoxic UV/visible range, is stable against light, and does not show any light-related findings of clinical or nonclinical concerns. Therefore, COBI was not tested for phototoxicity.

3.(iii).A.(6).1) EVG

3.(iii).A.(6).1).(a). Bovine Corneal Opacity and Permeability Test

- EVG (20% suspension in saline) was tested for an eye irritating effect. EVG had no effect either on corneal opacity or permiability, from which EVG was considered non-corrosive and not strongly irritating.

3.(iii).A.(6).1).(b). Skin irritation test in rabbits

- EVG was tested for skin irritation in female NZW rabbits. As a result, EVG was considered not to have skin irritation potential.

3.(iii).A.(6).1).(c). Phototoxicity test in mice

- Phototoxicity following oral administration of EVG (2000 mg/kg) was evaluated in male ICR mice, with enoxacin as the positive control. Neither erythema nor oedema was noted in the EVG group, from which EVG was considered non phototoxic.

45) For drug concentration in milk, see "3.(ii).A.(4).2) COBI."

3.(iii).A.(6).2) COBI

3.(iii).A.(6).2).(a). Bovine Corneal Opacity and Permiability Test

- COBI (20% suspension in saline) was tested for an eye irritating effect. COBI had no effect on either corneal opacity or permiability, from which COBI was considered non-corrosive and not strongly irritating.

3.(iii).A.(6).2).(b). Skin irritation test in rabbits

- COBI was tested for skin irritation in female NZW rabbits. Very mild erythema was seen at the site where COBI patch was applied and persisted for 13 days even after patch removal in 1 of 3 animals. COBI was considered to have a mild skin irritaion potential.

3.(iii).A.(7) Other toxicity studies

3.(iii).A.(7).1) Antigenicity tests

3.(iii).A.(7).1).(a). Local lymph node assay of EVG in mice

- EVG was tested for skin sensitization in female CBA/Ca mice. Application of EVG (10%, 25%, 50%) to the ear auricle caused neither erythema nor lymphocyte growth in the auricular lymph node, from which EVG was considered non skin-sensitizing.

3.(iii).A.(7).1).(b). Local lymph node assay of COBI in mice

- COBI was tested for skin sensitization in female CBA/Ca mice. Application of COBI (2.5%, 5%, 10%) to the ear auricle caused neither erythema nor lymphocyte growth in the auricular lymph node, from which COBI was considered non skin-sensitizing.

3.(iii).A.(7).2) Immunotoxicity studies

3.(iii).A.(7).2).(a). One-month repeated oral dose immunotoxicity study of EVG in rats

- EVG (0 [vehicle], 300, 1000, 2000 mg/kg/day) was administered orally to male and female SD rats for 1 month. Animals were then subjected to test for sheep red blood cell (SRBC) immunity-induced antibody producibility and to lymphocyte subset analysis. Except decreased CD45RA + cell count in males of the 1000 mg/kg/day group, no test drug-related changes were noted, from which EVG was considered non-immunotoxic up to the dose of 2000 mg/kg/day.

3.(iii).A.(7).2).(b). One-month repeated oral dose T-cell-dependent antibody production study of COBI in rats

- COBI (0 [vehicle], 20, 50, 150 mg/kg/day) was administered orally to male and female SD rats for 1 month, and keyhole limpet hemocyanin (KLH) was administered intravenously to these animals on Day 5 of the treatment. T cell-dependent antibody producibility was investigated by measuring anti-KLH immunoglobulin over time. A decrease in anti-KLH IgG antibody titer was seen in females of the ≥50 mg/kg/day groups, and a tendency of decrease in anti-KLH IgG antibody titer was noted in males of the 150 mg/kg/day group as well. Microscopic observation showed a marked depletion of lymphocytes in the germinal center of the spleen in the ≥50 mg/kg/day groups. Immunohistochemical examination showed decreased KiB1R-positive B cell count in males of the ≥50 mg/kg/day groups and lymphocyte depletion in the germinal center of the spleen (males) and decreased PNA-positive B cell counts in the 150 mg/kg/day group. Based on the above results, the NOAEL in T-cell-dependent antibody production was determined to be 20 mg/kg/day in females and 50 mg/kg/day in males. Also, based on the findings in the spleen, the NOAEL regarding general toxicity was determined to be 20 mg/kg/day for both males and females.

3. (iii).A.(7).3) Studies on the mechanism of toxicity

- Lipid vacuoles in the lamina propria of the upper small intestine, which were seen in repeated dose toxicity studies of EVG in rats and dogs, were investigated for the reversibility and mechanism of action.

(a). In SD rats, the mechanism of EVG-induced lipid vacuole formation in the small intestine was investigated from the ultrafine structure of the droplets. Results suggested that the droplets were induced by intestinal epithelial cells.

(b). In SD rats, the effect of feeding time on the formation of intestinal lipid vacuoles was investigated. Results suggested that presence of food in the small intestine and a high local concentration of EVG were associated with lipid vacuole formation.

(c). EVG (2000 mg/kg/day) was administered orally to SD rats for 2 weeks, and animals were examined for lipid vacuole formation after a withdrawal period of 8 weeks. Results showed that lipid vacuoles were still present and suggested that they were disappearing and were filled with triglycerides.

(d). EVG (100 mg/kg/day) was administered orally to SD rats for 2 weeks, and recovery up until 16 weeks later was investigated. Results showed that lipid vacuoles were detected at 8 weeks into recovery period but not at 16 weeks later, demonstrating the reversibility.

(e). Following the oral administration of EVG (2000 mg/kg/day) to SD rats for 2 or 4 weeks, lipid vacuole formation in the small intestine was investigated histopathologically. Severe lipid vacuole formation was found at 2 weeks into treatment, but no aggravation occurred during the subsequent 2-week teatment.

(f). EVG was administered in diet (0%, 1%, 2%, 5%) to SD rats for 2 weeks. Severe lipid vacuole formation was found in the small intestine in the high concentration groups. Plasma EVG concentration was similar between the 2% and 5% groups, suggesting that the local EVG concentration was involved in the lipid vacuole formation.

3. (iii).A.(7).4) Toxicity studies on impurities

- In a 1-month repeated oral dose toxicity study of EVG (2000 mg/kg/day) in female SD rats, toxicity was compared between 2 different batches.[46)] The toxicity and the exposure level were similar between the 2 batches.

- In a 1-month repeated oral dose toxicity study of EVG (2000 mg/kg/day) in female SD rats, toxicity was compared between a pure product batch and another batch prepared from the same batch by adding impurities.[47)] Toxicity was similar between the 2 batches, while the exposure level to the EVG with impurities added was ≥2-fold that to the EVG from the pure batch, suggesting continuing absorption of EVG with impurities added.

- In a 1-month repeated oral dose toxicity study of COBI (100 mg/kg/day) in female SD rats, toxicity was compared between a pure product batch and another batch prepared from the same batch by adding impurities.[48)] No toxicologically significant difference was found, and the NOAEL was the same between the 2 batches.

- In a 1-month repeated oral dose toxicity study of EVG/COBI (30/30, 50/50 mg/kg/day) tablets in female SD rats, comparison of degraded and non-degraded tablets prepared from two different batches showed no toxicologically significant difference, suggesting the same NOAEL for the 2 batches.

46) Two batches with 99.1% purity and 96.2% purity
47) A batch with 98.8% purity and another batch with 95.6% purity prepared from the same batch by adding impurities
48) A batch with 99.4% purity and another batch with 95.1% purity prepared from the same batch by adding impurities

- Intermediates and potential impurities[49)] of EVG and COBI were evaluated *in silico*. No unique structural alerts associated with genotoxicity or carcinogenicity were detected.

3. (iii).A.(7).5) Other toxicity studies

- EVG and a non-steroidal anti-inflammatory drug (NSAID) were concomitantly administered to male ddY mice to evaluate a possible synergistic effect. In the positive control group receiving enoxacin/fenbufen 200/400 mg/kg, all animals developed clonic convulsions and died 2 hours after administration, whereas, in the EVG/fenbufen 2000/400 mg/kg group, neither convulsions nor death occurred.

- In the single oral dose TK study of EVG in beagle dogs, clinical signs and plasma exposure levels were similar among products with different excipients.[50)]

- Toxicity of COBI in concomitant use with GS-8374, a PI, was investigated in a single dose toxicity study in SD rats. Concomitant use did not aggravate the toxicity, and the NOAEL of COBI in concomitant use with GS-8374 (1000 mg/kg) was determined to be 50 mg/kg, the maximum dose administered in this study.

- Toxicity of COBI in concomitant use with GS-8374 was investigated in a 1-month repeated oral dose toxicity study in SD rats. Noted toxicity was considered to be due to GS-8374. The exposure level of COBI decreased due to the concomitant use, showing no accumulation. The NOAEL of COBI was determined to be 50 mg/kg, the maximum dose administered in this study.

- Toxicity of COBI in concomitant use with GS-8374 was investigated in a 1-month repeated oral dose toxicity study in beagle dogs. Results showed no toxicologically significant changes. The exposure level of COBI decreased initially by the concomitant use but increased until Week 4, suggesting the accumulation by repeated doses. The NOAEL of COBI was determined to be 15 mg/kg, the maximum dose administered in this study.

- An *in vivo* rat bone marrow micronucleus test of concomitant use of COBI with GS-8374 was conducted. GS-8374 (0 [vehicle], 2000 mg/kg) or GS-8374/COBI (500/50, 1000/50, 2000/50 mg/kg) was administered orally in a single dose to male and female SD rats. GS-8374 and GS-8374/COBI in all dose groups were negative.

3.(iii).B. Outline of the prior assessment by PMDA

- The carcinogenicity study of COBI in rats showed dose-dependent hyperplasia and fibrosis of the bile duct. Therefore, PMDA asked the prior assessment requestor to explain the possibility of increased incidence of tumor in the bile duct when EVG, a drug metabolized mainly in the liver, was concomitantly administered, and to explain the possibility of tumor risk being worsened by the combination of EVG, COBI, FTC, and TDF in the product.

  The prior assessment requestor explained as follows:
  Hyperplasia and fibrosis of the bile duct are age-related spontaneous lesions frequently found in old rats and are considered to be histopathological changes least likely to become malignant.[51)] In the carcinogenicity study of COBI in rats, the incidences of hyperplasia and

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49) EVG: GS-9207, GS-9208, GS-9209, GS-464364, GS-9205, GS-9238, GS-9239, GS-9240, GS-9305, GS-9321, GS-9538, GS-9189, GS-9320, GS-548743, GS-9215, GS-557402, GS-9286, GS-9535, GS-9302, GS-9303, GS-9304, GS-9351, GS-9356, GS-9262, GS-557405, GS-557398, GS-557458, GS-9482, GS-9317, GS-9665
COBI: GS-344433, GS-9389, GS-9397, GS-9402, GS-9404, GS-445739, GS-465430, GS-466544, GS-492986, GS-492987, GS-492988, GS-9390, GS-9398, GS-9407, GS-9429, GS-9454, GS-9455, GS-9612, GS-9654, GS-9655

50) For liquid products and capsules, exposure levels were compared between 2 formulations containing different excipients (liquid products, corn oil vs. 0.5% MC; capsules, sodium lauryl sulfate vs. sucrose fatty acid ester)

51) Boorman GA et al editors. *Pathology of the Fischer Rat*. 1990

fibrosis of the bile duct were slightly higher in the COBI groups in both males and females, but the changes were not obviously dose-dependent. EVG did not show any tendency of enhancing the hyperplasia/fibrosis of the bile duct in the 2-year carcinogenicity study in rats. Also, in the study of EVG/COBI (1000/30 mg/kg) in rats for 13 weeks, no bile duct-targeted toxic changes were noted. Above results suggest that the risk of biliary hyperplasia caused by COBI will not be further increased by concomitant use of EVG. Since the carcinogenicity study of COBI was conducted at the exposure level exceeding that achieved by the clinical dose in humans, EVG administration to humans is unlikely to increase the risk of tumor in the bile duct.

Thus, in the carcinogenicity studies of EVG and COBI submitted for prior assessment [see "3.(iii).A.(4) Carcinogenicity studies"] and in the carcinogenicity studies of FTC and TDF,[52)] none of EVG, COBI, FTC, or TDF increased the incidence of tumor at exposure levels exceeding that achieved by the clinical dose in humans. Therefore, they are unlikely to induce cancer in humans. In genotoxicity studies, COBI and FTC[52)] were tested negative for genotoxicity [see "3.(iii).A.(3) Genotoxicity studies"]. EVG was pseudo-positive in the *in vitro* chromosomal aberration test, but negative in the *in vivo* rat bone marrow micronucleus test, suggesting a low risk of genotoxicity [see "3.(iii).A.(3) Genotoxicity studies"]. TDF was positive in the TK study on mouse lymphoma and in the unscheduled DNA synthesis test, but negative in the mouse micronucleus test, from which TDF is considered to have a low risk of genotoxicity.[52)] Furthermore, in the TK study on mouse lymphoma, concomitant use of FTC and TDF showed neither an additive nor synergistic effect, which suggests that a combination therapy of EVG, COBI, FTC, and TDF has a low risk of genotoxicity. Thus, the combined administration of these drugs is least likely to increase the carcinogenic risk.

PMDA accepted the above explanation of the prior assessment requestor.

- PMDA asked the prior assessment requestor to explain (i) the clinical relevance of lipid vacuoles in the lamina propria of the small intestine found in rats and dogs and (ii) adverse events that could occur when lipid vacuoles accumulated after a long-term administration.

  The prior assessment requestor explained as follows:
  Results of various studies on the mechanism of toxicity in rats suggest that the amount of lipid vacuoles formed in the lamina propria of the small intestine depends on local EVG concentration in the small intestine and that food components in the gastrointestinal tract act as factors contributing to the formation of lipid vacuoles [see "3.(iii).A.(7).3) Studies on the mechanism of toxicity"]. Given that lipid vacuoles are caused by changes in the process of lipid absorption in the small intestine and that lipid vacuoles formed in dogs as well, the possibility of lipid vacuoles being formed in humans as well cannot be excluded. However, prominent formation of lipid vacuoles in rats was found only at ≥1000 mg/kg, and the local EVG concentration in the small intestine at the dose of 1000mg/kg is estimated to be 979 times that achieved following the administration of the clinical dose (150 mg) in humans.[53)] Therefore, EVG, when administered to humans at the clinical dose, is unlikely to elevate the local concentration so high that it causes prominent formation of lipid vacuoles as in rat small intestine. Rats in the high dose group (2000 mg/kg) in the carcinogenicity study of EVG did not show any clinical signs or changes in body weight or food intake supposedly related to lipid vacuoles formed in the lamina propria of the small

52) Data submitted in the initial application for Emtriva Capsules 200 mg or Viread Tab. 300 mg
53) In rats, the estimated local EVG concentration in the small intestine was 11.94 mg/mL when the drug was administered at a dose causing prominent formation of lipid vacuoles (≥1000 mg/kg by gavage in Study JTK303-TX-021, 5% mixed by diet in Study JTK303-TX-028). In humans, the estimated local EVG concentration in the small intestine following the administration at the clinical dose (150 mg) was 0.0122 mg/mL.

intestine, nor did they show any injurious changes in the small intestine or inflammatory changes in response to lipid vacuoles [see "3.(iii).A.(4).1).(b) Two-year repeated oral dose carcinogenicity study in rats"]. Based on the above, lipid vacuoles are unlikely to occur in humans, and lipid vacuole-induced events are unlikely to occur even in a long-term administration.

PMDA accepted the above explanation of the prior assessment requestor.

- PMDA asked the prior assessment requestor to explain the possible toxicity caused by administration of the proposed drug product, taking account that the ratio of exposure level of COBI's NOAEL to that of COBI's clinical dose in humans is small (safety margin of 0.1-7.2 times).

  The prior assessment requestor explained as follows:
  In the repeated oral dose toxicity studies of COBI (up to 13 weeks in mice, up to 26 weeks in rats, up to 39 weeks in dogs), toxic effects were noted in the target organs of liver (mice, rats, dogs) and thyroid gland (rats). Effects on the liver in mice and rats and on thyroid gland in rats are frequently found in rodents treated with liver metabolic enzyme inducers. Therefore, thyroid follicular cell adenoma and thyroid follicular cell carcinoma seen in the 2-year carcinogenicity study in rats are unlikely to be relevant to humans [see "3.(iii).A.(2).2) COBI" and "3.(iii).A.(4).2) COBI"]. Changes in the liver (increased weight, hepatocyte hypertrophy, high value of ALP) found in the 9-month repeated oral dose toxicity study in dogs were very mild in severity. They were not accompanied by changes in ALT or AST level or by any cytotoxic changes such as liver degeneration, and recovered after withdrawal, which suggest that the changes have least toxicological significance [see "3.(iii).A.(2).2).(g) Nine-month repeated oral dose toxicity study in dogs"]. Changes in thyroid gland, which were noted only in rats, were increased thyroid weight, changes in thyroid hormone levels (decreased T_4, increased TSH), and hyperplasia/hypertrophy of thyroid follicular cells found in the 26-week repeated oral dose study in rats. Thyroid follicular cell carcinoma was noted in 1 male. Thyroid follicular cell adenoma and/or thyroid follicular cell carcinoma were found in the 2-year carcinogenicity study in rats as well, and only the incidence of these two tumors increased due to COBI administration. These changes in thyroid gland are considered to be adaptive responses related to the changes in the liver. Thus, liver metabolic enzymes and the imbalance of thyroid hormones were considered involved as the primary causes.[54)] Effects on thyroid gland appear to be specific to rodents; COBI increases the risk of thyroid tumor in rats but is unlikely to have similar effects in humans. COBI induces liver metabolic enzymes by species-specifically activating rat PXR, whereas enzyme induction does not occur by this mechanism in humans. Therefore, in humans, COBI is least likely to induce secondary thyroid tumor by liver the metabolic enzyme induction and the thyroid hormone imbalance. In clinical studies conducted so far of COBI and the proposed drug product, no clinically significant adverse events related to thyroid gland or liver function have been found[55)] [see "4.(ii).B.(3) Safety evaluation"]. Although the safety margin of COBI, seen in nonclinical studies, was not wide, the safety of the proposed drug product is fully ensured by a series of nonclinical studies because the effects at doses exceeding the NOAEL were all very mild, and the effects on the liver and thyroid gland are species-specific and cannot be extrapolated to humans.

54) Burns-Naas LA et al. *Hum Exp Toxicol*. 2005;24(12):643-654, Capen CC. *Toxicol Pathol*, 2001;29(1):8-33, Cohen SM et al. *Toxicol Sci*. 2004;78(2):181-186, Wu KM, Farrelly JG. *Am J Ther*. 2006;13(2):141-144, McClain RM. *Mutat Res*. 1995;333(1-2):131-142, Meek ME et al. *Crit Rev Toxicol*. 2003;33(6):591-653

55) The study on thyroid function has demonstrated that there are no significant changes from baseline in TSH, T3, T4, or immunoglobulin (Study GS-US-236-0104). The studies on liver function have shown that the incidences of increased ALT, AST, γ-GTP, alkaline phosphatase, and bilirubin are equal to or less than those in the comparator group and that there are no significant difference in their severity (Studies GS-US-236-0102 and GS-US-236-103).

PMDA accepted the above explanation of the prior assessment requestor.

4. Clinical data

4.(i) Human pharmacokinetics and pharmacodynamics

4.(i).A. Summary of the submitted data

- Data from 1 BA study of EVG and from 3 BA studies of the coformulated EVG/COBI/FTC/TDF (including 1 food effect study) were submitted as data of biopharmaceutical studies. The following clinical pharmacokinetic data were submitted: of EVG, 12 phase I studies in healthy adult subjects (including 2 studies in Japanese healthy adult male subjects) and 1 each of phase I/II and phase II study in patients with HIV-1 infection; of COBI, 7 phase I studies in healthy adult subjects; of EVG/COBI, 6 phase I studies in healthy adult subjects; and of the coformulated EVG/COBI/FTC/TDF, 4 phase I studies in healthy adult subjects (including 3 BA studies described above), and 1 phase II and 2 phase III studies in patients with HIV-1 infection. Pharmacokinetic parameter values are expressed in means unless otherwise specified. In the following sections other than "4.(i).A.(1) Bioequivalence and food effect," the combination tablet comprising EVG, COBI, FTC, and TDF is defined as "the EVG/COBI/FTC/TDF combination," regardless of the types of coformulations developed.

4.(i).A.(1) Bioequivalence and food effect

4.(i).A.(1).1) EVG

- Study GS-US-183-0140 was conducted to evaluate the relative BA of EVG between EVG 125 mg tablets (EVG formulation for phase II studies) and EVG 150 mg tablets (EVG formulation for phase III studies[56]), both in concomitant uses with RTV 100 mg. As a result, the ratios of the least squares means and its 90% confidence intervals (CI) were 1.09 [1.03, 1.15] for AUC_{tau}, 1.05 [0.99, 1.12] for C_{max}, and 1.10 [0.99, 1.23] for trough concentration (C_{tau}).

4.(i).A.(1).2) EVG/COBI/FTC/TDF combination

- In Study GS-US-236-0101, combination tablets containing EVG (150 mg), COBI (100 or 150 mg), FTC (200 mg), and TDF (300 mg) were administered, and the relative BA of each component was compared with that found in concomitant use of EVG (150 mg) and RTV (100 mg) or in concomitant use of FTC (200 mg) and TDF (300 mg). Results were as shown in the following table.

[56] Prior to the phase III study (Study GS-US-183-0145) conducted in previously treated patients withHIV-1 infection using RAL as the comparator to evaluate the efficacy and safety of EVG/RTV, the drug substance and the drug product of EVG were optimized from the EVG formulation for phase II studies (i.e., formulation used in Study GS-US-183-0105). The resulting product with the new formulation containing the drug substance manufactured in the new manufacturing process was used for EVG formulation for phase III studies.

Table. Pharmacokinetic parameters of each component in EVG/COBI/FTC/TDF combination, EVG + RTV, or FTC + TDF, and ratios of their least squares mean to those of comparative formulations

	EVG/COBI/FTC/TDF combination	Comparator	Ratio of least squares mean [90% CI]
EVG	COBI 100 mg formulation	EVG + RTV	–
AUC_{tau} (ng·h/mL)	21,102.1 (25.4)	22,514.3 (23.4)	0.93 [0.89, 0.98]
C_{max} (ng/mL)	2246.6 (26.3)	2498.0 (32.1)	0.92 [0.86, 0.98]
C_{tau} (ng/mL)	282.3 (60.4)	408.5 (40.5)	0.63 [0.57, 0.71]
EVG	COBI 150 mg formulation	EVG + RTV	–
AUC_{tau} (ng·h/mL)	26,986.9 (29.4)	22,514.3 (23.4)	1.18 [1.10, 1.26]
C_{max} (ng/mL)	2660.5 (27.6)	2498.0 (32.1)	1.08 [1.00, 1.16]
C_{tau} (ng/mL)	489.8 (52.9)	408.5 (40.5)	1.10 [0.95, 1.27]
COBI	COBI 150 mg formulation	EVG/COBI/FTC/TDF (containing COBI 100 mg)	–
AUC_{tau} (ng·h/mL)	10,405.0 (35.2)	5153.5 (31.7)	1.95 [1.77, 2.16]
C_{max} (ng/mL)	1566.1 (29.7)	855.4 (27.6)	1.79 [1.65, 1.95]
C_{tau} (ng/mL)	22.7 (107.2)	7.6 (124.4)	2.35 [2.11, 2.61]
FTC	COBI 150 mg formulation	FTC + TDF	–
AUC_{tau} (ng·h/mL)	11,526.9 (19.4)	9326.7 (22.1)	1.27 [1.15, 1.40]
C_{max} (ng/mL)	1858.9 (22.5)	1603.5 (26.1)	1.21 [1.07, 1.37]
C_{tau} (ng/mL)	100.5 (26.8)	80.1 (25.9)	1.26 [1.18, 1.36]
TFV	COBI 150 mg formulation	FTC + TDF	–
AUC_{tau} (ng·h/mL)	3011.8 (20.4)	2552.6 (22.6)	1.18 [1.14, 1.22]
C_{max} (ng/mL)	331.5 (28.9)	251.8 (24.9)	1.30 [1.22, 1.38]
C_{tau} (ng/mL)	64.7 (26.0)	52.0 (25.4)	1.24 [1.19, 1.29]
RTV	–	EVG + RTV	–
AUC_{tau} (ng·h/mL)	–	5746.5 (38.4)	–
C_{max} (ng/mL)	–	1033.1 (47.8)	–
C_{tau} (ng/mL)	–	37.6 (52.2)	–

Mean (coefficient of variation [CV] %)

- Study GS-US-236-0110 was conducted to compare BA of components of the coformulated EVG/COBI/FTC/TDF for the phase II study with that of the coformulated EVG/COBI/FTC/TDF for the phase III studies (i.e., the commercial formulation).[57)] The ratios of the least squares means and the 90% CI of AUC_{tau}, C_{max}, and C_{tau} of EVG between the above two formulations were 0.95 [0.91, 1.00], 0.97 [0.91, 1.03], and 0.96 [0.88, 1.05], respectively; and of COBI, 0.99 [0.96, 1.02], 0.96 [0.93, 0.98], and 0.96 [0.87, 1.06], respectively.

- In Study GS-US-236-0105, the coformulated EVG/COBI/FTC/TDF for the phase III studies was administered, and the effect of food on the pharmacokinetics of each component was investigated. Results were as shown in the following table.

57) During the early stage of the development of the EVG/COBI/FTC/TDF combination, COBI was formulated using ethanol solution (formulation for phase II studies, used in Study GS-US-236-0140). Prior to the start of the phase III studies (Studies GS-US-236-0102 and GS-US-236-0103), the formulation containing a mixture of COBI and a solid carrier (silicon dioxide) was developed for use in phase III studies and for marketing.

Table. Effect of food on pharmacokinetic parameters of EVG, COBI, FTC, and TFV

Dietary conditions	C_{max}	AUC_{inf}	AUC_{last}
EVG			
Light meal vs. fasting	1.22 [1.08, 1.38]	1.34 [1.19, 1.51]	1.36 [1.21, 1.54]
High calorie/high fat diet vs. fasting	1.56 [1.38, 1.76]	1.87 [1.66, 2.10]	1.91 [1.70, 2.16]
High calorie/high fat diet vs. light meal	1.28 [1.14, 1.45]	1.39 [1.23, 1.57]	1.40 [1.24, 1.58]
COBI			
Light meal vs. fasting	1.04 [0.94, 1.14]	1.03 [0.90, 1.17]	1.03 [0.90, 1.18]
High calorie/high fat diet vs. fasting	0.76 [0.68, 0.84]	0.83 [0.73, 0.95]	0.82 [0.72, 0.94]
High calorie/high fat diet vs. light meal	0.73 [0.66, 0.81]	0.81 [0.71, 0.92]	0.80 [0.70, 0.92]
FTC			
Light meal vs. fasting	0.95 [0.86, 1.05]	0.95 [0.91, 1.00]	0.94 [0.90, 0.99]
High calorie/high fat diet vs. fasting	0.96 [0.87, 1.06]	0.96 [0.92, 1.00]	0.96 [0.91, 1.00]
High calorie/high fat diet vs. light meal	1.01 [0.91, 1.11]	1.00 [0.96, 1.05]	1.01 [0.97, 1.06]
TFV			
Light meal vs. fasting	1.20 [1.04, 1.39]	1.24 [1.18, 1.30]	1.25 [1.19, 1.31]
High calorie/high fat diet vs. fasting	1.03 [0.89, 1.20]	1.23 [1.17, 1.29]	1.25 [1.19, 1.31]
High calorie/high fat diet vs. light meal	0.86 [0.74, 1.00]	1.00 [0.95, 1.05]	1.00 [0.95, 1.05]

Ratio of least squares means [90% CI]

4.(i).A.(2) Studies in healthy adult subjects

4.(i).A.(2).1) EVG

- In a phase I study (Study GS-US-183-0102), EVG 100 mg was administered orally to healthy adult subjects in a single dose or multiple doses for 10 days with or without concomitant use of RTV. The exposure level of EVG under the steady state in multiple dose administration without concomitant use of RTV (AUC_{tau} = 719.3 ng·h/mL on Day 10) was lower by approximately 20% than the exposure level after a single dose administration (AUC_{inf} = 908.1 ng·h/mL on Day 1). This suggested that multiple dosing resulted in the self-induction of metabolic enzymes (CYP3A) by EVG. [for CYP induction by EVG, see "3.(ii).A.(5).1) EVG"]. When EVG was concomitantly administered with RTV, EVG exposure level was increased by the CYP3A4 inhibitory action of RTV (AUC_{tau} = 6167.3 ng·h/mL on Day 1 of EVG + RTV, AUC_{tau} = 14,302.1 ng·h/mL on Day 10 of EVG + RTV), accompanied by an increase in $t_{1/2}$ (median $t_{1/2}$ was 3.1 hours on Day 1 and 3.5 hours on Day 10 of EVG; 18.2 hours on Day 1 and 9.5 hours on Day 10 of EVG + RTV).

4.(i).A.(2).2) COBI

- In phase I studies (Studies GS-US-216-0101 and GS-US-216-0113), COBI (50, 100, 200, 300, 400 mg) was administered orally to healthy adult subjects in a single dose or multiple doses for 14 days. After single dosing, the median t_{max} was within the range of 3.3 to 4.3 hours, showing no significant difference among dose groups, whereas C_{max} was 61.6, 343, 1200, 2340, and 4110 ng/mL, respectively. Also, AUC_{inf} was 243, 1650, 8420, 20,800, and 39,900 ng·h/mL, respectively, with both parameters showing more than dose-proportional increases. The median $t_{1/2}$ was 1.4, 2.7, 4.2, 5.2, and 4.8 hours, respectively, showing a dose-dependent increase. The prior assessment requestor explained that the increases were caused by the inhibitory effect of COBI on CYP3A4, the enzyme involved in the metabolism of COBI [see "3.(ii).A.(3).2) COBI]. As regards the exposure level of COBI after multiple dosing of COBI (50, 100, 200 mg), C_{max} was 170, 563, and 1850 ng/mL, respectively, and AUC_{tau} was 827, 3440, and 16,100 ng·h/mL, respectively. Both parameters showed more than dose-proportional increases, as was the case with single dosing.

4.(i).A.(2).3) EVG/COBI/FTC/TDF combination

- In phase I studies (Studies GS-US-236-0105 and GS-US-236-0110), the EVG/COBI/FTC/TDF combination was administered orally to healthy adult subjects in a single dose or multiple doses. The exposure level of EVG was similar between the single dosing (C_{max} = 1762.1 ng/mL, AUC_{inf} = 21,123.3 ng·h/mL) and the multiple dosing (C_{max} = 1919.3 ng/mL, AUC_{tau} = 22,485.9 ng·h/mL), whereas C_{tau} was 355 ng/mL (C_{24h}) in single dosing and 508 ng/mL in multiple dosing, showing an increase after multiple dosing. The exposure level of COBI was higher in the multiple dosing (C_{max} = 1528.0 ng/mL, AUC_{tau} = 11,288.2 ng·h/mL) than in the single dosing (C_{max} = 1243.1 ng/mL, AUC_{inf} = 8092.3 ng·h/mL). The exposure level of FTC was similar between single dosing (C_{max} = 1811.5 ng/mL, AUC_{inf} = 10,738.6 ng·h/mL) and multiple dosing (C_{max} = 1932.1 ng/mL, AUC_{tau} = 12,491.0 ng·h/mL). The exposure level of TFV was higher in the multiple dosing (C_{max} = 485.6 ng/mL, AUC_{tau} = 4066.2 ng·h/mL) than in the single dosing (C_{max} = 386.3 ng/mL, AUC_{inf} = 3139.0 ng·h/mL).

4.(i).A.(3) Studies in patients with HIV-1 infection

4.(i).A.(3).1) EVG

- In the phase I/II study (Study GS-US-183-0101), EVG (200, 400, 800 mg; twice daily), EVG (800 mg once daily), or EVG (50 mg) + RTV (100 mg) once daily was administered orally to patients with HIV-1 infection in a single dose or multiple doses. The median $t_{1/2}$ of EVG was 2.53 to 3.80 hours following the administration of EVG alone and 8.86 hours following the concomitant use of EVG and RTV, showing that the $t_{1/2}$ was increased by concomitant use of RTV. Steady-state AUC[58)] after twice daily administration of EVG (200, 400, 800 mg) was lower by 31% (2820 ng·h/mL on Day 1, 1950 ng·h/mL on Day 10) at 200 mg, by 23% (3050 ng·h/mL on Day 1, 2340 ng·h/mL on Day 10) at 400 mg, and by 52% (7410 ng·h/mL on Day 1, 3570 ng·h/mL on Day 10) at 800 mg than the AUC after the single dosing. This suggested CYP3A self-induction by EVG. Steady-state AUC[58)] of EVG in multiple use of EVG/RTV was 34% higher than the AUC in the single dosing (6590 ng·h/mL on Day 1, 8840 ng·h/mL on Day 10).

- In the phase II study (Study GS-US-183-0105), multiple doses of EVG (20, 50, 125 mg) were orally administered concomitantly with RTV (100 mg) to patients with HIV-1 infection. At Week 8, C_{max} of EVG was 265.79, 753.71, and 1442.20 ng/mL, respectively, and AUC_{tau} was 3029.25, 8701.86, and 16,789.54 ng·h/mL, respectively. The exposure level of EVG increased in a dose-proportional manner with the increase of dose from 20 to 50 mg, but in a less than dose-proportional manner from 50 to 125 mg.

4.(i).A.(3).2) COBI

- Pharmacokinetics of COBI after administration of COBI alone was not investigated in patients with HIV-1 infection [see "4.(i).A.(3).3) EVG/COBI/FTC/TDF combination"].

4.(i).A.(3).3) EVG/COBI/FTC/TDF combination

- In the phase II study (Study GS-US-236-0104) and the phase III studies (Studies GS-US-236-0102 and GS-US-236-0103), the EVG/COBI/FTC/TDF combination was administered to patients with HIV-1 infection. At steady state, AUC_{tau} of EVG was 23,000 ng·h/mL, C_{max} was 1730 ng/mL, and C_{tau} was 451 ng/mL, with the exposure level being similar to that in healthy adult subjects treated with EVG/COBI/FTC/TDF combination (Study GS-US-236-0110).[59)] AUC_{tau} of COBI was 8300 ng·h/mL, C_{max} was 1140 ng/mL, and C_{tau} was 49.1 ng/mL, with AUC_{tau} and C_{max} being lower than those in healthy adult

58) AUC represents AUC_{inf} on Day 1 and AUC_{tau} on Day 10.
59) AUC_{tau}, 22,485.9 ng·h/mL; C_{max}, 1919.3 ng/mL; C_{tau}, 508.2 ng/mL

subjects (Study GS-US-236-0110).[60] The prior assessment requestor explained that, although contributing factors of the differences were unclear, the COBI dose posed no particular problem because the exposure level of COBI does not significantly affect the exposure level of EVG, based on the results of PPK analysis[61] using the plasma EVG concentration data in the phase I studies using EVG/COBI and the phase II and III studies using EVG/COBI/FTC/TDF combination (8273 measuring points in a total of 580 subjects consisting of 161 healthy adult subjects and 419 patients with HIV-1 infection[62] [for justification of dosage and administration, see "4.(i).B Outline of the prior assessment by PMDA" and "4.(ii).B.(5) Dosage and administration"]. AUC_{tau} of FTC was 12,700 ng·h/mL, C_{max} was 1880 ng/mL, and C_{tau} was 142 ng/mL, and AUC_{tau} of TFV was 4360 ng·h/mL, C_{max} was 450 ng/mL, and C_{tau} was 98.7 ng/mL. Thus, the exposure levels of FTC and TFV were similar to those in healthy adult subjects (Study GS-US-236-0110).[63]

- PPK analysis of 9 clinical studies[62] using EVG/COBI or EVG/COBI/FTC/TDF combination showed that pharmacokinetics of EVG administered as EVG/COBI/FTC/TDF combination was not affected by age, sex, race, subjects studied (healthy adult subjects, patients with HIV-1 infection), body weight, BMI, creatinine clearance (CL_{cr}), superinfection with hepatitis B or C virus, or exposure level of COBI. PPK data suggested the possible effect of body surface area on the exposure level of EVG. However, when subjects were classified into quartiles[64] by body surface area and EVG exposure level was calculated and compared among the quartiles,[65] any clinically significant association was not found between EVG exposure level and body surface area, although an association was suggested in the PPK analysis.

- PPK analysis[66] of plasma COBI concentration data (a total of 9584 measuring points in 504 subjects)[67] in the phase I studies using COBI and in the phase II and III studies using EVG/COBI/FTC/TDF combination was conducted. As a result, the pharmacokinetics of COBI administered as EVG/COBI/FTC/TDF combination was not affected by age, sex, race, BMI, body surface area, dosage form, underlying therapy, CL_{cr}, subjects studied (healthy adult subjects, patients with HIV-1 infection), or superinfection by hepatitis B or C virus. PPK data suggested the possible effect of body weight on COBI exposure level. However, in the data set used for PPK analysis, subjects with body weight below 5 percentile (57 kg) and above 95 percentile (101 kg) had AUC_{tau} of 14,063 ng·h/mL and 7507 ng·h/mL, respectively. The both of them exceeded the AUC_{tau} (5153.5 ng·h/mL) obtained at 100 mg of COBI, the dose showing the approximately maximum CYP3A4 inhibitory action [see "4.(i).A.(1).2) EVG/COBI/FTC/TDF combination"]. Based on the above, the prior assessment requestor explained that, in both body weight ranges, COBI exposure level was sufficiently high for inhibiting CYP3A, and therefore body weight was unlikely to have any clinically significant association with the COBI exposure level.

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60) AUC_{tau}, 11,288.2 ng·h/mL; C_{max}, 1528.0 ng/mL; C_{tau}, 45.5 ng/mL

61) NONMEM version 7.2 was used.

62) Results of the following studies were used: 6 phase I studies (Studies GS-US-236-0105, GS-US-236-0110, GS-US-236-0101, GS-US-216-0123, GS-US-236-0106, and GS-US-216-0116), 1 phase II study (Study GS-US-236-0104), and 2 phase III studies (Studies GS-US-236-0102 and GS-US-236-0103).

63) For FTC, AUC_{tau} was 12,491.0 ng·h/mL, C_{max} was 1932.1 ng/mL, and C_{tau} was 116.4 ng/mL.
For TFV, AUC_{tau} was 4066.2 ng·h/mL, C_{max} was 485.6 ng/mL, and C_{tau} was 84.1 ng/mL.

64) Body surface area $\leq$1.81 m^2 (108 subjects in the group 1); 1.82 to 1.95 m^2 (111 subjects in the group 2); 1.96 to 2.07 m^2 (98 subjects in the group 3); and >2.07 m^2 (102 subjects in the group 4)

65) In group 1, AUC_{tau} was 26,100 ng·h/mL; C_{max}, 1960 ng/mL; C_{tau}, 515 ng/mL. In group 2, AUC_{tau} was 21,700 ng·h/mL; C_{max}, 1670 ng/mL; C_{tau}, 411 ng/mL. In group 3, AUC_{tau} was 22,000 ng·h/mL; C_{max}, 1650 ng/mL; C_{tau}, 440 ng/mL. In group 4, AUC_{tau} was 21,800 ng·h/mL; C_{max}, 1630 ng/mL; C_{tau}, 438 ng/mL

66) NONMEM version 7.2 was used.

67) Data of the following studies were used: 11 phase I studies (Studies GS-US-216-0115, GS-US-216-0116, GS-US-216-0119, GS-US-216-0120, GS-US-216-0121, GS-US-216-0122, GS-US-216-0123, GS-US-216-0124, GS-US-236-0105, GS-US-236-0106, and GS-US-236-0110); 2 phase II studies (Studies GS-US-216-0105 and GS-US-236-0104); and 3 phase III studies (Studies GS-US-216-0114, GS-US-236-0102, and GS-US-236-0103).

4.(i).A.(4) Pharmacokinetics of EVG in Japanese subjects

- In a phase I study (Study XAX1-1), EVG (100, 200, 400, 800 mg) was administered orally in a single dose to Japanese healthy adult male subjects. AUC_{inf} was 538, 798, 1451, and 2524 ng·h/mL, respectively, and C_{max} was 108, 160, 264, and 455 ng/mL, respectively, showing less than dose-proportional increases. EVG (400 mg) was administered orally before or after a meal to evaluate the food effect. AUC_{inf} and C_{max} in the fed states were 3942 ng·h/mL and 903 ng/mL, respectively, which were higher than in the fasted states (AUC_{inf}, 1451 ng·h/mL; C_{max}, 264 ng/mL). Comparison of the data with those obtained in a phase I study (Study GS-US-183-0101) in foreign patients with HIV-1 infection using the drug product manufactured according to the same formulation as that in the above study [see "4.(i).A.(3).1) EVG"] showed no significant difference in EVG exposure level (C_{max}, AUC_{inf}) between Japanese and Caucasians subjects.

4.(i).A.(5) Effect of renal function on pharmacokinetics of EVG and COBI concomitantly administered

- In Study GS-US-216-0124, EVG (150 mg) + COBI (150 mg) was administered once daily for 7 days to subjects with severe renal impairment (CL_{cr}[68)] <30 mL/min) and to subjects with normal renal function (CL_{cr} ≥90 mL/min), and pharmacokinetics of these drugs were investigated. Results were as shown in the following table. Based on the results, the prior assessment requestor considered that the differences in the exposure levels of EVG and COBI were not clinically significant between subjects with renal impairment and subjects with normal renal function. Also, the prior assessment requestor explained that it is therefore unnecessary to adjust the dose of EVG or COBI in patients with HIV-1 infection who have renal impairment.

Table. Pharmacokinetic parameters of EVG and COBI concomitantly administered to subjects with renal impairment and ratio of their least squares means to those in subjects with normal renal function

	Subjects with severe renal impairment	Subjects with normal renal function	Ratio of least squares mean [90% CI]
EVG			
AUC_{tau} (ng·h/mL)	26,044.6 (24.3)	34,597.3 (26.9)	0.76 [0.63, 0.91]
C_{max} (ng/mL)	2224.1 (26.7)	3348.6 (33.9)	0.67 [0.55, 0.83]
C_{tau} (ng/mL)	531.2 (42.3)	761.1 (38.9)	0.69 [0.52, 0.92]
COBI			
AUC_{tau} (ng·h/mL)	18,552.9 (36.8)	14,212.4 (24.4)	1.25 [0.99, 1.60]
C_{max} (ng/mL)	2149.4 (35.0)	1709.3 (22.4)	1.22 [1.00, 1.50]
C_{tau} (ng/mL)	165.7 (128.2)	97.0 (61.0)	1.13 [0.57, 2.24]

Mean (CV%)

- Meanwhile, the prior assessment requestor took account of the facts that patients with CL_{cr} of <70 mL/min were not included in the phase II study (Study GS-US-236-0104) or phase III studies (Studies GS-US-236-0102 and GS-US-236-0103) of EVG/COBI/FTC/TDF combination, and that, for FTC and TDF, dose adjustment is required in patients with CL_{cr} of <50 mL/min but such adjustment is impossible with EVG/COBI/FTC/TDF combination, which is formulated as combination tablets. As a result, the prior assessment requestor explained that caution will be provided that treatment with EVG/COBI/FTC/TDF combination should not be started in patients with CL_{cr}<70 mL/min and that administration of EVG/COBI/FTC/TDF combination should be discontinued if CL_{cr} decreases <50

68) Calculated using Cockroft-Gault equation:
CL_{cr} = ([140 – age [years] × body weight [kg] × [0.85 in female])/(serum creatinine [mg/dL] × 72)

mL/min after treatment start.[69)]

4.(i).A.(6) Effect of hepatic function on pharmacokinetics of EVG and COBI concomitantly administered

- In Study GS-US-183-0133, EVG (150 mg) + COBI (150 mg) was administered once daily for 10 days to subjects with moderate hepatic impairment (Child-Pugh class B) and to subjects with normal hepatic function, and pharmacokinetics of these drugs were investigated. Results were as shown in the following table. The prior assessment requestor took account of the following results that showed no significant difference in the exposure level of EVG and COBI between subjects with normal hepatic function and subjects with moderate hepatic impairment and that both FTC and TDF are excreted mainly from the kidney. As a result, the prior assessment requestor explained that the dose of EVG/COBI/FTC/TDF combination need not be adjusted in patients with mild to moderate hepatic impairment. The prior assessment requestor also explained that caution will be provided that no pharmacokinetic data of EVG and COBI are available for patients with severe hepatic impairment (Child-Pugh class C).

Table. Pharmacokinetic parameters of EVG and COBI concomitantly administered to subjects with moderate hepatic impairment and ratios of their least squares means to those in subjects with normal hepatic function

	Subjects with moderate hepatic impairment	Subjects with normal hepatic function	Ratio of least squares mean [90% CI]
EVG			
AUC_{tau} (ng·h/mL)	29,802.7 (40.6)	21,278.5 (27.8)	1.35 [1.03, 1.77]
C_{max} (ng/mL)	2822.9 (33.7)	1951.5 (29.8)	1.41 [1.09, 1.83]
C_{tau} (ng/mL)	740.9 (65.3)	370.2 (43.7)	1.80 [1.11, 2.91]
COBI			
AUC_{tau} (ng·h/mL)	9903.8 (33.9)	9844.4 (36.8)	1.00 [0.76, 1.31]
C_{max} (ng/mL)	1152.1 (33.4)	1293.8 (29.6)	0.86 [0.65, 1.13]
C_{tau} (ng/mL)	90.7 (75.5)	41.0 (75.2)	2.08 [1.17, 3.68]

Mean (CV%)

4.(i).A.(7) Drug-drug interaction studies

- Data of 12 clinical drug interaction studies of EVG and COBI were submitted. The following table shows results of studies on the interactions of EVG or COBI with other drugs.

[69)] A clinical study (Study GS-US-236-0118) is ongoing in patients with moderate renal impairment ($50 \leq CL_{cr} \leq 79$ mL/min) in the U.S.

Table. Studies on interactions of EVG or COBI with other drugs

Concomitant drug	Treatment group including EVG or COBI	No. of subjects	Ratio of pharmacokinetic parameter values of EVG, COBI, or concomitant drugs in concomitant use to those without concomitant use* [90% CI]			
			Drug	C_{max}	AUC_{tau}	C_{tau}
RTV 100 mg single dose	EVG 100 mg BID	12	EVG	NC	8.58 [7.11, 10.36]	NC
RTV 100 mg BID		12	EVG	11.06 [9.25, 13.24]	19.94 [16.87, 23.57]	NC
Ketoconazole 200 mg BID	EVG 150 mg + RTV 100 mg QD	18	EVG	1.17 [1.04, 1.33]	1.48 [1.36, 1.62]	1.67 [1.48, 1.88]
Maalox Max (antacid) 20 mL 4 hours before EVG/RTV administration	EVG 50 mg + RTV 100 mg QD	8	EVG	0.95 [0.84, 1.07]	0.96 [0.88, 1.04]	1.04 [0.93, 1.17]
4 hours after EVG/RTV administration		10		0.98 [0.88, 1.10]	0.98 [0.91, 1.06]	1.00 [0.90, 1.11]
2 hours before EVG/RTV administration		11		0.82 [0.74, 0.91]	0.85 [0.79, 0.91]	0.90 [0.82, 0.99]
2 hours after EVG/RTV administration		10		0.79 [0.71, 0.88]	0.80 [0.75, 0.86]	0.80 [0.73, 0.89]
ATV 300 mg QD	EVG 200 mg + RTV 100 mg QD	33	EVG	1.85 [1.69, 2.03]	2.00 [1.85, 2.16]	2.88 [2.53, 3.27]
		33	ATV	0.84 [0.78, 0.91]	0.79 [0.74, 0.85]	0.65 [0.59, 0.73]
	EVG 85 mg + RTV 100 mg QD	20	EVG	0.91 [0.81, 1.02]	1.07 [0.95, 1.21]	1.38 [1.18, 1.61]
		20	ATV	0.97 [0.87, 1.08]	0.89 [0.80, 0.99]	0.83 [0.72, 0.95]
	EVG 85 mg + COBI 150 mg QD	18	EVG	0.84 [0.62, 1.15]	1.17 [0.88, 1.56]	1.83 [1.17, 2.86]
		18	ATV	0.76 [0.59, 0.98]	0.90 [0.73, 1.12]	0.81 [0.56, 1.17]
LPV/RTV 400/100 mg QD	EVG 125 mg QD	14	EVG	1.52 [1.29, 1.79]	1.75 [1.50, 2.05]	2.38 [1.81, 3.13]
		13	LPV	0.99 [0.88, 1.12]	0.97 [0.85, 1.09]	0.92 [0.79, 1.08]
		13	RTV	1.14 [0.87, 1.49]	1.03 [0.87, 1.21]	0.88 [0.74, 1.05]
Desipramine 50 mg Single dosing on Day 10 of COBI treatment	COBI 150 mg QD	8	Desipramine	1.24 [1.08, 1.44]	1.65 [1.36, 2.02][a)]	NA
Digoxin 0.5 mg Single dosing on Day 10 of COBI treatment	COBI 150 mg QD	22	Digoxin	1.41 [1.29, 1.55]	1.08 [1.00, 1.17][a)]	NA
EFV 600 mg Single dosing on Day 10 of COBI treatment	COBI 150 mg QD	17	EFV	0.87 [0.80, 0.94]	0.93 [0.89, 0.97][a)]	NA
		16 [b)]		0.89 [0.83, 0.96]	0.92 [0.89, 0.96][a)]	NA
Rosuvastatin 10 mg single dose	EVG 150 mg + COBI 150 mg QD	10	EVG	0.94 [0.83, 1.07]	1.02 [0.91, 1.14]	0.98 [0.83, 1.16]
		10	Rosuvastatin	1.89 [1.48, 2.42]	1.38 [1.14, 1.67][a)]	1.43 [1.08, 1.89][c)]
Famotidine 40 mg QD At dinner time 12 hours after EVG/COBI treatment	EVG 150 mg + COBI 150 mg QD	10	EVG	1.02 [0.89, 1.17]	1.03 [0.95, 1.13]	1.18 [1.05, 1.32]
		10	COBI	1.04 [0.99, 1.08]	1.05 [1.02, 1.08]	1.15 [1.06, 1.26]
Simultaneous administration		16	EVG	1.00 [0.92, 1.10]	1.03 [0.98, 1.08]	1.07 [0.98, 1.17]
		16	COBI	1.06 [0.99, 1.13]	1.03 [0.97, 1.11]	1.11 [1.00, 1.24]
Omeprazole 20 mg QD At breakfast time 2 hours before EVG/COBI treatment	EVG 150 mg + COBI 150 mg QD	11	EVG	1.16 [1.04, 1.30]	1.10 [1.02, 1.19]	1.13 [0.96, 1.34]
		11	COBI	0.90 [0.82, 0.99]	0.92 [0.85, 1.01]	0.93 [0.74, 1.17]
At dinner time 12 hours after EVG/COBI treatment		11	EVG	1.03 [0.92, 1.15]	1.05 [0.93, 1.18]	1.10 [0.92, 1.32]
		11	COBI	0.94 [0.85, 1.05]	0.99 [0.89, 1.09]	1.02 [0.82, 1.28]

Concomitant drug	Treatment group including EVG or COBI	No. of subjects	Ratio of pharmacokinetic parameter values of EVG, COBI, or concomitant drugs in concomitant use to those without concomitant use* [90% CI]			
			Drug	C_{max}	AUC_{tau}	C_{tau}
Omeprazole 40 mg QD	EVG 50 mg + RTV 100 mg QD	9	EVG	0.93 [0.83, 1.04]	0.99 [0.91, 1.07]	0.94 [0.85, 1.04]
Norgestimate (hormonal oral contraceptive) 0.180, 0.215, and 0.250 mg QD	EVG/COBI/FTC/TDF 150/150/200/300 mg QD	15	Norelgestro-min	2.08 [2.00, 2.17]	2.26 [2.15, 2.37]	2.67 [2.43, 2.92]
Etinyl estradiol 0.025 mg QD		15	Etinyl estradiol	0.94 [0.86, 1.04]	0.75 [0.69, 0.81]	0.56 [0.52, 0.61]
Rifabutin 150 mg QD	EVG 150 mg + COBI 150 mg QD	12	EVG	0.91 [0.84, 0.99]	0.79 [0.74, 0.85]	0.33 [0.27, 0.40]
		12	Rifabutin d)	1.09 [0.98, 1.20]	0.92 [0.83, 1.03]	0.94 [0.85, 1.04]
		12	25-O-Desacetyl rifabutin d)	4.84 [4.09, 5.74]	6.25 [5.08, 7.69]	4.94 [4.04, 6.04]

*, Ratio relative to the value without concomitant use

a, AUC_{inf}; b, Excluding outlier; c, C_{last}; d, Compared with the dose-adjusted value obtained by rifabutin 300 mg QD alone

NA, Not applicable; NC, Not calculated; Didanosine EC, Didanosine enteric coated

4.(i).A.(8) Effects on ventricular repolarization

4.(i).A.(8).1) EVG

- In a phase I study (Study GS-US-183-0128), EVG (125, 250 mg) was orally administered concomitantly with RTV (100 mg) for 10 days using moxifloxacin as the positive control, and the effect on QTc interval was investigated. As a result, the upper limit of the 90% CI of the mean difference in the change of QTcF on Day 10 from baseline (Day 1) between EVG and the control was 5.1 to 7.6 msec following EVG (125 mg) + RTV (100 mg) administration and 4.1 to 8.1 msec following EVG (250 mg) + RTV (100 mg) administration. The results did not exceed 10 msec, the threshold stipulated by ICH E14 guideline, in either group at any time point, demonstrating that EVG and RTV, up to the dose of 250 mg + 100 mg, did not prolong QTc interval.

4.(i).A.(8).2) COBI

- In a phase I study (Study GS-US-216-0107), COBI (250, 400 mg) was administered orally in a single dose using moxifloxacin as the positive control, and the effect on QTc interval was investigated. As a result, the upper limit of the 90% CI of the mean difference in the change of QTcF from baseline (before administration) between COBI and the control was -5.3 to 3.9 msec following COBI 250 mg administration and -8.8 to 7.9 msec following COBI 400 mg administration. The results did not exceed 10 msec, the threshold stipulated by ICH E14 guideline, in either group at any time point, demonstrating that COBI, up to the dose of 400 mg, did not prolong QTc interval.

- In a phase I study (Study GS-US-216-0116), COBI (150 mg) was administered orally for 10 days, and the effect on the left ventricular function was evaluated using echocardiogram (ECHO) and electrocardiogram (ECG). The results showed that COBI did not induce any clinically significant change in the left ventricular function.

4.(i).B. Outline of the prior assessment by PMDA

- PMDA asked the prior assessment requestor to explain the appropriateness of the dose selection (combination ratio) of each active ingredient (EVG, COBI, FTC, TDF) of EVG/COBI/FTC/TDF combination from the pharmacokinetic point of view.

The prior assessment requestor explained as follows:

(a). Dose of EVG (150 mg)

In Study GS-US-183-0101 [see "4.(i).A.(3).1) EVG"], C_{tau} of EVG following EVG/RTV (50/100 mg) once daily administration was higher than that in other groups receiving EVG alone,[70)] and the decrease in plasma HIV-1 RNA level was similar to that following EVG (400 mg or 800 mg) twice daily administration.[71)] Also by taking account of the results of Study GS-US-183-0102 [see "4.(i).A.(2).1) EVG"], EVG, even by once daily administration at a low dose, was expected to exhibit a continuous anti-HIV effect by the concomitant use of RTV, which inhibits CYP3A, the major EVG-metabolizing enzyme. Also, in the phase II study (Study GS-US-183-0105) in patients with HIV-1 infection who had previously been treated with ARV, the time-weighted average change in plasma HIV-1 RNA level at each time point (Weeks 16, 24, and 48) from baseline in the EVG (50 mg) + RTV (100 mg) group and the EVG (125 mg) + RTV (100 mg) group was similar to that in the CPI/RTV group.[72)] In addition, a significant decrease was found at Weeks 16 and 24 in the EVG (125 mg) + RTV (100 mg) group compared with the CPI/RTV group. Based on the above results, the optimal dose of EVG under the concomitant use of RTV was determined to be 125 mg [see "4.(i).A.(3).1) EVG"]. Furthermore, in Study GS-US-183-0140 [see "4.(i).A.(1).1) EVG"], EVG exposure level following the concomitant use of the EVG formulation for phase III studies (EVG 150 mg tablets) with RTV 100 mg[73)] was similar to the level obtained following the concomitant use of the EVG formulation for phase II studies (EVG 125 mg tablets) with RTV 100 mg. Based on the above results, the optimal dose of EVG was finally determined to be 150 mg.

The results of Studies GS-US-183-0101 and GS-US-183-0105 in patients with HIV-1 infection suggested that C_{tau} was the pharmacokinetic parameter of EVG most closely correlated with the decrease in plasma HIV-1 RNA level,[74)] and showed that, following the administration of EVG (150 mg) + RTV (100 mg), C_{tau} of EVG reached the concentration necessary to maximally decrease plasma HIV-1 RNA level.[75)] Also, results of PPK analysis[61)] confirmed that, when EVG/COBI/FTC/TDF combination was administered to patients with HIV-1 infection, C_{tau} of EVG almost reached the concentration necessary to maximally decrease plasma HIV-1 RNA level.

(b). Dose of COBI (150 mg)

In *in vitro* studies, COBI inhibited CYP3A to a similar extent to which RTV did, and no significant difference in inhibitory actions on various transporters between COBI and RTV was noted, but, unlike RTV, COBI did not induce various metabolic enzymes [see "3.(ii).A.(5).2) COBI"]. In a phase I study (Study GS-US-216-0101) in

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70) EVG 200 mg BID group, 30.73 ng/mL; EVG 400 mg BID group, 48.68 ng/mL; EVG 800 mg QD group, 13.62 ng/mL; EVG 800 mg BID group, 47.98 ng/mL; EVG/RTV 50/100 mg QD group, 135.00 ng/mL

71) EVG 200 mg BID group, 1.48 $\log_{10}$ copies/mL; EVG 400 mg BID group, 1.94 $\log_{10}$ copies/mL; EVG 800 mg QD group, 0.98 $\log_{10}$ copies/mL; EVG 800 mg BID group, 1.91 $\log_{10}$ copies/mL; EVG/RTV 50/100 mg group, 1.99 $\log_{10}$ copies/mL

72) CPI/RTV, RTV-boosted comparative protease inhibitor (CPI)

73) In Study GS-US-183-0113, RTV (20, 50, 100, 200 mg) was concomitantly administered with EVG (125 mg) to determine the RTV dose for boosting EVG. The clearance of EVG and MDZ decreased with the increase in the dose of RTV but was not different between 100 mg and 200 mg, from which the optimum dose of RTV for boosting EVG was considered to be 100 mg.

74) Based on the results of Study GS-US-183-0101, PK-PD analysis was performed to investigate the correlation between the decrease in plasma HIV-1 RNA level and pharmacokinetic parameters of EVG (C_{max}, C_{tau}, AUC_{tau}).

75) PK-PD analysis was performed based on the combined results of Studies GS-US-183-0101 and GS-US-183-0105 to investigate the correlation between the decrease in plasma HIV-1 RNA level and C_{tau} of EVG.

healthy adult subjects, midazolam (MDZ), a substrate of CYP3A, was orally administered, and the CYP3A-inhibitory action of COBI (50, 100, 200 mg) or RTV (100 mg) was investigated using oral clearance of MDZ as the index. The results suggested that the CYP3A-inhibitory effect of COBI almost reached the maximum level at the dose of 100 mg, and the inhibitory effect at this dose was similar to that obtained with RTV 100 mg. In a phase I study (Study GS-US-236-0101), 2 combination tablets containing 100 or 150 mg of COBI with fixed doses of EVG, FTC, and TDF (150 mg, 200 mg, and 300 mg, respectively) were administered. EVG exposure level (C_{tau}) following the administration of combination tablet containing COBI 100 mg was lower than that following the administration of EVG (150 mg) + RTV (100 mg), whereas C_{tau} following the administration of combination tablet containing COBI 150 mg was similar to that following the administration of EVG (150 mg) + RTV (100 mg) [see "4.(i).A.(1).2) EVG/COBI/FTC/TDF combination"]. Based on the above results, the dose of COBI in EVG/COBI/FTC/TDF combination was determined to be 150 mg.

(c). Doses of FTC and TDF (200 mg and 300 mg, respectively)
FTC and TDF neither induce nor inhibit CYPs, and clinical studies conducted so far did not show any data that suggested FTC or TDF had transporter related drug-drug interactions. Therefore, FTC and TDF are unlikely to significantly affect the pharmacokinetics of EVG or COBI. In a phase I study (Study GS-US-183-0103) in healthy adult subjects, drug-drug interactions between EVG + RTV and FTC/TDF were investigated. The results showed that FTC/TDF did not affect the pharmacokinetics of EVG + RTV.[76)] Based on the above results and on the results of Study GS-US-236-0101 [see "4.(i).B.(b). Dose of COBI (150 mg)"], neither FTC nor TDF affected the pharmacokinetics of EVG or COBI. In phase I studies (Studies GS-US-236-0101 and GS-US-236-0110), TFV exposure level was shown to be increased by the concomitant use of EVG and COBI.[77)] However, the exposure level of TFV when administered as EVG/COBI/FTC/TDF combination is similar to that when administered concomitantly with other ARVs with which safety and efficacy of TFV has been established. Therefore, the increase in exposure level of EVG is not clinically significant and it is appropriate to use FTC and TDF at the same doses (200 mg and 300 mg, respectively) as those used for approved drugs.

PMDA accepts the above explanation of the prior assessment requestor. The clinical efficacy and safety will be discussed in "4.(ii).B.(2) Efficacy evaluation, and 4.(ii).B.(3) Safety evaluation."

- PMDA asked the prior assessment requestor to discuss the factors that cause higher EVG exposure level in Japanese and Asian subjects than in Caucasian subjects when EVG is administered alone[78)] or as EVG/COBI/FTC/TDF combination,[79)] and asked for the view on whether or not the difference in the exposure level could affect the safety of EVG/COBI/FTC/TDF combination.

  The prior assessment requestor explained as follows:
  Even if the exposure level of EVG following the administration of EVG/COBI/FTC/TDF combination is slightly higher in Japanese subjects, the difference in exposure levels is

76) In this study, the exposure level of EVG (C_{max} = 1106.3 ng/mL, AUC_{tau} = 14,149.1 ng·h/mL) following the concomitant use of EVG (50 mg) + RTV (100 mg) with FTC/TDF (200/300 mg) was similar to that (C_{max} = 1123.9 ng/mL, AUC_{tau} = 13,814.0 ng·h/mL) obtained following the administration of EVG + RTV alone.
77) AUC_{tau} increased by 18% to 26%, C_{tau} by 24% to 28%, and C_{max} by 30% to 50%.
78) Phase I study in Japanese healthy adult male subjects (Study XAX1-1) and phase I/II study in foreign patients with HIV-1 infection (Study GS-US-183-0101)
79) Phase II study (Study GS-US-236-0104) and phase III studies (Studies GS-US-236-0102 and GS-US-236-0103) in foreign patients with HIV-1 infection

unlikely to affect the safety of EVG/COBI/FTC/TDF combination, for the following reasons: (i) among the covariates (age, sex, race, subjects studied, body weight, BMI, body surface area, CL_{cr}, superinfection with hepatitis B or C virus, COBI exposure level) investigated in PPK analysis in 9 clinical studies[61)] in which EVG/COBI or EVG/COBI/FTC/TDF combination was administered, body surface area was shown to affect the clearance (CL/F) of EVG, but the effect is considered to be of little clinical significance [see "4.(i).A.(3).3) EVG/COBI/FTC/TDF combination"]; (ii) results of Study XAX1-1 and Study GS-US-183-0101 suggested that C_{max} and AUC_{inf} in Japanese subjects were similar to those in foreign subjects; (iii) AUC_{tau} and C_{max} of EVG in subjects who experienced headache, diarrhoea, or nausea were similar to those in subjects who did not experience these adverse events; and (iv) there was no correlation between EVG exposure level and increased serum creatinine level following the administration of EVG/COBI/FTC/TDF combination.

PMDA asked the prior assessment requestor to explain the possible increase in the exposure level of each active ingredient other than EVG following the administration of EVG/COBI/FTC/TDF combination to Japanese subjects, and to explain the effect of such increase on the safety of EVG/COBI/FTC/TDF combination.

The prior assessment requestor explained as follows:
The exposure level of COBI following the administration of EVG/COBI/FTC/TDF combination is unlikely to be higher in Japanese subjects than in foreign subjects, for the following reasons: (i) as a result of PPK analysis of COBI [see "4.(i).A.(3).3) EVG/COBI/FTC/TDF combination"], race was not regarded as a covariate for COBI exposure level; (ii) pharmacokinetic profiles of each active ingredient suggested that EVG, FTC, and TFV would have only a minimal effect on the pharmacokinetics of COBI; and (iii) as shown above, the pharmacokinetic profile of EVG is considered not to be significantly different between Japanese and foreign subjects, and it has been confirmed from post-marketing clinical studies that the pharmacokinetic profiles of TDF, FTC, and TDF/FTC combination tablets are not significantly different between Japanese and foreign subjects. In addition, in phase II and III studies of EVG/COBI/FTC/TDF combination, increased exposure levels of COBI did not cause changes either in the incidences of bilirubin-related adverse drug reactions or other adverse drug reactions such as nausea and diarrhoea, or in the incidence of serum creatinine increased. Based on these results, even if the exposure level of COBI is higher in Japanese subjects than in foreign subjects, the difference in exposure level of COBI is unlikely to affect the safety of EVG/COBI/FTC/TDF combination.

Also, regarding FTC and TDF, post-marketing clinical studies did not show any significant difference in the pharmacokinetic profiles of these drugs between Japanese and foreign subjects, as shown above, and concomitant use of EVG and COBI did not cause any clinically significant increase in the exposure level of either drug. Based on the above results, the exposure levels of FTC and TFV following administration of EVG/COBI/FTC/TDF combination is not significantly different between Japanese and foreign subjects.

PMDA considers as follows:
Based on the data currently available, each active ingredient of EVG/COBI/FTC/TDF combination is unlikely to show any clinically significant difference in pharmacokinetics between Japanese and foreign subjects following administration, and is thus unlikely to affect safety of EVG/COBI/FTC/TDF combination. However, since pharmacokinetic data following administration of EVG/COBI/FTC/TDF combination have not been obtained from Japanese subjects, it is necessary to continue to collect data after the market launch,

evaluate the pharmacokinetics of each active ingredient, and provide the data to healthcare providers in the clinical settings. Safety of EVG/COBI/FTC/TDF combination in Japanese patients will be discussed in "4.(ii).B.(3).3) Safety in Asian patients."

- Regarding UGT1A1 and UGT1A3, enzymes involved in EVG metabolism, PMDA asked the view of the prior assessment requestor on the possibility of drug-drug interactions not only with UGT1A1 inhibitors (ketoconazole, ATV) but also with drugs with UGT1A1-inducing activities, as well as the possibility of UGT1A3-mediated drug-drug interactions.

  The prior assessment requestor explained as follows:
  CYP3A, UGT1A1, and UGT1A3 contribute to the metabolism of EVG, but the contribution of each molecular species is unknown. However, when the contribution of oxidative metabolism and glucuronidation metabolism in the total clearance of EVG were calculated based on the result of *in vitro* test using human liver microsomes, the contribution was 99.3% and 0.69%, respectively. This suggested that the contribution of glucuronidation metabolism was extremely low. These results suggest that concomitant use of drugs with UGT1A1-inducing activity is unlikely to significantly decrease the exposure level of EVG.

  PMDA accepted the above explanation of the prior assessment requestor.

- PMDA asked the prior assessment requestor to explain the effects of EVG, its main metabolites, COBI, and its main metabolites on the cardiovascular system, based on the results of safety pharmacology studies [see "3.(i).A.(3) Safety pharmacology"] and of clinical studies.

  The prior assessment requestor responded as follows:
  The concentrations of EVG that inhibited hERG current (10 μM) and that did not (1 μM) were 393 and 39 times higher, respectively, than the concentration of free EVG calculated from the clinical EVG exposure level of 1731 ng/mL[80)] (concentration of unbound EVG adjusted for protein-binding rate [99.34%] was 0.0255 μM). Investigation of M4 and M1 showed that M4 did not inhibit hERG even at 100 μM, whereas M1 inhibited hERG current at ≥30 μM (IC_{50} = 81 ± 8 μM, mean ± standard deviation [SD]). In the QT study of EVG (Study GS-US-183-0128), following the concomitant administration of EVG (125 mg, 250 mg) and RTV (100 mg) for 10 days, C_{max} of M4 was 112.1 and 178.2 ng/mL (<5% of EVG), respectively, and C_{max} of M1 was 29.2 ng/mL (<2% of EVG) at most. These concentrations of M1 and M4 were lower than those that inhibited hERG current. In the phase III studies (Studies GS-US-236-0102 and GS-US-236-0103) of EVG/COBI/FTC/TDF combination containing EVG and COBI, hot flush and hypertension were noted in 1 patient each as cardiovascular adverse drug reactions, and both were mild in severity. In QT study of EVG (Study GS-US-183-0128), no clinically significant changes were found either [see "4.(i).A.(8).1) EVG].

  In the clinical pharmacology study with ^{14}C-labeled COBI (Study GS-US-216-0111), the main component in plasma was COBI (98.6% of total radioactivity), with least metabolites detected. In QT study of COBI (Study GS-US-216-0107), C_{max} following a single dose of COBI (400 mg) was 4027.2 ng/mL, which was higher than the clinical exposure level of COBI (1224.5 ng/mL). Although QTcF shortened and PR interval prolonged were seen, these abnormalities were slight and considered to be of no clinical significance [see "4.(i).A.(8).2) COBI"].

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80) C_{max} based on PPK analysis of the data of the phase III studies (Studies GS-US-236-0102 and GS-US-236-0103) and a phase II study (Study GS-US-236-0104)

PMDA confirmed from the submitted safety pharmacology data that although EVG and COBI may cause cardiovascular adverse events such as QT prolonged, the reported events occurred only at higher concentration than the clinical exposure levels of EVG and COBI. Also, taking account of the currently available data including those of the phase III studies and the QT study of EVG/COBI/FTC/TDF combination, EVG/COBI/FTC/TDF combination was considered to have no significant safety problem regarding cardiovascular disorder including QT interval prolonged. However, since safety of EVG/COBI/FTC/TDF combination in Japanese patients has not been investigated to date, PMDA considers that it is necessary to reinvestigate the risk of QT interval prolonged by EVG/COBI/FTC/TDF combination in Japanese patients and to take measures such as promptly providing the information to healthcare providers in the clinical settings when new findings become available.

4.(ii) Efficacy and safety data

4.(ii).A. Summary of the submitted data

- Data of the following clinical studies in patients with HIV-1 infection were submitted: 1 phase I/II study (Study GS-US-183-0101) and 1 phase II study (Study GS-US-183-0105) of EVG; and 1 phase II study (Study GS-US-236-0104) and 2 phase III studies (Studies GS-US-236-0102 and GS-US-236-0103) of EVG/COBI/FTC/TDF combination. Also, data of 27 phase I studies in foreign healthy adult subjects (4 studies of EVG/COBI/FTC/TDF combination [131 subjects in total], 12 studies of EVG alone [392 subjects in total], and 11 studies of COBI alone [374 subjects in total]) were submitted. In addition, data of 2 phase I studies of EVG in Japanese healthy adult male subjects (48 subjects in total) were submitted.

- Data of 1 phase II study and 2 phase III studies of EVG/COBI/FTC/TDF combination in patients with HIV-1 infection are summarized in the following table.

Table. Summary of phase II and phase III studies in patients with HIV-1 infection

Study (phase)	Study method	Patients studied	Dosage and administration of study drug (No. of patients treated)	Treatment duration	Primary efficacy endpoint	Results
GS-US-236-0104 (II)	Randomized, double blind, active controlled study	Adult patients with HIV-1 infection[a] not previously treated with anti-HIV drugs	(i) EVG/COBI/FTC/TDF, 150/150/200/300 mg QD (48) (ii)EFV/FTC/TDF, 600/200/300 mg QD (23)	96 weeks[b]	Percentage of patients with HIV-1 RNA <50 copies/mL at Week 24	(i) 89.6% (ii)87.0%
GS-US-236-0102 (III)	Randomized, double blind, active controlled study	Adult patients with HIV-1 infection[a] not previously treated with anti-HIV drugs	(i) EVG/COBI/FTC/TDF, 150/150/200/300 mg QD (348) (ii)EFV/FTC/TDF, 600/200/300 mg QD (352)	96 weeks	Percentage of patients with HIV-1 RNA <50 copies/mL at Week 48	(i) 87.6% (ii)84.1%
GS-US-236-0103 (III)	Randomized, double blind, active controlled study	Adult patients with HIV-1 infection[a] not previously treated with anti-HIV drugs	(i) EVG/COBI/FTC/TDF, 150/150/200/300 mg QD (353) (ii)ATV/RTV 300/ 100 mg + FTC/TDF 200/300 mg, QD (355)	96 weeks	Percentage of patients with HIV-1 RNA <50 copies/mL at Week 48	(i) 89.5% (ii)86.8%

QD, Once daily; EVG/COBI/FTC/TDF combination, 150/150/200/300 mg; EFV/FTC/TDF, 600/200/300 mg; Atripla, a combination drug (unapproved in Japan)

a) HIV-1 RNA ≥5000 copies/mL

b) The study was conducted under randomized, double-blind conditions during the first 60 weeks, followed by open-label treatment with EVG/COBI/FTC/TDF combination in both groups during the subsequent 36 weeks.

4.(ii).B. Outline of the prior assessment by PMDA

4.(ii).B.(1) Clinical positioning and significance of combination

- The prior assessment requestor explained the clinical positioning of EVG/COBI/FTC/TDF combination and the significance of this combination, as follows:
  In ARV therapy of patients with HIV infection, it is extremely important to continuously suppress the level of HIV RNA below the detection limit over a long period of time in order to prevent the rebound of HIV RNA and induction of drug-resistant virus. For this purpose, it is most important to maintain a high adherence to dosage regimen of ARV therapy. In fact, results of prospective studies in patients with HIV infection showed that treatment with a dosage regimen of less frequent administration achieved favorable compliance.[81)] Also, patients were reported to be more satisfied with treatment with a dosage regimen of less frequent administration and fewer pill counts.[82)] In Japan, a combination product comprising a backbone[83)] and a key drug[84)] which can treat HIV-1 infection by 1 pill once daily administration is not approved. Thus, there is a clinical significance in introducing EVG/COBI/FTC/TDF combination as a new and convenient treatment option for treatment-naïve patients with HIV-1 infection. EVG, contained in EVG/COBI/FTC/TDF combination, is a novel INSTI and requires only once daily concomitant administration with a booster, while RAL, a drug with a similar mechanism of action, requires twice daily administration. In a phase III study (Study GS-US-183-0145)[85)] in patients with HIV-1 infection previously treated with ARV therapy, EVG + RTV was shown to be non-inferior to RAL and the incidence of adverse events was similar between the 2 therapies. As regards COBI, in a phase III study (Study GS-US-216-0114)[86)] in treatment-naïve patients with HIV-1 infection, no significant difference was noted in the incidence of adverse events between the ATV + COBI group and the ATV + RTV group. EVG/COBI/FTC/TDF combination, which contains these ingredients, showed a similar efficacy to EFV/FTC/TDF 600/200/300 mg and ATV/RTV + FTC/TDF in the respective phase III studies (Study GS-US-236-0102 and Study GS-US-236-0103) in treatment-naïve patients with HIV-1 infection [see "4.(ii).B.(2) Efficacy evaluation"], and did not pose any particularly significant safety problems [see "4.(ii).B.(3) Safety evaluation"], suggesting that EVG/COBI/FTC/TDF combination is a useful treatment option for treatment-naïve patients with HIV-1 infection.

  PMDA asked the prior assessment requestor to explain the treatment option in case of treatment failure with EVG/COBI/FTC/TDF combination, since no other combination drug comprising a key drug and a backbone drug has been approved in Japan.

  The prior assessment requestor responded as follows:
  If administration of EVG/COBI/FTC/TDF combination has resulted in virologic failure and a mutation resistant to active ingredients of EVG/COBI/FTC/TDF combination is detected, administration of EVG/COBI/FTC/TDF combination should be discontinued and the new treatment regimen should be selected in the following manner.

  1) If EVG-resistant mutation is detected

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81) Gallant JE et al. *N Engl J Med*. 2006;19;354(3):251-260, Molina JM et al. *AIDS Res Hum Retroviruses*. 2007;23(12):1505-1514
82) Stone VE et al. *J Acquir Immune Defic Syndr*. 2004;36(3):808-816
83) NRTI, FTC/TDF and ABC/3TC
84) NNRTI, EFV; PI, ATV/RTV and DRV/RTV; INSTI, RAL
85) A randomized, double-blind comparative study in which EVG 150 mg + RTV 100 mg QD (EVG 85 mg + RTV 100 mg QD in subjects receiving ATV or LPV) or RAL 400 mg twice daily (BID) was concomitantly administered with a backbone drug to patients with HIV-1 infection previously treated with ARV, and non-inferiority of EVG + RTV to RAL was investigated using the percentage of patients with plasma HIV-1 RNA <50 copies/mL at Week 48.
86) A randomized, double-blind comparative study in which ATV 300 mg + COBI 150 mg QD or ATV 300 mg + RTV 100 mg QD is concomitantly administered with FTC/TDF 200/300 mg to treatment-naïve patients with HIV-1 infection, and non-inferiority of ATV + COBI to ATV + RTV is investigated using the percentage of patients with plasma HIV-1 RNA <50 copies/mL at Week 48 (currently ongoing).

Main EVG-resistant mutations detected in patients with virologic failure after treatment with EVG/COBI/FTC/TD combination were T66I, E92Q, Q148R, and N155H. RAL is an INSTI, as EVG is, and had decreased efficacy against EVG-resistant mutants except T66I, thus showing cross-resistance [see "3.(i).A.(1).1) EVG" and "4.(ii).B.(2).3) Emergence of resistant mutations and effect on the efficacy"]. Therefore, if EVG-resistant mutation other than T66I is detected, RAL should not be included as a candidate for substitution; instead, a substitute should be selected from among ARV drugs of other classes (PI, NNRTI).

2) If mutations resistant to TDF and/or FTC were detected
If mutations resistant to TDF and/or FTC were detected, consideration should be given to replacing NRTI(s) against which resistant mutation is detected. For example, if only FTC-resistant mutation is detected, TDF is still effective. Therefore, ARV therapy including TDF alone may be a treatment option. If mutants remain sensitive to EVG/COBI, a regimen containing EVG/COBI might be a treatment option. However, since neither EVG nor COBI has been approved as a single drug at present, consideration should be given to changing the treatment regimen to RAL, another INSTI, or to drugs of other classes (PI, NNRTI).

- PMDA considers as follows:
In the treatment of HIV infection, it is important to continuously control HIV RNA level below the detection limit. Decreasing the frequency of administration and the number of pills for daily dosing in the long-term treatment is expected to reduce the burden of patients and increase the compliance. Also, since no other combination product comprising a key drug and a backbone drug has been approved in Japan, it is of clinical significance that EVG, COBI, FTC, and TDF enables once daily administration of 1 combination tablet. Results of the phase III studies (Studies GS-US-236-0102 and GS-US-236-0103) showed that EVG/COBI/FTC/TDF combination was effective against untreated HIV-1 infection and did not show any particularly significant safety problem, suggesting that EVG/COBI/FTC/TDF combination is a new treatment option for untreated HIV-1 infection. However, EVG/COBI/FTC/TDF combination is a combination product containing EVG, FTC, and TDF at fixed doses and neither EVG nor COBI is approved at present. Therefore, information on substitutive treatment options, in case any resistance mutation to any one of the active ingredients emerges, should be adequately provided to healthcare providers in clinical settings [for the effects of resistant mutations on efficacy, see "4.(ii).B.(2).3) Emergence of resistant mutations and effect on the efficacy"].

4.(ii).B.(2) Efficacy evaluation

4.(ii).B.(2).1) Efficacy

- The prior assessment requestor explained as follows:
Findings on the primary efficacy endpoint (percentage of patients with HIV-1 RNA <50 copies/mL at Week 48) and virologic failures in the phase III studies (Studies GS-US-236-0102 and GS-US-236-0103) of the EVG/COBI/FTC/TDF combination in treatment-naïve patients with HIV-1 infection were as shown in the following table. The lower limit of the 95.2% CI of the difference in primary efficacy point between the EVG/COBI/FTC/TDF combination group and the comparator groups (EFV/FTC/TDF group in Study GS-US-236-0102, ATV/RTV + FTC/TDF group in Study GS-US-236-0103) exceeded the pre-defined non-inferiority margin (-12%). Thus, the EVG/COBI/FTC/TDF combination is non-inferior to EFV/FTC/TDF or to ATV/RTV + FTC/TDF, demonstrating the efficacy against treatment-naïve patients with HIV-1 infection.

Table. Percentage of patients with HIV-1 RNA <50 copies/mL at Week 48 and patients with virologic failure in phase III studies (Studies GS-US-236-0102 and GS-US-236-0103) (ITT [intention-to-treat] population)

	Study GS-US-236-0102		Study GS-US-236-0103	
	EVG/COBI/ FTC/TDF group (N = 348)	EFV/FTC/ TDF group (N = 352)	EVG/COBI/ FTC/TDF group (N = 353)	ATV/RTV + FTC/TDF group (N = 355)
Patients with HIV-1 RNA <50 copies/mL	305 (87.6)	296 (84.1)	316 (89.5)	308 (86.8)
Between-group difference (%) [95.2% CI] a)	3.6 [-1.6, 8.8]		3.0 [-1.9, 7.8]	
Patients with virologic failure	25 (7.2)	25 (7.1)	19 (5.4)	19 (5.4)
HIV-1 RNA ≥50 copies/mL	13 (3.7)	11 (3.1)	7 (2.0)	8 (2.3)
Discontinuation due to lack of efficacy	4 (1.1)	2 (0.6)	4 (1.1)	0
Among discontinuation due to other reasons, patients with HIV-1 RNA ≥50 copies/mL at the last examination	8 (2.3)	12 (3.4)	8 (2.3)	11 (3.1)

Number of patients (%)

a) Mantel-Haenszel analysis stratified by baseline HIV-1 RNA level (≤100,000 copies/mL, >100,000 copies/mL) In each study, 2 interim analyses were performed, with α of 0.001 being consumed in each analysis.

- PMDA considers as follows:
  In the phase III studies (Studies GS-US-236-0102 and GS-US-236-0103) in treatment-naïve patients with HIV-1 infection, EVG/COBI/FTC/TDF combination was shown to be non-inferior to EFV/FTC/TDF and to ATV/RTV + FTC/TDF in the primary efficacy endpoint, i.e., the percentage of patients with HIV-1 RNA <50 copies/mL at Week 48. Therefore, EVG/COBI/FTC/TDF combination is effective against patients with HIV-1 infection previously untreated with ARV. The phase III studies (Studies GS-US-236-0102 and GS-US-236-0103) are currently ongoing with the treatment period extended up to 192 weeks under blinded conditions. As soon as the results after Week 48 are summarized, they should be promptly reported to PMDA and appropriately provided to healthcare providers in the clinical settings.

4.(ii).B.(2).2) Factors affecting efficacy

- The prior assessment requestor explained as follows:
  In the phase III studies (Studies GS-US-236-0102 and GS-US-236-0103) in treatment-naïve patients with HIV-1 infection, efficacy (percentage of patients with HIV-1 RNA <50 copies/mL at Week 48) by patient characteristics (baseline HIV-1 RNA level, baseline CD4-positive T-lymphocyte count, race, sex) was as shown in the following table. Baseline HIV-1 RNA level, race, or sex did not have any particular effects on the efficacy of EVG/COBI/FTC/TDF combination, and results were similar to those in the comparator groups. In patients with baseline CD4-positive T-lymphocyte count ≤200 cells/μL, the efficacy was lower in EVG/COBI/FTC/TDF combination group than in the comparator groups. However, the efficacy in the patient subgroup with baseline CD4-positive T-lymphocyte count >50 cells/μL and ≤200 cells/μL was not significantly different from the efficacy in other patient subgroups (>200 cells/μL and ≤350 cells/μL, >350 cells/μL) or in the comparator groups. In the patient subgroup ≤50 cells/μL, plasma HIV-1 RNA level exceeded 100,000 copies/mL in all 19 patients in EVG/COBI/FTC/TDF combination group, which resulted in a greater number of early drop-outs than in the comparator group (7 patients in EVG/COBI/FTC/TDF combination group, 1 patient in the EFV/FTC/TDF group). This difference appears to have caused the apparent lower efficacy of EVG/COBI/FTC/TDF combination in the patient subgroup ≤200 cells/μL.

Table. Percentage of patients with HIV-1 RNA <50 copies/mL at Week 48, classified by patient characteristics in each of phase III studies (Studies GS-US-236-0102 and GS-US-236-0103) and in Pooled analysis [ITT population]

		Study GS-US-236-0102		Study GS-US-236-0103	
		EVG/COBI/FTC/TDF combination group	EFV/FTC/TDF group	EVG/COBI/FTC/TDF combination group	ATV/RTV + FTC/TDF group
Baseline HIV-1 RNA level (copies/mL)	≤100,000	89.6 (206/230)	85.2 (201/236)	92.6 (188/203)	89.7 (192/214)
	>100,000	83.9 (99/118)	81.9 (95/116)	85.3 (128/150)	82.3 (116/141)
Baseline CD4-positive T-lymphocyte count (cells/μL)	≤200	74.4 (32/43)	82.4 (42/51)	79.6 (43/54)	84.6 (33/39)
	>200 and ≤350	86.6 (97/112)	84.4 (81/96)	93.4 (114/122)	88.7 (110/124)
	>350	91.2 (176/193)	84.4 (173/205)	89.8 (159/177)	85.9 (165/192)
Race	Caucasians	89.3 (191/214)	87.7 (199/227)	90.0 (225/250)	86.6 (240/277)
	Blacks/African Americans	83.0 (88/106)	74.7 (68/91)	83.3 (60/72)	85.1 (40/47)
	Asians	83.3 (5/6)	80.0 (8/10)	100.0 (17/17)	82.4 (14/17)
	Other	95.5 (21/22)	87.5 (21/24)	100.0 (14/14)	100.0 (14/14)
Sex	Male	87.9 (270/307)	84.2 (266/316)	90.1 (292/324)	87.3 (276/316)
	Female	85.4 (35/41)	83.3 (30/36)	82.8 (24/29)	82.1 (32/39)

% (number of patients)

- PMDA confirmed that there are no background factors that have any particular effects on the efficacy based on the currently available information, and that there is no significant difference in efficacy between Asian patients and non-Asian patients. However, since there were only a limited number of patients in subgroups for some background factors, information on the efficacy in patients classified by background should be collected continuously after the market launch and new findings, if available, should be provided to healthcare providers in the clinical settings.

4.(ii).B.(2).3) Emergence of resistance mutations and its effect on the efficacy

- The prior assessment requestor explained the emergence of resistance mutations and its effect on efficacy as follows:
  In the phase III studies (Studies GS-US-236-0102 and GS-US-236-0103) and the phase II study (Study GS-US-236-0104), the percentage of subjects with virologic failure or with HIV-1 RNA >400 copies/mL at Week 48 or at the early termination of the study drug was 3.6% (27 of 749 subjects) in EVG/COBI/FTC/TDF combination group, 4.8% (18 of 375 subjects) in the EFV/FTC/TDF group, and 2.3% (8 of 355 subjects) in the ATV/RTV + FTC/TDF group. One or more major mutations related to resistance to EVG, FTC, or TDF, the active ingredients of EVG/COBI/FTC/TDF combination, were found in 13 subjects in EVG/COBI/FTC/TDF group, 8 subjects in the EFV/FTC/TDF group, and 0 subjects in the ATV/RTV + FTC/TDF group. Of the 13 subjects who showed resistance in EVG/COBI/FTC/TDF combination group, 11 subjects had reduced sensitivity to EVG, 12 subjects to FTC, and 2 subjects to TDF. Of the 11 subjects with resistant mutation to EVG, 10 subjects showed resistant mutation to FTC (M184I/V) as well. The reason why INSTI-resistant mutation is accompanied by M184V/I resistance is unknown, but this appears to be a class-specific phenomenon, based on the report of a similar phenomenon with RAL.[87)]
  In the sensitivity evaluation test equipped with the major resistance mutations identified in EVG-resistant isolates (T66I, E92Q, Q148R, N155H), decreased efficacy of RAL was found against the EVG-resistant mutations except T66I, suggesting cross-resistance between RAL and EVG [for details, see "3.(i).A.(1).1) EVG"]. There were only a limited

87) Lennox JL et al. *Lancet*. 2009;374(9692):796-806, Lnnox JL et al. *J Acquir Immune Defic Syndr*. 2010;55(1):39-48, Eron JJ et al. *Lancet Infect Dis*. 2011;11(12):907-915

number of patients with virologic failure in EVG/COBI/FTC/TDF combination group and, in case resistant mutation to EVG occurs, drugs of other classes such as PI and NNRTI may be used. Therefore, emergence of cross-resistance to EVG and RAL is considered to have only a minor effect on treatment at clinical practice. However, information on cross-resistance between EVG and RAL will be provided via the package insert.

- PMDA considers as follows:
  The investigation of the cases associated with emergence of resistance after treatment with EVG/COBI/FTC/TDF combination showed that the frequency of mutation resistant to EVG was not high. However, many of the major mutations resistant to EVG noted in the phase III studies (Studies GS-US-236-0102 and GS-US-236-0103) were identical to those resistant to RAL, suggesting cross-resistance between EVG and RAL. This information should be included in the package insert, etc., to adequately provide caution to healthcare providers in the clinical settings. Also, the profile of mutations resistant to EVG has not been fully identified to date. Therefore, information on the emergence of mutations resistant to EVG and the site of the resistant mutations should be collected continuously in and out of Japan after the market launch as well, and new findings, if available, should be appropriately provided to healthcare providers in the clinical settings.

4.(ii).B.(3) Safety evaluation

- PMDA performed the following evaluation on the safety of EVG/COBI/FTC/TDF combination based on the results of the phase III studies (Studies GS-US-236-0102 and GS-US-236-0103) and a phase II study (Study GS-US-236-0104), and concluded that the safety of EVG/COBI/FTC/TDF combination is generally acceptable. In this application, data of 2 phase I studies (Studies XAX1-1 and XAX1-2) in which EVG was administered to healthy adult male subjects were submitted as the safety data in the Japanese population. Although no particular safety problems have been noted, there are no data available in the clinical studies of EVG/COBI/FTC/TDF combination in Japanese patients. Therefore, it is necessary to continue collecting safety information in Japanese patients treated with EVG/COBI/FTC/TDF combination after the market launch. Also, since there is no sufficient safety information on the long-term use of EVG/COBI/FTC/TDF combination, the results of the phase III studies (Studies GS-US-236-0102 and GS-US-236-0103) after Week 48, as soon as they are summarized, should be promptly reported to PMDA and appropriately supplied to healthcare providers in the clinical settings.

4.(ii).B.(3).1) Summary of safety

- The prior assessment requestor explained that adverse events[88)] reported as of Week 48 (or Week 60) in the phase III studies (Studies GS-US-236-0102 and GS-US-236-0103) and in a phase II study (Study GS-US-236-0104) were as shown in the following table.

88) Events with an incidence of ≥5% in any treatment group are shown. For a phase II study (Study GS-US-236-0104), data at Week 60 are shown.

Table. Summary of safety as of Week 48 (or Week 60) in EVG/COBI/FTC/TDF combination and comparator groups in phase III studies (Studies GS-US-236-0102 and GS-US-236-0103) and in phase II study (Study GS-US-236-0104)

	EVG/COBI/FTC/TDF group [a)] (N = 749)	EFV/FTC/TDF group[b)] (N = 375)	ATV/RTV + FTC/TDF group[c)] (N = 355)
All adverse events	694 (92.7)	355 (94.7)	333 (93.8)
Treatment-related adverse events	343 (45.8)	250 (66.7)	203 (57.2)
Ocular icterus	2 (0.3)	0	51 (14.4)
Diarrhoea	170 (22.7)	70 (18.7)	97 (27.3)
Nausea	146 (19.5)	50 (13.3)	69 (19.4)
Vomiting	41 (5.5)	16 (4.3)	24 (6.8)
Flatulence	28 (3.7)	5 (1.3)	29 (8.2)
Fatigue	98 (13.1)	49 (13.1)	45 (12.7)
Pyrexia	26 (3.5)	19 (5.1)	14 (3.9)
Jaundice	0	1 (0.3)	31 (8.7)
Upper respiratory tract infection	106 (14.2)	44 (11.7)	58 (16.3)
Nasopharyngitis	53 (7.1)	21 (5.6)	28 (7.9)
Sinusitis	41 (5.5)	33 (8.8)	18 (5.1)
Bronchitis	49 (6.5)	22 (5.9)	18 (5.1)
Back pain	42 (5.6)	14 (3.7)	13 (3.7)
Headache	109 (14.6)	38 (10.1)	44 (12.4)
Dizziness	42 (5.6)	89 (23.7)	25 (7.0)
Somnolence	11 (1.5)	29 (7.7)	4 (1.1)
Abnormal dreams	70 (9.3)	103 (27.5)	14 (3.9)
Insomnia	65 (8.7)	51 (13.6)	18 (5.1)
Depression	57 (7.6)	41 (10.9)	23 (6.5)
Cough	42 (5.6)	17 (4.5)	28 (7.9)
Oropharyngeal pain	29 (3.9)	27 (7.2)	18 (5.1)
Rash	52 (6.9)	47 (12.5)	22 (6.2)

Number of patients (%)

a) Pooled analysis of the phase III studies (Studies GS-US-236-0102 and GS-US-236-0103) and a phase II study (Study GS-US-236-0104)

b) Pooled analysis of a phase III study (Study GS-US-236-0102) and a phase II study (Study GS-US-236-0104)

c) A Phase III study (Study GS-US-236-0103)

- There was no significant difference in the incidence of adverse events as of Week 48 (or Week 60) between EVG/COBI/FTC/TDF combination and the comparator groups in the phase III studies (Studies GS-US-236-0102 and GS-US-236-0103) and in a phase II study (Study GS-US-236-0104). Based on the above findings, PMDA concluded that there is no particular safety problem up to 48-week treatment with EVG/COBI/FTC/TDF combination. Renal impairment caused by EVG/COBI/FTC/TDF combination is discussed separately in the following section.

4.(ii).B.(3).1).(a). Renal impairment

- The prior assessment requestor explained renal impairment associated with EVG/COBI/FTC/TDF combination as follows:
  In the phase III studies (Studies GS-US-236-0102 and GS-US-236-0103) and in a phase II study (Study GS-US-236-0104), kidney-related adverse events[89)] were reported by 5 patients in EVG/COBI/FTC/TDF combination group (renal failure in 4 patients, Fanconi syndrome acquired in 1 patient), 1 patient in the EFV/FTC/TDF group (renal failure), and 1 patient in the ATV/RTV + FTC/TDF group (nephropathy toxic). Except renal failure in 2 patients and Fanconi syndrome acquired in 1 patient in EVG/COBI/FTC/TDF combination group, the outcomes of all other adverse events were reported as improved or

89) Fanconi syndrome, Fanconi syndrome acquired, renal failure, renal failure acute, renal tubular disorder

resolved. Treatment discontinuations due to kidney-related adverse events or laboratory abnormalities were reported by 8 patients in EVG/COBI/FTC/TDF combination group (serum creatinine in 4 patients, renal failure in 3 patients, Fanconi syndrome acquired in 1 patient) and by 1 patient in the ATV/RTV + FTC/TDF group (nephropathy toxic).

In EVG/COBI/FTC/TDF combination group, more kidney-related adverse events were reported than in the control groups, and serum creatinine increased was found in 7.7% (57 of 749 patients) in EVG/COBI/FTC/TDF combination group, 1.1% (4 of 372 patients) in the EFV/FTC/TDF group, and 4.8% (17 of 352 patients) in the ATV/RTV + FTC/TDF group. However, in a phase I study (Study GS-US-216-0121) in which COBI (150 mg) was administered to healthy adult subjects for 7 days, in despite of changes in CL_{cr} from baseline, no change was found in actual glomerular filtration rate (aGFR) or in cystatin C-derived glomerular filtration rate (cysGFR). In light of these findings, the serum creatinine increased noted in EVG/COBI/FTC/TDF combination group is considered to be due to the inhibition by COBI of creatinine secretion from the renal tubules. In the pooled analysis of the phase II study (Study GS-US-216-0105) and a phase III study (Study GS-US-216-0114) in which ATV/COBI + FTC/TDF or ATV/RTV + FTC/TDF was administered to patients with HIV-1 infection, the incidence of kidney-related adverse drug reactions was similar between the ATV/COBI + FTC/TDF group (0.3% [1 of 394 patients]) and the ATV/RTV + FTC/TDF group (1.9% [7 of 377 patients]). The incidence was not significantly different from that reported for FTC/TDF.[90)]

- PMDA considers as follows:
  Although there were not many kidney-related adverse events or laboratory abnormalities in the phase III studies (Studies GS-US-236-0102 and GS-US-236-0103) or in the phase II study (Study GS-US-236-0104), the incidence was higher in EVG/COBI/FTC/TDF combination group than in the control groups, and adverse events leading to study drug discontinuation were also reported. As regards the serum creatinine increased, although it may be due to the inhibition by COBI of creatinine secretion from the renal tubules, other kidney-related adverse events were also noted more frequently in EVG/COBI/FTC/TDF combination group than in the control groups. In light of these observations and other currently available information, the possibility cannot be excluded that the risk of renal impairment is increased by the combined administration of COBI and FTC/TDF. Therefore, it is necessary to provide adequate caution to perform renal function tests before and after the start of treatment with EVG/COBI/FTC/TDF combination. Also, after the market launch, information should be continuously collected on the incidence of kidney-related adverse events and laboratory abnormalities (including outcome) found after dosing of EVG/COBI/FTC/TDF combination, and on the effect on the risk of kidney-related adverse events after dosing of COBI. Findings in the ongoing and planned clinical studies, etc., should be provided to healthcare providers in the clinical settings as soon as they are summarized.

4.(ii).B.(3).2) Occurrence of liver-related adverse events in patients superinfected with hepatitis virus

- The prior assessment requestor explained the occurrence of liver-related adverse events in patients superinfected with hepatitis virus as follows:
  The pooled analysis of the phase III studies (Studies GS-US-236-0102 and GS-US-236-0103) and the phase II study (Study GS-US-236-0104) of EVG/COBI/FTC/TDF combination showed that, among the patients treated with EVG/COBI/FTC/TDF combination, 10 patients had HIV-1/HBV superinfection and 35 patients had HIV-1/HCV superinfection, and none had HIV-1/HBV/HCV superinfection. Adverse events of

90) Smith KY et al. *AIDS Res Ther*. 2008;5(1):5, Smith KY et al. *AIDS*. 2009;23(12):1547-1556

hepatobiliary system found among these patients were hepatomegaly in 1 patient with HIV-1/HBV superinfection and liver injury in 1 patient with HIV-1/HCV superinfection. In the phase I study (Study GS-US-183-0133) in which EVG (150 mg) + COBI (150 mg) was administered to patients with moderate hepatic impairment (Child-Pugh class B), occurrence of adverse events was not significantly different from that in subjects with normal hepatic function.

- PMDA considers as follows:
  At present, there is no tendency of increased occurrence of liver-related adverse events in patients superinfected with hepatitis virus. However, since liver-related adverse events may occur at a higher frequency in patients superinfected with hepatitis virus, it is necessary to provide the same cautions for EVG/COBI/FTC/TDF combination as for similar drugs. Also, relevant information should be continuously collected after the market launch.

4.(ii).B.(3).3) Safety in Asian patients

- The prior assessment requestor explained the difference in the safety of EVG/COBI/FTC/TDF combination among different ethnic groups in the pooled analysis (at Week 48) of the phase III studies (Studies GS-US-236-0102 and GS-US-236-0103) and a phase II study (Study GS-US-236-0104) in terms of the incidence of adverse events as follows: 87.5% (21 of 24 patients) in Asian patients, 90.5% (172 of 190 patients) in Black/African American patients, and 93.4% (464 of 497 patients) in Caucasian patients in EVG/COBI/FTC/TDF combination group; 100.0% (10 of 10 patients) in Asian patients, 92.7% (89 of 96 patients) in Black/African American patients, and 95.1% (233 of 245 patients) in Caucasian patients in the EFV/FTC/TDF group; and 94.1% (16 of 17 patients) in Asian patients, 91.5% (43 of 47 patients) in Black/African American patients, and 94.2% (261 of 277 patients) in Caucasian patients in the ATV/RTV + FTC/TDF group. Thus, there was no significant difference in the safety among ethnic groups in each treatment group, and adverse events noted in Asian patients were similar to those noted in the entire EVG/COBI/FTC/TDF combination group; no events were frequently found particularly in Asian patients. In the Japanese phase I studies (Studies XAX1-1 and XAX1-2) in which EVG (100-800 mg) was administered in a single dose to Japanese healthy adult male subjects, adverse events were seen in 6.7% (2 of 30 subjects)[91)] in each study, but they were all mild and resolved without treatment, suggesting that there are no particularly significant safety problems.

- PMDA considers that the explanation of the prior assessment requestor is generally acceptable. However, since only a limited number of Asian patients have been studied, information on the safety in Japanese patients treated with EVG/COBI/FTC/TDF combination should be collected continuously after the market launch.

4.(ii).B.(4) Indications

- PMDA considers as follows:
  Results of the phase III studies (Studies GS-US-236-0102 and GS-US-236-0103) and a phase II study (Study GS-US-236-0104) showed that EVG/COBI/FTC/TDF combination is effective against HIV-1 infection and does not pose any particularly significant safety problems [see "4.(ii).B.(2) Efficacy evaluation" and "4.(ii).B.(3) Safety evaluation"]. Taking account of these results, the indication for EVG/COBI/FTC/TDF combination should be "HIV-1 infection" as proposed by the prior assessment requestor. Clinical studies of EVG/COBI/FTC/TDF combination were conducted in patients with HIV-1 not

91) Three adverse events (ALT increased, AST increased, anorexia) were reported in Study XAX1-1 and 6 adverse events (pharyngitis, white blood cell count increased, neutrophil count increased, lymphocyte count decreased, CRP increased, diarrhoea) in Study XAX1-2.

previously treated with anti-HIV drugs. This fact should be appropriately provided to healthcare providers in clinical settings.

4.(ii).B.(5) Dosage and administration

- The dosage and administration proposed by the prior assessment requestor was as follows: the usual adult dosage is one tablet (containing 150 mg of elvitegravir, 150 mg of cobicistat, 200 mg of emtricitabine, and 300 mg of tenofovir disoproxil fumarate) administered orally once daily during or immediately after a meal.

  The prior assessment requestor explained the rationale as follows:
  In Study GS-US-236-0105, the effect of food on the pharmacokinetics of each active ingredient was investigated after dosing of EVG/COBI/FTC/TDF combination. As a result, EVG exposure level after fed administration was higher than that achieved after fasted administration. COBI exposure level after fed administration was similar to or slightly lower than that after fasted administration, but did not affect the boosting effect on EVG. FTC and TFV exposure levels did not show any consistently significant difference between fed and fasted administration. It was expected from these results that administration during or immediately after food intake would result in a high EVG exposure level and a high trough concentration would be maintained [see "4.(i).A.(1).2) EVG/COBI/FTC/TDF combination"]. Therefore, in a phase II study (Study GS-US-236-0104) and in the phase III studies (Studies GS-US-236-0102 and GS-US-236-0103), EVG/COBI/FTC/TDF combination was administered during or immediately after food intake. Appropriateness of the dose selection (combination ratio) of active ingredients of EVG/COBI/FTC/TDF combination was explained in "4.(i).B Outline of the prior assessment by PMDA." Since the above dosage has been shown to be effective and safe, it will not pose any significant problems. Caution statements to the following effect will be included: (i) since the dose of components in EVG/COBI/FTC/TDF combination cannot be adjusted, treatment with EVG/COBI/FTC/TDF combination should not be started in patients with renal impairment with CL_{cr} <70 mL/min and the administration of EVG/COBI/FTC/TDF combination should be discontinued if CL_{cr} has decreased <50 mL/min after the start of treatment [see "4.(i).A.(5) The effect of renal function on pharmacokinetics in concomitant use of EVG and COBI"]; and (ii) no pharmacokinetics or safety data of EVG/COBI/FTC/TDF combination are available for patients with severe hepatic impairment (Child-Pugh class C) [see "4.(i).A.(6) Effect of hepatic function on pharmacokinetics in concomitant use of EVG and COBI"].

- PMDA accepts the above explanation of the prior assessment requestor. However, taking account of the facts that no efficacy or safety data in Japanese patients are available at present, and that there is only limited information on the safety of EVG/COBI/FTC/TDF combination in patients with renal or hepatic impairment, relevant information should be continuously collected after the market launch and appropriately provided to healthcare providers in the clinical settings. When new findings are obtained from ongoing or planned clinical studies, the information should also be provided to the healthcare providers.

4.(ii).B.(6) Special patient populations

4.(ii).B.(6).1) Pediatric use

- The prior assessment requestor explained the pediatric use of EVG/COBI/FTC/TDF combination as follows:
  In Japan, there were only 17 patients newly infected with HIV who were aged ≤19 years in 2011,[92)] and there is currently no plan to develop the product for pediatric use. However, in foreign countries, 2 clinical studies (Study GS-US-236-0112 in patients aged ≥12 and

92) AIDS Surveillance Report 2011 (January 1 to December 31), National AIDS Surveillance Committee, MHLW (http://api-net.jfap.or.jp/status/2011/11nenpo/nenpo_menu.htm)

<18 years, Study GS-US-236-0119 in patients aged ≥6 and <18 years) are planned. In Japan, use-results surveys have revealed that 1 child was treated with TDF before August 2012 and experienced non-serious adverse drug reactions (blood triglycerides increased, diabetes mellitus) but they resolved or improved without interruption of ARV therapy. Thus, to date, there have been few reports on pediatric use and no safety concerns requiring special measures. Therefore, caution will be provided that safety of EVG/COBI/FTC/TDF combination in children has not been established, as are the cases with TDF, FTC and FTC/TDF.

- PMDA considers as follows: it is unavoidable at present to withhold developing the product for pediatric use in Japan; however, data of planned foreign clinical studies in pediatric patients should be submitted to PMDA as soon as they become available; additionally, consideration should be given to developing the product for pediatric use, taking account of the use conditions of the product for pediatric patients in and out of Japan.

III. Overall Evaluation

PMDA conducted the above evaluations based on the data submitted for prior assessment, and has concluded that the efficacy and safety of EVG/COBI/FTC/TDF combination have been confirmed. Conclusions on the following points will be finalized, taking account of comments raised in the Expert Discussion.

- Clinical positioning of EVG/COBI/FTC/TDF combination and significance of this combination
- Efficacy and safety of EVG/COBI/FTC/TDF combination
- Indications of EVG/COBI/FTC/TDF combination
- Dosage and administration of EVG/COBI/FTC/TDF combination

Review Report (1)

February 19, 2013

I. Product Submitted for Registration

[Brand name]	Stribild Combination Tab.
[Non-proprietary name]	Elvitegravir/Cobicistat/Emtricitabine/Tenofovir Disoproxil Fumarate
[Applicant]	Japan Tobacco Inc.
[Date of application]	December 6, 2012
[Dosage form/Strength]	Each tablet containing 150 mg of elvitegravir, 150 mg of cobicistat, 200 mg of emtricitabine, and 300 mg of tenofovir disoproxil fumarate (245 mg as tenofovir disoproxil)
[Proposed indication]	HIV-1 infection
[Proposed dosage and administration]	The usual adult dosage is one tablet (containing 150 mg of elvitegravir, 150 mg of cobicistat, 200 mg of emtricitabine, and 300 mg of tenofovir disoproxil fumarate) administered orally once daily during or immediately after a meal.

II. Content of the Review

The Pharmaceuticals and Medical Devices Agency (PMDA) sought the comments from the Expert Discussion based on the Prior Assessment Report (1). The expert advisors for the Expert Discussion were nominated based on their declarations etc., concerning the product submitted for registration, in accordance with the provisions of the "Rules for Convening Expert Discussions, etc., by Pharmaceuticals and Medical Devices Agency" (PMDA Administrative Rule No. 8/2008 dated December 25, 2008).

The Expert Discussion supported the conclusion of PMDA described in Prior Assessment Report (1). PMDA held additional discussions on the following points and took necessary measures.

(1) Post-marketing investigations

1) Post-marketing surveillance

The applicant plans to conduct a post-marketing surveillance by participating in the Joint Investigation Consortium of HIV related drugs (HRD).

PMDA sought the comments from the Expert Discussion on what types of information should be mainly collected in the post-marketing surveillance, to which the following opinion was provided from expert advisors.

- Since EVG/COBI/FTC/TDF combination contains COBI, a novel pharmacokinetic booster, a focus should be placed on collecting the information on the drug-drug interaction between EVG/COBI/FTC/TDF combination and other drugs.

PMDA, based on the opinion of the expert advisors, instructed the applicant to continue to collect the following information and to provide the obtained information to healthcare providers in clinical settings in an appropriate manner.

- Interactions with other drugs, foods, and beverages, and their effects on the safety
- Emergence of resistance mutations to EVG, FTC, or TDF (including the studies on the profile of resistance mutations to EVG and on cross-resistance with RAL)

- Inducibility of development of resistant bacteria by EVG and cross-resistance with quinolone antibacterial drugs
- Efficacy and safety of EVG/COBI/FTC/TDF combination in Japanese patients with HIV infection (including efficacy by patient characteristics)
- Occurrences of renal disorder-related adverse events and laboratory abnormalities after administration of EVG/COBI/FTC/TDF combination
- Occurrences of adverse events after administration of EVG/COBI/FTC/TDF combination in patients superinfected with hepatitis virus
- Safety of EVG/COBI/FTC/TDF combination in patients with renal or hepatic impairment

PMDA also instructed the applicant to actively collect information on the pharmacokinetic data of EVG/COBI/FTC/TDF combination, if measured in the post-marketing surveillance, and to investigate the relationship between blood concentrations of EVG/COBI/FTC/TDF combination and adverse drug reactions as closely as possible.

The applicant agreed to the above instructions.

2) Pharmacokinetics in Japanese subjects

Since there are no pharmacokinetic data of EVG/COBI/FTC/TDF combination in Japanese subjects at present, PMDA asked the applicant to conduct a post-marketing clinical study on pharmacokinetics of EVG/COBI/FTC/TDF combination in Japanese subjects.

The applicant explained that, after the approval, they would promptly conduct a Japanese pharmacokinetic study (i) to investigate the pharmacokinetics of EVG, COBI, FTC, and TDF following a single dose of EVG/COBI/FTC/TDF combination to Japanese healthy adult male subjects, (ii) to evaluate the effect of food intake on the pharmacokinetics of EVG/COBI/FTC/TDF combination, and (iii) to assess the safety, and that the information obtained from the study would be provided to healthcare providers in clinical settings in an appropriate manner.

PMDA accepted the above explanation of the applicant.

(2) Additional study data submitted

1) Data obtained at Week 96 in phase III studies (Studies GS-US-236-0102 and GS-US-236-0103)

Data up until Week 96 in the phase III studies (Studies GS-US-236-0102 and GS-US-236-0103), which were being compiled at the time of the preparation of the Prior Assessment Report (1), were submitted at the time of this application as a part of the clinical study report. The applicant explained that the efficacy as of Week 96 was similar to that as of Week 48, as shown in the following table, and that occurrences of adverse events were not significantly different between as of Week 96 and as of Week 48, demonstrating that EVG/COBI/FTC/TDF combination is safe and well tolerated.

Table. Percentage of patients with HIV-1 RNA <50 copies/mL and with virologic failure at Week 48 and Week 96 in phase III studies (Studies GS-US-236-0102 and GS-US-236-0103) [ITT population]

	Study GS-US-236-0102		Study GS-US-236-0103	
	EVG/COBI/ FTC/TDF group (N = 348)	EFV/FTC/ TDF group (N = 352)	EVG/COBI/ FTC/TDF group (N = 353)	ATV/RTV + FTC/TDF group (N = 355)
Week 48				
Patients with HIV-1 RNA <50 copies/mL	305 (87.6)	296 (84.1)	316 (89.5)	309 (87.0)[b)]
Between-group difference (%) [95.2% CI] [a)]	3.6 [-1.6, 8.8]		2.7 [-2.1, 7.5]	
Patients with virologic failure	25 (7.2)	25 (7.1)	19 (5.4)	18 (5.1)
HIV-1 RNA ≥50 copies/mL	13 (3.7)	11 (3.1)	7 (2.0)	7 (2.0)
Treatment discontinuation due to lack of efficacy	4 (1.1)	2 (0.6)	4 (1.1)	0
Among discontinuation due to other reasons, patients with HIV-1 RNA ≥50 copies/mL at the last examination	8 (2.3)	12 (3.4)	8 (2.3)	11 (3.1)
Week 96				
Patients with HIV-1 RNA <50 copies/mL	293 (84.2)	287 (81.5)	294 (83.3)	292 (82.3)
Between-group difference (%) [95.2% CI] [a)]	2.7 [-2.9, 8.3]		1.1 [-4.5, 6.7]	
Patients with virologic failure	22 (6.3)	27 (7.7)	24 (6.8)	26 (7.3)
HIV-1 RNA ≥50 copies/mL	4 (1.1)	7 (2.0)	7 (2.0)	11 (3.1)
Treatment discontinuation due to lack of efficacy	6 (1.7)	5 (1.4)	4 (1.1)	1 (0.3)
Among discontinuation due to other reasons, patients with HIV-1 RNA ≥50 copies/mL at the last examination	12 (3.4)	15 (4.3)	13 (3.7)	14 (3.9)

Number of patients (%)

a) Mantel-Haenszel analysis stratified by baseline HIV-1 RNA level (≤100,000 copies/mL, >100,000 copies/mL) In each study, 2 interim analyses were performed, with α of 0.001 being consumed in each analysis.

b) In the Week 96 report, 1 patient was added to the Week 48 report submitted for the prior assessment. This patient, who had a plasma HIV-1 RNA level of ≥50 copies/mL at Week 48 (Day 352), was found to have <50 copies/mL upon repeated assay after 20 days (Day 372). Therefore, the case of this patient was changed from virologic failure to treatment success.

PMDA confirmed that its review results concerning efficacy and safety of EVG/COBI/FTC/TDF combination remain unchanged.

2) Other study results

The applicant explained that the following clinical study reports were also compiled although not included in the data package for submission in the U.S.: (i) 1 drug-drug interaction study and (ii) 5 *in vitro* studies to investigate the possibility of each active ingredient of EVG/COBI/FTC/TDF combination serving as a substrate or an inhibitor of drug transporters in the kidney and liver, using transporter-expressing cells, as required by the U.S. FDA at the time of approval; and (iii) 1 phase I study of the effect of COBI on the renal function as required by the European Medicines Agency (EMA). The outline of each study was as shown below.

(i) A drug-drug interaction study (Study GS-US-216-0125) was conducted in healthy adult subjects to investigate the pharmacokinetics of methadone, buprenorphine (BUP), and naloxone (NLX) following the concomitant administration of EVG (150 mg) + COBI (150 mg) with methadone (80-120 mg) or with BUP/NLX (16/4-24/6 mg). Results were as shown in the following table.

Table. Results of study on drug-drug interactions between EVG + COBI and methadone or BUP/NLX (Study GS-US-216-0125)

Concomitant drugs	EVG + COBI	N	Ratio of pharmacokinetic parameters of the concomitant drugs when used with EVG + COBI to those when used without EVG + COBI [90% CI]			
			Drug	C_{max}	AUC_{tau}	C_{tau}
Methadone 80-120 mg QD	EVG 150 mg + COBI 150 mg QD	11	*R*-Methadone	1.01 [0.91, 1.13]	1.07 [0.96, 1.19]	1.10 [0.95, 1.28]
		11	*S*-Methadone	0.96 [0.87, 1.06]	1.00 [0.89, 1.12]	1.02 [0.89, 1.17]
BUP/NLX 16/4-24/6 mg QD		17	BUP	1.12 [0.98, 1.27]	1.35 [1.18, 1.55]	1.66 [1.43, 1.93]
		17	norBUP[b)]	1.24 [1.03,1.49]	1.42 [1.22, 1.67]	1.57 [1.31, 1.88]
		17	NLX	0.72 [0.61, 0.85]	0.72 [0.59, 0.87]	—[a)]

a) C_{tau} of NLX was below the detection limit.
b) Norbuprenorphine

(ii) Results of *in vitro* studies using drug transporter-expressing cells have shown that EVG and COBI are not substrates for OCT1; FTC not for OCT2, OAT1, or MRP2; TFV not for OCT2; and that FTC is a substrate for OAT3. IC_{50} values of EVG and FTC against MRP2 were >20 μM and >100 μM, respectively, showing no inhibitory activity at the maximum concentrations tested.

(iii) A phase I study (Study GS-US-236-0130) was conducted in healthy adult subjects to evaluate the effect on renal function of 30-day multiple dose of COBI (150 mg), TDF (300 mg), COBI (150 mg) + TDF (300 mg), or 1 tablet of EVG/COBI/FTC/TDF combination. Groups receiving COBI (COBI group, COBI + TDF group, EVG/COBI/FTC/TDF combination group) showed serum creatinine increased,[93)] CL_{cr} decreased,[94)] and increased urinary excretion of phosphorus[95)] during the treatment period compared with the TDF group, but showed little changes in aGFR, renal plasma flow, or renal blood flow from baseline.[96)] The applicant explained that effects of COBI on the renal function were similar to those in the phase I study (Study GS-US-216-0121) [see "4.(ii).B.(3).1).(a). Renal impairment" of the Prior Assessment Report (1)].

Based on the review results of the additional study data which were not included in the clinical data package of this application but submitted later, PMDA confirmed that the data do not affect the efficacy or safety evaluation of EVG/COBI/FTC/TDF combination. PMDA instructed the applicant to provide the data of the drug-drug interaction study to healthcare providers in clinical settings in an appropriate manner, via the package insert etc. Also, because only limited information concerning the effects of EVG/COBI/FTC/TDF combination on the renal function-related safety is available, PMDA instructed the applicant to continuously collect information concerning the effects of COBI on renal function after the market launch.

93) The mean change in serum creatinine level from baseline was 0.16 to 0.22 mg/dL in the groups receiving COBI, alone or in combination with other drugs, and 0.04 to 0.07 mg/dL in the TDF group.
94) The mean ratio of CL_{cr} to the placebo group was 79.75% to 86.26% in the groups receiving COBI, alone or in combination with other drugs, and 91.94% to 93.36% in the TDF group.
95) The mean change in urinary excretion rate of phosphorus ([serum creatinine (mg/dL) × urinary phosphoric acid (mg/dL)]/[serum phosphoric acid (mg/dL) × urine creatinine (mg/dL)] × 100) from baseline was 1.7% to 5.6% in the groups receiving COBI, alone or in combination with other drugs, and 1.8% to 3.0% in the TDF group.
96) The mean ratio of aGFR to the placebo group was 89.66% to 96.92% in the groups receiving COBI, alone or in combination with other drugs, and 95.91% to 96.23% in the TDF group. The mean ratio of renal plasma flow rate to the placebo group was 91.94% to 102.81% in the groups receiving COBI, alone or in combination with other drugs, and 94.99% to 96.85% in the TDF group. The mean ratio of renal blood flow rate to the placebo group was 91.66% to 104.51% in the groups receiving COBI, alone or in combination with other drugs, and 92.87% to 96.58% in the TDF group.

The applicant agreed to the above instructions of PMDA.

III. Results of Compliance Assessment Concerning the Data Submitted in the New Drug Application and Conclusion by PMDA

1. PMDA's conclusion on the results of document-based GLP/GCP inspections and data integrity assessment

A document-based compliance inspection and data integrity assessment were conducted in accordance with the provisions of the Pharmaceutical Affairs Act for the data submitted in the new drug application. As a result, there were no particular problems. Thus, PMDA concluded that there should be no problem with conducting a regulatory review based on the submitted application documents.

2. PMDA's conclusion on the results of GCP on-site inspection

GCP on-site inspection was conducted in accordance with the provisions of the Pharmaceutical Affairs Act for the data submitted in the new drug application (5.3.3.1-8, 5.3.3.1-9). As a result, PMDA concluded that there should be no problem with conducting a regulatory review based on the submitted application documents.

IV. Overall Evaluation

As a result of the above review, PMDA concludes that the product may be approved for the following indication and dosage and administration, with the following conditions for approval. Since this product is an orphan drug, the re-examination period is 10 years. Elvitegravir and cobicistat among the drug substances, as well as the drug product, are classified as powerful drugs, and the product is not classified as a biological product or a specified biological product.

[Indication]	HIV-1 infection
[Dosage and administration]	The usual adult dosage is one tablet (containing 150 mg of elvitegravir, 150 mg of cobicistat, 200 mg of emtricitabine, and 300 mg of tenofovir disoproxil fumarate) administered orally once daily during or immediately after a meal.

[Conditions for approval]
The applicant iwa required to:

1. Request physicians to obtain patients' informed consent to the use of the product after having thoroughly informed the patients that the collection of additional data on the efficacy and safety of the product is still ongoing, in light of the fact that a pharmacokinetic study on the product is planned to be conducted in Japan.

2. Submit periodical reports on the progress status of the pharmacokinetic study to be conducted in Japan, as well as the results and analysis of the study promptly after the study completion. The results and analyses of ongoing or planned foreign clinical studies should also be submitted promptly after the study completion.

3. Conduct the post-marketing surveillance which must, as a general rule, cover all patients treated with the product in Japan, until the expiration of the re-examination period, and collect information on the use status of the product (patient characteristics, efficacy and safety [including the efficacy and safety of the product in concomitant use with other drugs], data on drug-drug interactions), thereby submitteing periodical reports. Also, the final results of the surveillance should be submitted in support of the application for re-examination.