4.2. Repeated Dose Toxicity Studies

4.2.1. Darunavir

In a 2-week study in mice, the NOAEL was not established in this preliminary study but at 50 or 150 mg/kg/day, the changes were minimal. In a 3-month repeated dose study, no NOAEL was established.

In rats, the oral (gavage) toxicity of DRV alone was evaluated in studies up to 6-months at doses up to 500 mg/kg/day. The oral toxicity of various combinations of DRV and RTV were also evaluated in 3 studies up to 6-months at doses up to 1000/75 mg/kg/day DRV/RTV.

In the rat studies with DRV alone, no deaths associated with DRV occurred, but there were a number of accidental gavage deaths that were considered related to the viscous and irritant nature of the vehicle (polyethylene glycol [PEG]400). There were no relevant clinical signs or effects on body weight or food consumption and no abnormalities were detected during ophthalmoscopy. The hematopoietic system was affected, as demonstrated by a limited decrease (about 10%) in red blood cell (RBC) counts and related parameters, and an increase in reticulocytes (up to 79%) and bilirubin levels (up to 7-fold) at 500 mg/kg/day. These changes are indicative of an increased RBC turnover, which was confirmed microscopically by the presence of extramedullary hematopoiesis in the spleen. Activated partial thromboplastin time (APTT) was prolonged at 100 and 500 mg/kg/day (by about 50%), but was not associated with any gross or microscopic evidence of bleeding. There was also an increase in platelet counts at 500 mg/kg/day. Liver weight increases correlated with hepatocellular hypertrophy and sometimes with vacuolation. The hepatocellular hypertrophy is considered to reflect an adaptive response to enzyme induction after administration of a xenobiotic, rather than a direct adverse effect of DRV. Besides bilirubin, several clinical biochemistry parameters (lipids and proteins at 500 mg/kg/day) were altered, which was considered secondary to the liver effects. Diffuse follicular hypertrophy and hyperplasia in the thyroid at 1000 mg/kg/day were observed in females in different studies, but not in the 6-month study. It is possible that this is a rodent specific adaptive response to liver enzyme induction and an enhanced metabolism of thyroid hormones. An ex vivo-liver enzyme induction and inhibition study showed that DRV was an inducer of CYP3A4 isoenzyme and UGT, in line with the liver and thyroid findings. No relevant changes were seen in the 3- and 6-month studies at the NOAEL of 20 mg/kg/day. In rats, the exposures based on plasma DRV AUC values at the NOAEL dose from the 6-month study were between 0.03- to 0.04-fold, relative to that observed in humans at the therapeutic dose (D/C/F/TAF 800/150/200/10 mg once daily; see Table 3).

In rat studies of DRV in combination with RTV, similar changes to those with DRV alone were observed, but there were differences in the detail of the changes. In the 6-month combination study, 33 animals were found dead or killed before scheduled necropsy. The majority of deaths were considered accidental (due to twice daily gavage dosing of irritant formulations), but the cause of 12 deaths was not evident. As most of these 12 animals were dosed at 75 mg/kg/day RTV, a relationship with RTV cannot be excluded. A reduction of body weight gain was evident in male animals given 500/75 and 1000/75 mg/kg/day, but not in animals given RTV alone, indicating an effect of DRV or the combination and the MTD had been achieved. In addition to

the hematopoietic changes observed in all treated groups (20/50, 100/50, 500/75 and 1000/75 mg/kg/day), APTT and prothrombin time (PT) initially increased and decreased thereafter. Increases in liver transaminase activities, centrilobular hypertrophy accompanied by multinucleated hepatocytes and some single cell necrosis were observed as a result of an increase in DRV exposure and presence of RTV. The transaminase increases seen with RTV showed an inverse relationship with the DRV dose which might be related to decreases in RTV systemic exposure at higher DRV dose levels. In addition, histopathological changes (increased incidence/severity of islet fibrosis/siderocytes in males) were observed in the pancreas after treatment with DRV/RTV. In these studies, it was not possible to establish a NOAEL. The combination of DRV with RTV resulted in sustained systemic exposure to DRV. In addition, the exacerbation of changes related to DRV was dose related and no new target organs were identified. It is considered that there is evidence of a small increase in effect of the combination of DRV and RTV in the 6-month study on RBC parameters, liver and thyroid. These changes appear to reflect the sustained systemic exposure to DRV in the presence of RTV and were similar to the effects seen with each compound alone. In the 6-month study, exposure to DRV expressed as $AUC_{0\text{-}24h}$ was in the range of 11.2 to 318 µg.h/mL at the end of the study. These exposures were between 0.11- and 3.2-fold, relative to that observed in humans at the therapeutic dose (D/C/F/TAF FDC; see Table 3).

In dogs, the oral (gavage) toxicity of DRV alone was evaluated in studies up to 12-month duration at doses up to 360 mg/kg/day. Various combinations of DRV with RTV were investigated in studies up to 2-week duration.

In the dog studies with DRV alone, there were only limited effects up to the highest dose of 120 mg/kg/day. In dogs, after 120 mg/kg/day for 6 months there was no significant toxicology effect. After 12 months of treatment a limited response in the liver was observed. In the 12-month study, the major change was limited liver enlargement (13% to 22%) in some animals, which occurred generally in the absence of histopathological observations. At the end of the treatment, there was only increased hepatocellular pigment and vacuolation observed with a limited increase in alkaline phosphatase (ALP) (up to 45%) levels at 60 and 120 mg/kg/day. In the 12-month dog study, the NOAEL was 30 mg/kg/day, based on the absence of any relevant liver enzyme and histopathological changes. At NOAEL dose, the exposures based on plasma DRV AUC values were between 0.21- to 0.32-fold, relative to that observed in humans at the therapeutic dose (D/C/F/TAF 800/150/200/10 mg once daily; see Table 3). In this study at 120 mg/kg/day at the end of the dosing period, DRV exposures, expressed as mean $AUC_{0\text{-}24h}$, were 130 and 100 µg.h/mL in males and females respectively. These exposures were higher (up to 1.3-fold) relative to that observed in humans at the therapeutic dose (D/C/F/TAF FDC).

In order to increase exposure to DRV, two 2-week studies were conducted with various combinations of DRV with RTV. Dogs did not tolerate DRV co administered with RTV well. Vomiting and a body weight decrease were observed. As these clinical effects would be unacceptable in a chronic study, no further studies with DRV/RTV in dogs were conducted.

4.2.2. Cobicistat

In a 4-week non-pivotal toxicity study using wild type mice, the NOAEL was considered to be 100 mg/kg/day. In the 13-week mouse study, the NOAELs were considered to be 5 mg/kg/day in males and 50 mg/kg/day in females.

In the 4-week and 26-week oral dose rat studies, increases in liver and thyroid weights were associated with CYP3A enzyme induction, hepatocellular hypertrophy, thyroid hormone changes (decreased thyroxine [T4]; increased thyroid stimulating hormone [TSH]) and thyroid follicular cell hyperplasia/hypertrophy. These findings were reversible, and were not considered adverse. However, 1 high dose male animal had a follicular cell carcinoma in the thyroid in the 26-week study. The liver and thyroid effects are considered adaptive changes, are commonly seen in rodents with microsomal enzyme inducers, and are considered secondary to microsomal enzyme induction and thyroid hormone imbalance (decreases in T4 and increases in TSH), respectively [5, 6, 63, 32, 10, 33, 14, 4]. Hematological and clinical chemistry changes were not considered adverse. Hematological changes (not exceeding 10% versus controls) included slightly lower mean values for erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin, and slightly higher mean platelet counts. Serum chemistry changes observed after 13 and/or 26 weeks of dosing included slightly higher mean gamma glutamyltransferase, cholesterol, total protein, albumin, globulin, and calcium. After a 13-week recovery period, cholesterol and total protein values remained slightly higher in high-dose females, whereas other values were generally comparable to control values, indicating reversibility. Urinalysis and urine chemistry changes, noted primarily in high-dose rats at 100 mg/kg/day, included slightly higher electrolyte excretion and slightly lower electrolyte concentrations, consistent with findings of lower urine osmolality, higher urine volume and/or pH. These changes showed no progression after long term dosing, were reversible, were not associated with remarkable clinical chemistry changes, including serum creatinine and blood urea nitrogen (BUN), and were without histopathological correlates in the kidney. The NOAEL for COBI in the 26-week rat study is considered to be 30 mg/kg/day, based on significant decreases in body weight and food consumption, slight changes in hematological parameters, and increases in urine volume at 100 mg/kg/day. In rats, the exposures based on plasma AUC values at the NOAEL doses in the longest duration studies were approximately 1.1- to 1.5-fold higher than the AUC in humans at the therapeutic dose (D/C/F/TAF FDC) (see Table 4).

In dogs, apart from salivation and vomiting associated with dosing, treatment with COBI was well tolerated at doses up to 15 mg/kg/day in the 4-week study, and up to 10 mg/kg/day in the 39-week study. Changes in the thymus and adrenal gland in dogs observed in high dose animals after 13-weeks of dosing were absent after 39-weeks of dosing, and were considered stress related, and not a direct effect of COBI. In dogs administered 20 mg/kg/day for 39-weeks, clinical signs (salivation, emesis, fecal changes), decreases in body weight and food consumption, non-adverse changes in clinical pathology parameters, and minimal, adaptive changes in the liver (increased weights, hypertrophy) were noted. After 39-weeks of dosing at 10 mg/kg/day, effects were limited to minimal hepatocellular hypertrophy in males, and slightly increased liver weights in females. Clinical pathology changes in the 28-day study included minimal-to-mild increases in bilirubin, alanine aminotransferase (ALT), and ALP activities. In

the 39-week study, slightly higher platelets counts, slightly higher ALP, and slightly lower total protein and albumin were observed; these changes were reversible following cessation of dosing. Urinalysis and urine chemistry changes, noted in female dogs at 20 mg/kg/day, included slightly higher electrolyte excretion and slightly lower electrolyte concentrations, consistent with findings of lower urine osmolality, higher urine volume and/or pH. These changes showed no progression after long term dosing, were reversible, were not associated with remarkable clinical chemistry changes, including serum creatinine and BUN, and were without histopathological correlates in the kidney. In dogs, a greater incidence of bilirubinuria was noted in males at 20 mg/kg/day during the 39-week study; no changes were observed at recovery. Based on these findings, the NOAEL for COBI when administered daily by oral gavage to dogs for up to 39 weeks is 10 mg/kg/day. In dogs, the exposures based on plasma COBI AUC values at the NOAEL doses in the longest duration studies were approximately 1.9- to 2.2-fold higher than the AUC in humans at the therapeutic dose (D/C/F/TAF FDC) (see Table 4).

4.2.3. Emtricitabine

A series of GLP oral repeat-dose toxicity studies were conducted with FTC in mice (4 weeks and 26 weeks), rats (13 weeks), and cynomolgus monkeys (4 weeks, 13 weeks, and 52 weeks).

Effects associated with the administration of FTC in the toxicology studies were confined to high-dose groups. Changes in RBC parameters, interpreted as a mild, reversible anemia occurred at the highest dose in several studies (ie, 1- and 6-month mouse; 3-month rat; and 12-month monkey). The NOELs for the longest treatment period in each species were 500 mg/kg/day in mice (6 months), 600 mg/kg/day in rats (3 months), and 200 mg/kg/day in monkeys (12 months). The exposures based on plasma AUC values at the NOEL doses in the animals were approximately 26-fold (mice), 30-fold (rats), and 8-fold (monkeys) higher than the AUC in patients treated with FTC at 200 mg once daily in the D/C/F/TAF FDC.

4.2.4. Tenofovir Alafenamide

The repeat-dose oral toxicity of TAF has been studied in mice, (2 weeks and 13 weeks), rats (6-7 days, 4 weeks and 26 weeks), dogs (4-weeks and 39 weeks) and monkeys (4 weeks). In chronic studies, kidneys (karyomegaly, tubular degeneration), and bone (atrophy of metaphyseal cancellous bone) were the primary target organs. TAF also appeared to increase biochemical markers of bone turnover and decrease serum 1, 25-dihydroxy- and 25-hydroxyvitamin D_3 at doses in rats and dogs. Renal tubular karyomegaly was observed in rats and dogs orally administered TAF. Focal areas of minimal renal cortical tubular basophilia and associated minimal nuclear karyomegaly were present in rats administered 400 mg/kg/day for 4 weeks and 100 mg/kg/day for 26 weeks. Renal tubular karyomegaly and/or basophilia were observed in dogs administered 3 and 10 mg/kg/day for 4 weeks and dogs administered 6 or 18/12 mg/kg/day for at least 13 weeks. Renal cortical tubular degeneration/regeneration findings were limited to animals administered 6 or 18/12 mg/kg/day for at least 13 weeks in the 39-week dog toxicity study. Similar findings of renal cortical tubular degeneration/regeneration and karyomegaly were present in dogs administered either 6 or 18/12 mg/kg/day for 39 weeks. These changes were minimal to slight in affected males and females at 6 mg/kg/day. In high-dose males (18/12 mg/kg/day) the severity ranged from mild to moderate. Similar lesions (karyomegaly and

tubular degeneration) but of only minimal severity were also present in 2 males administered 2 mg/kg/day of TAF for 39 weeks. After a 13-week recovery period, treatment-related histology changes were still observed in the kidney but were of reduced incidence and severity. Atrophy of metaphyseal cancellous bone was observed in rats administered TAF at 100 mg/kg/day for 26 weeks. TAF also increased biochemical markers of bone turnover and decrease serum 1,25-dihydroxy- and 25-hydroxyvitamin D_3 in rats (≥25mg/kg/day) and dogs (≥37.5 mg/kg/day for 6 days). In the 39-week dog study, bone mineral density changes at 18/12 mg/kg/day were most likely due to body weight loss, but these changes were accompanied by a slight but significant decrease in serum 1,25-dihydroxyvitamin D_3 in males only and a significant increase in 25-hydroxyvitamin D_3 in females only.

Tenofovir alafenamide administered by oral gavage for up to 13 weeks to mice at ≥ 10 mg/kg/day resulted in adverse degenerative (olfactory) and acute inflammatory (infiltrate neutrophil) changes in the nasal mucosa. Because these changes were not observed in rats, dogs or monkeys for longer durations of administration, the relevance to humans is unknown and the risk of nasal inflammation in humans is very low.

In the 39-week dog study, TAF had no discernible electrocardiographic effect at the low dose of 2 mg/kg/day. There was some evidence at 6 and 18/12 mg/kg/day for an effect to slightly prolong PR intervals (~13-24%) which was associated with significant decreases in T3 [27, 53]. After the 13-week recovery period, serum T3 values returned to levels similar to the control group animals at the end of the study. No PR prolongation or any change in ECG results occurred in the safety pharmacology study that evaluated a TAF dose up to 100 mg/kg or in the thorough QT study (Genvoya® NDA207561/GS-US-120-0107).

At 18/12 mg/kg/day in dogs, the highest dose tested, a minimal infiltration of histiocytes was present in some organs (eye [choroid plexus, ciliary body], lung, and spleen) in some animals. In-life ophthalmologic examinations were normal. The histiocytic infiltration observed in multiple organs was most likely an indirect drug effect due to general debilitation and was not observed in other repeat dose toxicity studies. There were no drug-related effects on ophthalmic exams or microscopic exams of ocular tissue observed in repeat-dose toxicity studies in mice (up to 13 weeks), rats (up to 26 weeks), and non-human primates (4 weeks) or in the 4-week dog toxicology study. The minimal to slight infiltration of mononuclear cells in the ocular posterior uvea occurred only in dogs administered the highest dose of TAF where compound-related toxicities occurred on clinical condition, weight gain, serum chemistry, bone mineral density, and other organ histopathology. Distribution of ^{14}C-TAF to eyes has been assessed in mice, rats, and dogs. Melanin binding has specifically been assessed by comparing distribution in pigmented and non-pigmented mice (C57 black and CD-1, respectively) and rats (Long Evans and Sprague-Dawley, respectively). ^{14}C-TAF-related radioactivity distributed poorly to the eyes of rats and dogs (C_{max} in eyes < 8% that observed in plasma). Transient exposure to low levels of ^{14}C-TAF-related radioactivity was observed in the eyes of rats decreasing to undetectable levels at 8 hours postdose. No difference in distribution was observed between Sprague-Dawley and Long Evans rats, including in the skin and eyes, suggesting no binding to melanin. The distribution of ^{14}C-TAF to eyes in mice was higher than other species studied (C_{max} in eyes 15%-20% that observed in plasma). More persistent exposures in eye lens, eye uveal tract, and

eyes were observed in C57 black mice compared to CD-1 mice. However, no difference in distribution between pigmented and non-pigmented skin was observed illustrating that ^{14}C-TAF-related radioactivity was not selectively associated with melanin-containing tissues. The posterior uveitis in dogs administered the highest dose of TAF occurred at 3.7- and 17-fold higher exposure to TAF and TFV, respectively, than that observed in human subjects administered a 25-mg dose of TAF and does not correlate with the tissue distribution where TAF was found to poorly penetrate across the blood brain and blood retinal barrier in dogs. Because TAF has poor penetration across the blood brain and blood retinal barrier in dogs, it is unlikely that TAF directly caused the observed histiocytic infiltration in the posterior uvea. Based on the evidence from tissue distribution and toxicology studies, it was concluded that the risk of posterior uveitis in humans is very low.

No new or more marked toxicities occurred in two 14-day rat toxicology studies and a 4-week dog study with the combination of FTC and TAF.

4.3. Genotoxicity Studies

4.3.1. Darunavir

In vitro and in vivo genotoxicity tests have shown that DRV has no genotoxic potential.

4.3.2. Cobicistat

No significant effects were observed in genotoxicity.

4.3.3. Emtricitabine

Emtricitabine was not genotoxic in the reverse mutation bacterial test (Ames test), mouse lymphoma, or mouse micronucleus assays.

4.3.4. Tenofovir Alafenamide

Tenofovir alafenamide was not genotoxic in a battery of in vitro and in vivo assays. The in vitro assays included gene mutation assays with bacterial strains (*Salmonella typhimurium*, *Escherichia coli*, and a L5178Y gene mutation assay in mouse lymphoma cells. The in vivo evaluation consisted of a mouse bone marrow micronucleus study at oral doses of 500, 1000, and 2000 mg/kg.

4.4. Carcinogenicity

4.4.1. Darunavir

DRV was evaluated for carcinogenic potential by oral gavage administration to mice and rats up to 24-months. Daily doses up to 1000 mg/kg were administered to mice and doses up to 500 mg/kg were administered to rats. A dose-related increase in the incidences of hepatocellular adenomas and carcinomas was observed in males and females of both species. Thyroid follicular cell adenomas were noted in male rats. Administration of DRV did not cause a statistically significant increase in the incidence of any other benign or malignant neoplasm in mice or rats. The observed hepatocellular findings in rodents were associated with liver enzyme induction and

are considered to be of limited relevance to humans. At the highest tested doses, the systemic exposures (based on AUC) were between 0.5- and 0.6-fold (mice) and 0.91-fold (rats), of that observed in humans at the therapeutic dose (D/C/F/TAF FDC) (see Table 3).

4.4.2. Cobicistat

In the 104-week carcinogenicity study in mice with COBI, no drug-related increase in tumor incidence was observed at exposures 9- to 20- times (males and females, respectively) the human systemic exposure at the therapeutic daily dose (see Table 4). In the 104-week carcinogenicity study in rats, increases in follicular cell adenomas and/or carcinomas in the thyroid gland were observed at doses of 25 and 50 mg/kg/day in males, and at 30 mg/kg/day in females. The follicular cell findings are considered to be rat-specific, secondary to hepatic microsomal enzyme induction and thyroid hormone imbalance, and are not relevant for humans [63, 10, 33]. At the highest doses tested in the rat carcinogenicity study, systemic exposures were approximately 2.5 times the human systemic exposure at the therapeutic daily dose (see Table 4). In rats, COBI induces hepatic CYP3A activity due to a species-specific activation of PXR, which does not occur in humans. The observed toxicity profile on the thyroid is rodent-specific and it is unlikely that COBI presents a risk to the human thyroid. These effects, associated with liver enzyme induction, bear no relevance for man as a similar association between liver enzyme induction and carcinogenesis does not exist in man.

4.4.3. Emtricitabine

In long-term carcinogenicity studies of FTC, no drug-related increases in tumor incidence were found in mice at doses up to 750 mg/kg/day (25-fold the human systemic exposure at the therapeutic dose of 200 mg/day) or in rats at doses up to 600 mg/day/day (29-fold the human systemic exposure at the therapeutic dose).

4.4.4. Tenofovir Alafenamide

Per separate agreements with the FDA ( ) and with the EMA ( ), Summaries of the carcinogenicity studies with TDF are provided below.

Long-term oral carcinogenicity studies of TDF in mice and rats were carried out at exposures up to approximately 140 times (mice) and 21 times (rats) at those observed in humans (D/C/F/TAF FDC). Female mice showed a low incidence of duodenal tumors at the highest dose of 600 mg/kg/day. Rats did not show any carcinogenic potential in the long-term study.

4.5. Reproductive Toxicity Studies

4.5.1. Darunavir

The reproduction and developmental toxicity studies demonstrated that there was no effect of DRV or DRV/RTV on fertility or early embryonic development in rats and DRV showed no teratogenic potential in mice, rats, and rabbits. In embryo-fetal development studies, the

NOAELs were considered at 1000 mg/kg in mouse and rabbits where exposures were approximately 0.64-fold and 0.06-fold higher, respectively than the human therapeutic exposures (see Table 3). In rats, the NOAEL for maternal toxicity was considered to be less than 600/100 mg/kg/day DRV/RTV and the NOAEL for embryo-fetal development was considered to be 600/100 mg/kg/day (DRV/RTV) where maternal exposures were approximately 2.0-fold (Day of gestation (GD) 11) and 2.6-fold (GD17) higher, than the human therapeutic exposure (see Table 3).

In the pre- and postnatal development study in rats, DRV caused a transient reduction in pup body weight gain, which was attributed to exposure to DRV during lactation.The toxicity of DRV was also investigated in a juvenile study where male and female rats received dose levels up to 500 mg/kg/day from 23 days of age until sexual maturity (50-day old). In addition, several single and repeated oral dose studies were conducted in juvenile rats as young as 5 days of age. In studies where dosing commenced at less than 23 days of age (equivalent to less than 2 years of age in humans), a NOAEL was not established. Mortality was observed at most dose levels (from 20 mg/kg/day up to 1000 mg/kg/day) and in some cases convulsions and other apparent central nervous system effects preceded these deaths. High plasma, liver and brain exposure values were observed, which were both dose- and age-dependent and considerably greater than those observed in adult rats. These findings were attributed to the ontogeny of CYP liver enzymes involved in the metabolism of DRV and the immaturity of the blood brain barrier.

4.5.2. Cobicistat

No adverse effects were observed in a rat fertility study with COBI; the NOEL for reproductive parameters was 100 mg/kg/day at exposures approximately 1.7-fold higher than human therapeutic exposures. No teratogenic effects were observed in rat and rabbit developmental toxicity studies. In rats at 125 mg/kg/day, increases in post-implantation loss and decreased fetal weights were associated with significant maternal toxicity (adverse clinical signs, decreased body weight and food consumption). The NOEL/NOAELs in the rat and rabbit studies were 50 and 100 mg/kg/day, respectively, where exposures were approximately 1.7 and 4.1 fold higher, respectively, than human therapeutic exposures (see Table 4). In the pre/postnatal study, the maternal NOAEL for general toxicity was 30 mg/kg/day, and the NOAEL for reproduction in the dams and viability and growth of the offspring was 75 mg/kg/day, the highest dose tested. In the juvenile toxicity phase of the pre/postnatal study in rats with COBI, the NOAEL for toxicity of COBI is 75 mg/kg/day for juvenile rats where exposures were 2.5-fold higher (for males and females) than therapeutic adult human exposures at the therapeutic dose (D/C/F/TAF FDC).

4.5.3. Emtricitabine

Emtricitabine did not affect fertility in male rats or in male and female mice. There were no adverse effects in embryo-fetal development studies in mice. In the pre/postnatal study in mice, first generation (F_1) dams at 1000 mg/kg/day had slightly longer estrous cycles than controls, but fertility was normal in the offspring exposed daily from before birth (in utero) through sexual maturity.

Repeat-dose studies with FTC have not shown effects in developing organ systems, and reproductive and developmental NOELs for FTC were at exposure levels well above human exposures. Emtricitabine is approved for use in infants (aged 4 months of age or older), children, adolescents, and adults. No specific juvenile toxicity studies are considered warranted with FTC.

4.5.4. Tenofovir Alafenamide

Reproductive tissues were examined in repeat-dose toxicology studies in the rat, dog, and monkey. There were no treatment-related histologic alterations or changes in organ weights in the rat and the dog following chronic daily dosing, or in the monkey following 28 days of daily oral administration.

The TAF fumarate (GS-7340-03) oral rat fertility study data indicate dose related decreases in body weight gain in males and females, but no drug related changes occurred in male or female fertility endpoints including mating index, fertility index, sperm motility, sperm concentration, number of corpora lutea, implantation sites, early and late resorptions and live fetuses at doses up to 160 mg free base equivalents/kg/day. The TAF NOAEL for reproductive and early embryonic toxicity was 160 mg/kg/day.

There was no effect on fetal viability or fetal development in pregnant rats administered doses of GS-7340-02 up to 250 mg/kg/day, or in pregnant rabbits administered GS-7340-02 up to 100 mg/kg/day. The highest doses were maternally toxic. In the rat, decreased fetal body weight associated with some minor transitory delays in the rate of ossification was observed at 250 mg/kg/day, a maternally toxic dose. At the NOAEL for embryo-fetal development of approximately 100 mg/kg/day in rats, AUC_{tau} values for TAF and TFV on Day 17 were 0.2 and 17.4 µg.h/mL, respectively. At the NOAEL for embryo-fetal development of 100 mg/kg/day in rabbits, AUC_{tau} values for TAF and TFV on Day 20 were 11.0 and 27.3 µg·h/mL, respectively (Table 6).

Tenofovir DF, but not TAF, has been tested in a perinatal/postnatal study. Per agreement with the FDA ([redacted]) [redacted] There was an alteration of the estrous cycle in female rats in the perinatal study in rats. The NOEL for behavioral, reproductive, and development toxicity was 150 mg/kg/day. Maternally toxic doses (≥450 mg/kg/day) had effects on pup survival, pup body weights, and sexual maturation.

Although no specific juvenile toxicity studies have been conducted with TAF or TDF, data are available from efficacy studies of TFV in simian immunodeficiency virus (SIV)-infected and non-infected rhesus macaques [51, 59, 58]. These studies included 12 gravid rhesus macaques, and more than 85 infant and juvenile rhesus macaques treated from ages ranging from 1 day to 7.5 years at initiation of dosing. This age range covers the human equivalent of prenatal, infant, juvenile and adolescent phases of growth. The duration of treatment ranged from 12 weeks to 13 years. Clinically relevant renal and bone pathology (including reduced bone mineral density, joint swellings, and bone fractures) occurred only in animals in which TFV was chronically administered at 30 mg/kg/day by daily subcutaneous injection. Exposure levels

(TFV AUC = 150 µg·h/mL) at this dose were more than 442-fold higher than those of adults after a 10 mg dose of TAF. Effects in rhesus monkeys were reversible by decreasing or stopping exposure. Administration of lower doses of TFV (10 mg/kg/day, ~15 µg·h/mL) did not cause renal dysfunction or abnormal bone density or growth.

Tenofovir administered to newborn or infant rhesus monkeys at doses of 4 to 30 mg/kg/day did not cause adverse effects in short-term studies (up to 12 weeks). However, prolonged TFV treatment (generally more than 4 months of daily treatment at 30 mg/kg/day administered subcutaneously) resulted in a Fanconi-like syndrome with glucosuria, aminoaciduria, hypophosphatemia, growth restriction, and bone pathology (osteomalacia) [59]. Clinical, biochemical, and radiographic resolution/improvement occurred with dose reduction (from 30 to ≤10 mg/kg/day) or discontinuation of treatment.

Three animals (1 SIV-infected) were dosed chronically, beginning as neonates, with 10 mg/kg/day TFV administered subcutaneously. After more than 5 years of treatment, there were no clinical, radiographic, or dual-emission X-ray absorptiometry scan [59] findings of an adverse effect on bone. The mean AUC associated with this dosage (18 µg·h/mL) 53-fold greater than the human AUC_{ss} following a 10 mg/day dose of TAF.

4.6. Local Tolerance

4.6.1. Darunavir

Local tolerance studies showed no skin irritation and sensitization and eye irritation.

4.6.2. Cobicistat

Cobicistat was mildly irritating to skin, not a severe irritant to eyes, and showed no potential for phototoxicity.

4.6.3. Emtricitabine

No local tolerance studies have been conducted with FTC.

4.6.4. Tenofovir Alafenamide

Local toxicity studies concluded that TAF was predicted to be a noncorrosive/non-severe eye irritant, and nonirritating/noncorrosive to rabbit skin under semi-occluded conditions.

4.7. Other Toxicity Studies

4.7.1. Immunotoxicity

4.7.1.1. Darunavir

Darunavir formulated in PEG400 was administered daily, alone or in combination with RTV, in a 4-week rat study at doses of DRV alone up to 500 mg/kg/day, or DRV/RTV at 100/50 mg/kg/day. Changes in the hematopoietic system and liver (as seen in the repeated dose toxicity studies) were present but the immune response of treated animals, as measured by immunoglobulin (Ig) M production, was not affected. The NOAEL was 20 mg/kg/day.

4.7.1.2. Cobicistat

The immunotoxicity of COBI was evaluated in a 28 day T-cell dependent antibody response (TDAR) study in rats at doses up to 150 mg/kg/day. Immunosuppressive effects were noted in females at 50 and 150 mg/kg/day, based on a decreased response to keyhole limpet hemocyanin (KLH) immunization (lower anti-KLH IgG antibody titers). Decreased anti-KLH IgG responses in males did not reach statistical significance at 150 mg/kg/day. No COBI-related changes in the anti KLH IgM response in males and females were noted. In addition, clinical signs, decreases in body weight gain and/or food consumption, increases in liver and thyroid weights, and lymphoid depletion of germinal centers in the spleen were observed at 50 and/or 150 mg/kg/day. The NOEL for the TDAR is considered to be 20 mg/kg/day in females, and 50 mg/kg/day in males, and the NOAEL was 20 mg/kg/day in both sexes. Additional immunohistochemical analysis of spleens from all animals was conducted, as described in the ICH S8 Guideline, Immunotoxicity Studies for Human Pharmaceuticals [55]. Formalin-fixed, paraffin-embedded tissues (spleen) were evaluated for detection of B cells (KiB1R-positive), T cells (CD3-positive), and germinal centers (PNA-positive). Immunohistochemical findings observed in COBI-treated animals did not correlate with the decreases noted in anti-KLH IgG levels, as greater decreases in anti KLH IgG levels were observed in females versus males, but immunohistochemical trends were noted only in males. In females, there was a notable lack of any dose-response with respect to the immunohistochemical changes.

To further assess the potential for immunotoxicity associated with COBI, immune tissues (spleen, thymus, lymph nodes, and Peyers patches) from rats administered COBI in the 4-week and 26-week toxicity studies were subjected to a pathology peer-review. Evaluation was conducted according to recommendations for rat lymphoid tissue evaluation and included an estimate of the numbers of germinal centers in the spleen for each animal [24, 18, 28]. The Peer Reviewing Pathologist was in agreement with the overall interpretations and conclusion of the histopathology diagnoses and conclusions of the studies regarding the lack of treatment related effects on rat lymphoid tissues. The NOAELs for COBI in the 4-week and 26-week studies were 50 and 30 mg/kg/day, respectively.

In the 26-week rat study with COBI, peripheral blood immunophenotyping was conducted during Week 26 and prior to recovery sacrifice (Recovery Week 13). Total T cells, helper T cells, cytotoxic T cells, B cells and NK cells were quantified using flow cytometry. No adverse effects on mean immunophenotyping values were noted.

In the 39-week dog study with COBI, peripheral blood immunophenotyping (total T cells, helper T cells, cytotoxic T cells, and B cells) conducted during Week 26 revealed no treatment-related changes. Histopathological changes in immune system tissues were limited to minimal to moderate thymic involution in high dose (20 mg/kg/day) males at the 13-week interim sacrifice. The thymic changes after 13 weeks of dosing were attributed to stress (thin appearance, decreased body weight and food consumption, excessive salivation, emesis and abnormal feces) and not considered direct COBI-related effects. Further, these changes were no longer apparent in terminal sacrifice or recovery sacrifice animals suggesting tolerance to these stress-related

changes. The NOAEL after 39 weeks daily oral gavage dosing to dogs is considered to be 10 mg/kg/day.

In summary, for COBI, decreased IgG levels were noted in female rats at dose levels (≥50 mg/kg/day) above the NOAEL for systemic toxicity in long term studies (30 mg/kg/day). However, in standard toxicity studies with COBI in mice (up to 13 weeks dosing), rats (up to 26 weeks dosing) and dogs (up to 39 weeks dosing), and at higher dose levels and exposures, no signs of immune function changes have been observed.

4.7.1.3. Emtricitabine

The immunotoxicity of FTC was evaluated in a 28-day study in CD rats at doses up to 1000 mg/kg/day. There were no adverse effects of FTC during the dosing period and FTC did not affect the IgM antibody titers to sheep RBCs at any of the doses administered. The NOEL for immunotoxicity was 1000 mg/kg/day.

4.7.1.4. Tenofovir Alafenamide

Data from repeat-dose toxicity studies with TAF (hematology, lymphoid organ weights, microscopy of lymphoid tissues, bone marrow cellularity) did not suggest immunotoxic potential.

4.8. Drug Substance Impurities

All specified impurities for the DRV and COBI compounds are qualified according to the ICHQ3A guideline.

Based on their impurity profiles, the multiple GLP batches of FTC and TAF tested in the toxicology program are considered, in composite, to be representative of the GMP material and support the specified limits of impurities and degradation products proposed for commercial production

4.9. Toxicology Evaluation

4.9.1. Darunavir

4.9.1.1. Mortality

Lethality, related to DRV, could not be demonstrated following single or repeated oral and IV treatment in adult mouse, rat and dog. Throughout all of the rodent toxicology studies there were no mortalities associated with DRV, but animals were found dead or were killed prematurely during the various studies. Most these deaths were associated with the dosing procedures with single daily doses or twice-daily gavage dosing where RTV was used or, in some cases, blood collection procedures. With the former, the irritant and viscous nature of the vehicle, PEG400 was considered contributory. In the 6-month rat study with DRV administered in combination with RTV, the cause of death was not evident in some animals and a relationship between mortality and RTV treatment at 75 mg/kg/day, could not be excluded based on the distribution of

the deaths. In dogs, there were also isolated incidence of accidental deaths associated with gavage dosing but none were related to DRV treatment.

Mortality was reported in juvenile rats between day 12 and day 25 of age following single and repeated dosing at 40 mg/kg/day and above. The exact cause of death was not established and clinical signs observed prior to mortality were among others distended abdomens, unsteady gait, convulsions, reduced activity and labored breathing. In contrast, a DRV dose of 1000 mg/kg given at day 26 of age to rats was without clinical signs or mortality. Exposure in juvenile animals directly dosed was dose and age dependent and mortality seen at this age range might be attributed to high DRV exposure caused by the immaturity of liver enzymes. Exposure at 1000 mg/kg on day 12 was at least 3-fold higher than that observed in the clinic at the recommended dose.

4.9.1.2. Assessment of General Toxicity

The key target organ/systems identified in rodents following oral administration of DRV and/or RTV were the hematopoietic system, the blood coagulation system, liver and thyroid. No target organs of toxicity were identified in dogs.

The hematopoietic system was affected in rats and mice. These changes included a variable, but slight decrease (about 10%) in some or all of the RBC counts, hemoglobin and hematocrit. There was also an increase in reticulocytes and bilirubin levels. These changes are indicative of an increased RBC turnover, which was confirmed microscopically by the presence of extramedullary hematopoiesis in the spleen, sometimes accompanied by an increase in spleen weight. In rats, the NOAEL of this finding, after DRV alone was 20 mg/kg/day and corresponded to exposures ($AUC_{0\text{-}24h}$) ranged between 3 and 4 µg.h/mL. The RBC system has been identified as a target organ of toxicity in rodents with other protease inhibitors [60]. No changes in RBC-related parameters have been observed with DRV in dogs. No clinically relevant adverse effects on the hematopoietic system have been observed in clinical studies conducted to date with DRV (Mod2.7.4).

The blood coagulation system was affected [39]. In rats platelet counts were increased and there was a prolongation in the APTT. The NOAEL of this finding, after DRV alone was 20 mg/kg/day and corresponded to exposures ($AUC_{0\text{-}24h}$) ranged between 3 and 4 µg.h/mL. The increase in the APTT observed was not associated with any gross or microscopic evidence of bleeding. APTT and PT were decreased in the 6-month combination study with RTV in rats, whereas in dogs no such changes were seen. No clinically relevant adverse effects on the blood coagulation system have been observed in clinical studies conducted to date with DRV (Mod2.7.4).

No changes in the immune response were observed for DRV.

Liver changes were observed in rats and mice and these included increases in weight and hepatocellular hypertrophy sometimes with vacuolation of centrilobular hepatocytes at doses of 100 mg/kg/day and above. The hepatocellular hypertrophy is considered mild in nature and reflects an adaptive response to enzyme induction (CYP3A and UGT) seen after DRV

administration rather than a direct adverse effect [21]. In rats, several clinical biochemistry parameters were altered by treatment with DRV, mainly increases in cholesterol and proteins (e.g. total protein) and decreases in triglycerides. These lipid and protein changes were probably secondary to the effects on the liver. In dogs, changes in the liver were observed only after 12 months of treatment and were limited to a slight increase in liver weight associated with hepatocellular vacuolation and pigmentation at an AUC_{last} of 100 to 130 µg.h/mL. Increases in liver transaminases in animal studies were generally mild or absent. In the clinic, liver abnormalities, mainly increases of aspartate aminotransferase (AST) and ALT were generally mild to moderate, and drug-induced hepatitis (e.g. acute hepatitis, cytolytic hepatitis) have been reported with DRV/RTV (Mod2.7.4).

Thyroid homeostasis was altered to various extends in rats (and mice) as seen by diffuse follicular hypertrophy and hyperplasia. It is likely that this is a rodent-specific adaptive response to liver enzyme induction and an enhanced metabolism of thyroid hormones. Both CYP P450 and UGT are responsible for eliminating numerous substrates, both endogenous and exogenous, rendering them water-soluble and excretable in urine and bile. Increased elimination of thyroid hormones, especially T4, via UGT induction, may stimulate the thyroid and result in the changes seen. Both of these enzymes were induced in rats following repeated oral administration with DRV alone and in combination with RTV. Thyroid changes were not observed in the dog.

The combination of DRV with RTV in rats resulted in slight increased exposure to DRV. Given the complex nature of the combination studies and the potential effects of both compounds these results should be considered in comparison with other studies. If this is done then it is considered that there is evidence of a small increase in effect in the 6-month study with the combination of DRV and RTV on RBC parameters, liver and thyroid. These changes appear to reflect the sustained systemic exposure to DRV in the presence of RTV but were not of a different order of magnitude observed in animals with each compound alone. At doses of 500/75 and 1000/75 mg/kg/day exposure to DRV expressed as AUC_{last} was similar and in the range of 183 to 318 µg.h/mL, at the end of the study. These values were about 4 times higher than those observed with DRV alone. As the full clinical development of DRV was done with RTV, the comparative information on safety of DRV versus the DRV/RTV combination is only available from Phase I trials. These trials showed no synergistic effect.

4.9.1.3. Assessment of Genotoxicity and Carcinogenicity

Genotoxicity tests, in vitro and in vivo, have shown DRV to be free of genotoxic potential.

Darunavir was evaluated for carcinogenic potential by oral gavage administration to mice and rats up to 24-months. Daily doses of 150, 450 and 1000 mg/kg were administered to mice and doses of 50, 150 and 500 mg/kg were administered to rats. A dose-related increase in the incidences of hepatocellular adenomas and carcinomas were observed in males and females of both species. Thyroid follicular cell adenomas were noted in male rats. Administration of DRV did not cause a statistically significant increase in the incidence of any other benign or malignant neoplasm in mice or rats. The observed hepatocellular findings in rodents were associated with liver enzyme induction and are considered to be of limited relevance to humans [20, 22, 25].

Repeated administration of DRV to rats caused hepatic microsomal UGT induction and increased thyroid hormone elimination, which predispose rats, but not humans, to thyroid neoplasms. At the highest tested doses, the systemic exposures (based on AUC) were between 0.48- and 0.64-fold (mice) and 0.91-fold (rats), of that observed in humans at the recommended therapeutic dose (see Table 3).

4.9.1.4. Assessment of Reproduction and Development Toxicology

There was no evidence of an effect on fertility or early embryonic development and DRV showed no teratogenic potential.

In the rat pre- and postnatal development study, reduction in pup body weight gain associated with a slight delay in developmental milestones was observed with DRV alone (at 200 mg/kg/day and above) or in combination with RTV (at 1000/75(50) mg/kg/day) during lactation. This was due to exposure of pups to drug substances via the milk as shown in the dose range finding study. Sexual development, fertility or mating performance of offspring was not affected by maternal treatment with DRV alone or in combination with RTV or RTV alone. No post-weaning functions were affected at any dose level.

The juvenile data available to date show that in juvenile rats from 23 days of age DRV has a toxicity profile and exposure that is comparable to that observed in adult rats. In studies where dosing commenced at less than 23 days of age (equivalent to less than 2 years of age in humans) mortality was observed and in some animals, convulsions and other apparent CNS effects preceded these deaths. Within this age range, high plasma, liver and brain AUC values were observed, which were both dose- and age-dependent and considerably greater than those observed in adults. These findings were attributed to the immaturity of the liver enzymes and of the blood brain barrier. The above observations in juvenile rats provide evidence of a potential safety risk as a result of drug-brain accumulation in young children (below 3 years of age) [12, 25, 29].

4.9.1.5. Safety Margin

The animal/human exposure ratios can be found in Table 3.

Table 3: Estimated Safety Margins for DRV 800 mg Based on Exposure (AUC) at Animal NOAEL

Species Gender	Study Type	NOAEL Dose (mg/kg/day)	AUC_{0-t} (µg.h/mL)	Safety Margin[a]
Mice				
Male - Female	24-Month Carcinogenicity (Day 188)	1000 [b]	48.1- 63.8-	0.48 – 064X
Female (pf)	Embryo- Fetal Development (GD15)	1000	63.9	0.64 X
Rat				
Male - Female	26-week Toxicity	20	2.9 – 4.4	0.03 – 0.04X
Male - Female	24-Month Carcinogenicity (Day 184)	500 [b]	90.3 -89.5	0.91 X
Female (pf)	Embryo- Fetal Development	2x300 (DRV) + 2 x 50 (RTV)	202 (GD11) 261 (GD17)	2.0 X 2.6 X
Male - Female	26-week Combination Toxicity	20 [c] (DRV) + 50 (RTV)	11.2 – 13.4	0.11 – 0.13 X
Rabbit				
Female (pf)	DRF of Prenatal Development Toxicity (GD20)	1000	6.0	0.06 X
Dog				
Male - Female	12-month Toxicity	30	21.2 – 31.6	0.21 – 0.32 X

RTV, ritonavir; NOAEL, no observed adverse effect level, Pf : pregnant female
[a]: Human AUC_{0-24h} =99.3 µg.h/mL (Mod5.3.5.1/GS-US-299-0102 PK substudy); [b]: the highest dose tested; [c]: in this study no NOAEL was determined, this dose corresponded to the low dose.

4.9.2. Cobicistat

4.9.2.1. Assessment of General Toxicity, genotoxicity and carcinogenicity

No clinically relevant adverse effects were observed in the safety pharmacology, genotoxicity, reproductive, juvenile toxicity, and local tolerance studies with COBI.

Minimal changes in RBC parameters (not exceeding 10%) were noted in high-dose rats given 100 mg/kg/day in the 26-week study. There were no correlative effects (eg, symptoms of anemia, bone marrow suppression) and similar effects were not seen in mice dosed for 13 weeks or in dogs dosed for 39 weeks. Due to the minimal change and reversibility, these effects were not considered adverse.

In rats, increases (up to 38%) in mean platelet counts were noted in the 4-week study at doses ≥50 mg/kg/day, and in the 26-week study in males at ≥30 mg/kg/day and in females at 100 mg/kg/day. In dogs, increases in platelet counts (up to 43%) were noted after 13, 26 and 39 weeks dosing in high dose (20 mg/kg/day) females, and in 10 mg/kg/day females at Week 26 only. Further, in dogs, minimal decreases (up to 15%) in APTT were noted in 10 mg/kg/day females and in both sexes at 20 mg/kg/day. Similar changes were not observed in the 4-week dog study at doses up to 45/30 mg/kg/day. In all cases, there were no associated effects on bleeding, the changes were reversible, and they were not considered adverse. Since these changes only occurred at high doses, the relevance of these limited changes is questionable.

Slight but statistically significant increases in cholesterol were observed in the 13-week mouse study (up to 46% increase at dose levels ≥15 mg/kg/day) and in female rats in the 26 week study (up to 35% increase at dose levels ≥30 mg/kg/day. Cobicistat is a rat PXR inducer, with activation similar to known PXR inducers (RTV and miconazole). Induction of PXR can reduce Cyp7a1 transcription and cholesterol 7a-hydroxylase activity, which are involved in the conversion of cholesterol to bile acids [49]. Down-regulation of Cyp7a1 results in less cholesterol being converted to bile acids and consequently more free cholesterol. An approximate 6-fold induction of PXR was observed with COBI at 10 µM. In the 13-week mouse and 26-week rat toxicity studies, COBI C_{max} values associated with cholesterol changes were 2.7 to 11.4 µg/mL (3.5 to 14.7 µM), suggesting adequate PXR activation to explain the slight increase in cholesterol levels observed with COBI. At clinically-relevant concentrations, COBI would not activate human PXR. In Phase 3 studies with COBI-boosted ATV, mean values for fasting glucose and lipid parameters remained in the normal range.

Minor non-adverse changes in total protein, globulin, and albumin were observed in rats and dogs. In rats, increases in mean total protein were less than 10% in high dose males and females, with similar changes in albumin and globulin values. As expected, increases in serum calcium correlated with the increases in serum albumin. In rats, these changes can be considered secondary to the effects on the liver (increased weights). In dogs, decreases in total protein (less than 13%), albumin and globulin were observed in high dose animals in the 39-week study; these changes may have been secondary to decreases in food consumption in high dose animals. The relevance of these limited effects in high dose animals is questionable.

Results from a rat immunotoxicity study showed lower anti KLH IgG antibody titers in females at ≥ 50 mg/kg/day. There were no COBI-related changes in the anti KLH IgM antibody response at any dose in either sex. The NOEL for the T cell dependent antibody response is considered to be 20 mg/kg/day in females and 50 mg/kg/day in males. However, in standard 13-week mouse, 26-week rat and 39-week dog toxicity studies at doses up to 50, 100 and 20 mg/kg/day in mouse, rat and dog, respectively, microscopic changes suggestive of immunotoxicity were not been observed in lymphoid organs. Further, immunophenotyping of peripheral blood cells evaluated in the chronic rat and dog toxicity studies did not reveal any adverse effects, and there were no signs of potential immunosuppression as assessed by animal health status (ie, no signs of opportunistic infections) and clinical chemistry and hematological analyses. The clinical significance of the decrease in anti KLH IgG levels in a single study in female rats is unclear considering that no adverse effects on hematological parameters, IgG levels, or rate of infections considered related to study drug that could be suggestive of immunosuppression have been observed in clinical studies conducted with COBI, mean values for fasting glucose and lipid parameters remained in the normal range.

Liver effects in mice, rats, and dogs were qualitatively similar. In rodents, the predominant effects were increased weights, microsomal enzyme induction, and hepatocellular hypertrophy. In dogs, increased liver weights and hepatocellular hypertrophy were similarly observed. Elevations in liver enzyme levels (ALT and AST) were most prominent in mice, not notable in

rats, and only observed in high dose animals in the 4-week dog study (accompanied by hepatocyte vacuolation).

In the 13-week mouse study, mild to marked elevations in ALT and AST were noted in males at 15 and 50 mg/kg/day, respectively. These changes were associated with increases in liver weight, microsomal enzyme induction, and minimal hepatocellular hypertrophy at 50 mg/kg/day. Female mice were notably less sensitive; a marked elevation in ALT and AST was noted in only one high dose female (50 mg/kg/day). Similar liver findings were noted in the 4-week toxicity in HRAS wild type mice. In rats, increases in liver weights were associated with hepatocellular hypertrophy. These findings were reversible and were not considered adverse. The liver effects are considered adaptive changes, are commonly seen in rodents with microsomal enzyme inducers, and are considered secondary to microsomal enzyme induction [4, 14]. Cobicistat induces hepatic CYP3A activity in mice and in rats likely due to a species specific activation of rodent PXR (Section 3.3.2).

In dogs, minimal to mild increases in bilirubin, ALT, and ALP activities, increased liver weights, and hepatocyte vacuolation were noted after 4 weeks dosing at 45 or 45/30 mg/kg/day. In the 39-week study, there were no notable serum chemistry changes; however, minimal liver changes (hepatocellular hypertrophy) were observed in males at 10 mg/kg/day, and in both sexes at 20 mg/kg/day. These hepatic changes observed in the 39 week study are considered an adaptive response, and not adverse based on their minimal severity, the absence of degeneration, and their reversibility after cessation of dosing [4, 14].

Effects on the thyroid glands in rats in the 26-week study were characterized by decreases in T4 in males at 100 mg/kg/day, increases in TSH in 10 mg/kg/day females and in both sexes at 30 and 100 mg/kg/day, increased thyroid weights at 30 and 100 mg/kg/day, and thyroid follicular cell hypertrophy (in one female at 10 and 30 mg/kg/day, and in most male and female animals at 100 mg/kg/day). These findings were reversible and were not considered adverse. However, one high dose 100 mg/kg/day male in the 26-week study also had a follicular cell carcinoma in the thyroid.

In the 104-week rat carcinogenicity study, neoplastic findings in the thyroid consisted of significant positive trends in thyroid follicular adenoma, carcinoma, and combined adenoma and carcinoma in males and/or females, with significant increases in adenoma for males at 50 mg/kg/day, and in combined adenoma/carcinoma for males at ≥25 mg/kg/day and for females at 30 mg/kg/day. The increase in thyroid follicular tumors was associated with follicular cell hypertrophy at ≥25 and ≥15 mg/kg/day in males and females, respectively.

Thyroid effects were slightly more pronounced in females possibly due to the higher exposures achieved in this sex. There are no indications that COBI has a direct effect on the thyroid gland, or a particular affinity for thyroid tissue. These clinical and anatomic pathology changes are considered adaptive changes, secondary to hepatic microsomal enzyme induction and thyroid hormone imbalance. The thyroid effects are considered rodent specific, and predispose rats, but not humans, to thyroid neoplasms. It is unlikely that COBI presents a risk to the human thyroid

[5, 6, 10, 32, 33, 63]. No clinically relevant adverse effects on thyroid function have been observed in clinical studies conducted to date with COBI.

Urinalysis and urine chemistry changes, noted primarily in high-dose rats (at 100 mg/kg/day) and in dogs (at ≥30 mg/kg/day), included higher urine volume, lower urine specific gravity, and increases in electrolyte excretion. These changes showed no progression after long term dosing, were not associated with remarkable serum clinical chemistry changes, including serum electrolytes, serum creatinine and BUN, were without histopathological correlates, and were reversible. Although the mechanism associated with these urinalysis changes is not understood (there were no treatment-related changes in serum vasopressin or aldosterone levels in the 26-week rat study), similar findings have been reported with other structurally-related agents, including ATV [44] and RTV [7].

4.9.2.2. Assessment of Reproduction and Development Toxicology

No teratogenic effects were observed in rats and rabbit developmental toxicity studies with COBI. Exposures were approximately 1.7- and 4.1-fold higher, respectively, than human therapeutic exposure. In rats, ossification changes in the spinal column and sternebra of foetuses occurred at a dose that produced significant maternal toxicity.

4.9.2.3. Safety Margins

A comprehensive program of nonclinical studies with COBI has been conducted. In repeat-dose studies (up to 13 weeks in mice, up to 26 weeks in rats; up to 39 weeks in dogs), target organs identified were liver (mouse, rat, and dog) and thyroid (rat). The liver effects in mice and rats are considered adaptive changes, are commonly seen in rodents with microsomal enzyme inducers, and are considered secondary to microsomal enzyme induction [4, 14]. In dogs, the hepatic changes observed in the 39-week study are considered an adaptive response, and not adverse based on their minimal severity, the absence of degeneration, and their reversibility after cessation of dosing [4, 14]. The thyroid changes in rats are considered adaptive changes, secondary to hepatic microsomal enzyme induction and thyroid hormone imbalance [5, 6, 10, 32, 33, 63]. The thyroid effects are considered rodent specific and predispose rats, but not humans, to thyroid neoplasms.

These animal/human exposure ratios can be found in Table 4.

Table 4: Estimated Safety Margins for COBI 150 mg Based on Exposure (AUC) at Animal NOAEL

Species Gender	Study Type	NOAEL Dose (mg/kg/day)	AUC_{0-t} (µg.h/mL)	Safety Margin[a]
Mice				
Male - Female	104-week Carcinogenicity (week 29)	50 -100[b]	75 -174	8.6 -20 X
Rat				
Male - Female	26-week Toxicity	30	9.9 – 13.3	1.1 – 1.5 X
Male - Female	104-week Carcinogenicity (week 26)	50 – 30[b]	22.6 – 19.9	2.6 – 2.3 X
Female (Pf)	Embryo-Fetal Development (GD17)	50	14.8	1.7 X
Rabbit				
Female (Pf)	Embryo-Fetal Development (GD20)	100	35.7	4.1 X
Dog				
Male - Female	39-week Toxicity	10	19.6 – 16.8	2.2 – 1.9 X

COBI, cobicistat; NOAEL, no observed adverse effect level; Pf : pregnant female
[a]: Human AUC_{0-24h}=8.7 µg.h/mL (Mod5.3.5.1/GS-US-299-0102 PK substudy); [b]: the highest dose tested

While the safety margins are not large, effects above the NOAELs were minimal and some effects were species-specific. At doses above the NOAEL in male mice, liver changes (transaminase elevations and minimal hepatocellular hypertrophy) were observed; female mice were notably less sensitive. In rats, notable effects were limited to decreased body weight gain and food consumption, with slight changes in hematology, clinical chemistry, and urinalysis parameters, and adaptive liver and thyroid changes. In dogs, salivation and emesis, decreased body weight gain and food consumption, slight changes in some clinical chemistry parameters, and minimal adaptive changes in the liver were noted above the NOAEL.

4.9.3. Emtricitabine

4.9.3.1. Assessment of General Toxicity

The only significant effect of FTC identified at dose levels constituting large clinical multiples was a minor anemia.

4.9.3.2. Assessment of Genotoxicity and Carcinogenicity

No specific concerns were identified in the genotoxicity and carcinogenicity studies.

4.9.3.3. Assessment of Reproduction and Development Toxicology

Reproductive studies were conducted in rats, mice, and rabbits. Animal studies did not indicate harmful effects of FTC with respect to fertility, pregnancy, fetal parameters, parturition, or postnatal development.

4.9.3.4. Safety Margin

Cross-species comparisons of FTC exposure (expressed based on AUC_{ss} levels) for the major target organs are shown in Table 5.

The NOELs obtained in the toxicity studies represent systemic exposures in animals well in excess of those expected in humans administered the daily recommended dose of 200 mg.

Table 5: Estimated Safety Margins of Emtricitabine 200 mg Based on AUCss When Comparing Animal No-Effect-Level (NOEL)

Species Gender	Study Type	NOEL Dose (mg/kg/day)	AUC_{0-ss} (µg.h/mL)	Safety Margin[a]
Mice				
Male and Female	6 months	500	350[b]	167[b]
Male - Female	2 year Carcinogenicity (week 26)	750	287-322	24-27
Rat				
Male - Female	3 months	600	329-369	28- 31
Male - Female	2 year Carcinogenicity	600	327-404	27- 34
Female (Pf)	Embryo-Fetal Development (GD15 single dose)	1000	186°	16
Rabbit				
Female (Pf)	Embryo-Fetal Development (GD19)	100	87	7.3
Monkey				
Male - Female	52-week Toxicity (week 26)	200	97.2 – 97.8	8.1 – 8.2

NOEL, no observed effect level; Pf : pregnant female
[a]: Human AUC_{0-24h}=11.9 µg.h/mL (GS-US-299-0102 PK substudy); [b]: C_{max} (µg/mL) safety margin calculated based on human C_{max} =2.1 µg/mL (Mod5.3.5.1/GS-US-299-0102 PK substudy)

4.9.4. Tenofovir Alafenamide

4.9.4.1. Assessment of General Toxicity

4.9.4.1.1. Kidney

Renal tubular karyomegaly was observed in rats and dogs orally administered TAF. Focal areas of minimal renal cortical tubular basophilia and associated minimal nuclear karyomegaly were present in rats administered 400 mg/kg/day for 4 weeks and 100 mg/kg/day for 26 weeks. Renal tubular karyomegaly and/or basophilia were observed in dogs administered 3 and 10 mg/kg/day for 4 weeks and dogs administered 6 or 18/12 mg/kg/day for at least 13 weeks.

Renal cortical tubular degeneration/regeneration findings were limited to animals administered 6 or 18/12 mg/kg/day for at least 13 weeks in the 39-week dog toxicity study. Similar findings of renal cortical tubular degeneration/regeneration and karyomegaly were present in dogs administered 6 or 18/12 mg/kg/day for 39 weeks. These changes were minimal to slight in affected males and females at 6 mg/kg/day. In high-dose males (18/12 mg/kg/day) the severity ranged from mild to moderate. Similar lesions (karyomegaly and tubular degeneration) but of

only minimal severity were also present in 2 males administered 2 mg/kg/day of TAF for 39 weeks. After a 13-week recovery period, treatment-related histology changes were still observed in the kidney but were of reduced incidence and severity.

4.9.4.1.2. Bone

Atrophy of metaphyseal cancellous bone was observed in rats administered TAF at 100 mg/kg/day for 26 weeks. Tenofovir alafenamide also increased biochemical markers of bone turnover and decrease serum 1, 25-dihydroxyvitamin D_3 and 25-hydroxyvitamin D_3 in rats (≥25 mg/kg/day) and dogs (≥37.5 mg/kg/day for 6 days). In the 39-week dog study, bone mineral density changes at 18/12 mg/kg/day may have been secondary to body weight loss but these changes were accompanied by a slight but significant decrease in serum 1, 25-dihydroxyvitamin D_3 in males only and a significant increase in 25-hydroxyvitamin D_3 in females only.

4.9.4.1.3. Other

TAF administered by oral gavage for up to 13 weeks to mice at ≥10 mg/kg/day resulted in adverse degenerative (olfactory) and acute inflammatory (infiltrate neutrophil) changes in the nasal mucosa. Because these changes were not observed in rats, dogs or monkeys for longer durations of administration, the relevance to humans is unknown and the risk of nasal inflammation in humans is very low.

In the 39-week study in dogs, TAF had no discernible electrocardiographic effect at the low dose of 2 mg/kg/day. There was some evidence at 6 and 18/12 mg/kg/day for an effect to slightly prolong PR intervals (~13-24%) which was associated with significant decreases in T_3 [27, 53]. After the 13-week recovery period, serum T_3 values returned to levels similar to the control group animals at the end of the study. No PR prolongation or any change in ECG results occurred in the safety pharmacology study that evaluated a TAF dose up to 100 mg/kg or in the thorough QT study (Genvoya® NDA207561/GS-US-120-0107).

At 18/12 mg/kg/day in dogs, the highest dose tested, a minimal infiltration of histiocytes was present in some organs (eye (choroid plexus, ciliary body), lung, and spleen) in some animals. In-life ophthalmologic examinations were normal. The histiocytic infiltration observed in multiple organs was most likely an indirect drug effect due to general debilitation and was not observed in other repeat dose toxicity studies. There were no drug-related effects on ophthalmic exams or microscopic exams of ocular tissue observed in repeat dose toxicity studies in mice (up to 13 weeks), rats (up to 26 weeks), and nonhuman primates (4 weeks) or in the 4-week dog toxicology study. The minimal to slight infiltration of mononuclear cells in the ocular posterior uvea occurred only in dogs administered the highest dose of TAF where compound-related toxicities occurred on clinical condition, weight gain, serum chemistry, bone mineral density (BMD), and other organ histopathology. Distribution of ^{14}C-TAF to eyes has been assessed in mice, rats, and dogs. Melanin binding has specifically been assessed by comparing distribution in pigmented and non-pigmented mice (C57 black and CD-1, respectively) and rats (Long Evans and Sprague-Dawley, respectively). ^{14}C-TAF-related radioactivity distributed poorly to the eyes of rats and dogs (C_{max} in eyes <8% that observed in plasma). Transient exposure to low levels of ^{14}C-TAF-related radioactivity was observed in the eyes of rats decreasing to undetectable levels

at 8 hours postdose. No difference in distribution was observed between Sprague-Dawley and Long Evans rats, including in the skin and eyes, suggesting no binding to melanin. The distribution of ^{14}C-TAF to eyes in mice was higher than other species studied (C_{max} in eyes 15% - 20 % that observed in plasma). More persistent exposures in eye lens, eye uveal tract, and eyes were observed in C57 black mice compared to CD-1 mice. However, no difference in distribution between pigmented and nonpigmented skin was observed illustrating that ^{14}C-TAF-related radioactivity was not selectively associated with melanin-containing tissues. The posterior uveitis in dogs administered the highest dose of TAF occurred at 15- and 40-fold higher exposure to TAF and TFV, respectively, than that observed in human subjects administered a 10-mg dose of TAF and does not correlate with the tissue distribution where TAF was found to poorly penetrate across the blood brain and blood retinal barrier in dogs. Because TAF has poor penetration across the blood brain and blood retinal barrier in dogs it is unlikely that TAF directly caused the observed histiocytic infiltration in the posterior uvea. Based on the evidence from tissue distribution and toxicology studies, it was concluded that the risk of posterior uveitis in humans is very low.

4.9.4.2. Assessment of Genotoxicity and Carcinogenicity

TAF was not mutagenic or clastogenic. Because there is a lower TFV exposure in rats and mice after TAF compared to TDF, carcinogenicity studies were conducted only with TDF. TDF did not show any carcinogenic potential in a long-term oral carcinogenicity study in rats. A long-term oral carcinogenicity study in mice showed a low incidence of duodenal tumors, considered likely related to high local concentrations in the gastrointestinal tract at the high dose of 600 mg/kg/day. The mechanism of tumor formation in mice and potential relevance for humans are uncertain.

4.9.4.3. Assessment of Reproduction and Development Toxicology

Studies in animals have shown no evidence of teratogenicity or an effect on reproductive function. In offspring from rat and rabbit, dams treated with TAF during pregnancy, there were no toxicologically significant effects on developmental endpoints.

4.9.4.4. Safety Margins

Cross-species comparisons of exposure (expressed based on AUC_{ss} levels) for the major target organs are shown in Table 6.

Table 6: Estimated Safety Margins of TAF 10 mg Based on AUCss When Comparing Animal No-Adverse-Effect-Level (NOAEL)

Species Gender	Study /Dose duration	TAF NOAEL Dose (mg/kg/day)	AUC_{ss} (µg·h/mL) NOAEL	Margin Relative to Human AUC_{ss}
			TFV/ TAF	TFV[a]/TAF[b]
Mice				
Male and Female	13 Weeks	<10	<0.213/NC	<0.62/NA
Rat				
Male and Female	26-week Toxicity	25	3.8/NC	11/NA
Female (Pf)	Embryo-Fetal Development	84	17.4/0.2	51/1.5
Rabbit				
Female (Pf)	Embryo-Fetal Development	100	27.3/11	80/85
Dog				
Male and Female	39 weeks	2	1.2/0.08	3.5/0.62
Monkey				
Male and Female	4 weeks	≥30	≥5.9/1.0	>17/7.7

NOAEL, no observed adverse effect level; Pf : pregnant female

[a]: Human $AUC_{0\text{-}24h}$ of TAF =0.13 µg.h/mL Mod5.3.5.1/GS-US-299-0102 PK substudy; Human $AUC_{0\text{-}24h}$ of TFV =0.34 µg.h/mL Mod5.3.5.1/GS-US-299-0102 PK substudy

NA = not applicable; NC = insufficient data to calculate

5. INTEGRATED OVERVIEW AND CONCLUSIONS

This nonclinical overview supports the use of an FDC tablet including 800 mg DRV/150 mg COBI/ 200 mg FTC/10 mg TAF for the treatment of HIV-1 infection. A comprehensive package of nonclinical safety data is available for DRV, COBI, FTC and TAF.

After oral administration, DRV was rapidly absorbed and eliminated. Darunavir is primarily eliminated by metabolism via the CYP3A iso-enzyme. Several metabolic pathways were identified in vivo and they were qualitatively similar in tested species including human. Darunavir was an inhibitor of CYP3A4 at clinically relevant concentrations and also showed a concentration-dependent effect on CYP3A4 induction in vitro. This effect may have limited clinical relevance, as induction was significant in vitro at concentrations several fold higher than those attained in the clinic. Exposure in rodents including pregnant rats decreased following repeated oral administration due to the induction of CYP3A. RTV, a structural analogue of COBI and a potent MBI of CYP3A, had a modest effect on DRV exposure in mice (2-fold increase), rats (4-fold increase) including pregnant rats but had no clear effect in minipigs and dogs. The highest impact, however, was in rabbits where a 15-fold increase in exposure was seen. In humans, however, the impact was noticeable. Ritonavir markedly reduced the metabolism of DRV and consequently increased its oral bioavailability from 37% to 82%. The exposure to DRV in humans was equivalent when co-administered with COBI as a booster compared to RTV as a booster.

After moderate doses of COBI, oral bioavailability in nonclinical species was low due to metabolic instability and resulting high first-pass elimination. COBI is a MBI of human CYP3A enzymes. In rodents and dogs, COBI is a weak competitive inhibitor of CYP3A enzymes, and primarily acts as a species-specific CYP inducer in rodents via activation of PXR. Overall, the difference in boosting effect of COBI across species including human is similar to that for RTV.

Emtricitabine shows high passive permeability. Emtricitabine does not undergo extensive first-pass or systemic metabolism, and is eliminated primarily by renal excretion of unchanged drug. The total body clearance of FTC exceeds the glomerular filtration rate, suggesting the drug is actively secreted by renal tubules into the urine.

Tenofovir alafenamide is a prodrug of TFV which is intracellularly converted to its pharmacologically active TFV-DP by cellular enzymes including CatA in PBMCs or carboxylesterase 1 in hepatocytes. Although TAF was observed in mouse plasma in a dose dependent manner, the concentrations were limited. Tenofovir alafenamide was not detected in any of the rat studies. Consistent with dose-dependent permeability observed in vitro, the oral bioavailability of TAF increased with increasing dose in dogs and the observed oral bioavailability was 14.3% at the 10 mg/kg dose [1]. Hepatic extraction of TAF was estimated to be approximately 65% in dogs. Renal excretion is the primary systemic route of elimination of TFV in all preclinical species tested.

In vitro studies have indicated that the intracellular levels of TFV-DP and FTC triphosphate are unlikely to be affected by DRV and/or COBI. The major enzyme that converts TAF to TFV is CatA and carboxylesterase 1 in PBMCs and hepatocytes, respectively. Neither DRV nor COBI inhibits these enzymes in vitro. Further, the antiviral activity of TAF was not affected by DRV or COBI. Emtricitabine and TFV do not interact significantly with human drug-metabolizing CYP enzymes or UGTs as substrates, inhibitors, or inducers, in vitro or in humans in vivo. Low plasma concentrations of TAF make drug-drug interactions, through inhibition or induction of metabolism or transport, unlikely.
The only anticipated significant drug-drug interaction upon administration of the 4-drug combination is the intended inhibition of CYP3A activity by COBI and the consequent increase in DRV exposure. Cobicistat is also anticipated to increase the bioavailability of TAF through inhibition of intestinal P-gp. However, in clinic, the increase in TFV exposure, without a change in TAF exposure, upon multiple-dose coadministration of TAF with DRV/COBI suggests a mixed inhibitory/inductive effect on P-gp influencing TAF absorption (Mod2.7.2/Sec3.4.2.1). Additionally, the clinical drug-drug interaction potential of D/C/F/TAF is described in details in the Clinical Pharmacology summary (Mod2.7.2/Sec3.4.2.1).

In DRV repeat dose toxicity studies, the key target organs identified in rodents were the hematopoietic system, the blood coagulation system, liver, and thyroid. The effects observed in the liver and thyroid were consistent with the liver enzyme inducing property of DRV. Limited changes in liver were observed in dogs after 12 months of treatment. Given the species specificity of the effects, these findings are not of concern for humans. In a 6-month study in rats, the combination of DRV with RTV showed a small increase in effect on RBC count parameters, liver and thyroid in rats. These changes appear to reflect the sustained systemic

exposure to DRV in the presence of RTV, but were not of a different order of magnitude observed in rats with each compound alone. These hematological, liver, and thyroid changes appeared to be not clinically relevant, as in clinical practice, DRV in combination with RTV or COBI is generally safe and well tolerated in HIV-infected patients. Minor anemia changes related to FTC and COBI administration were identified at dose levels constituting multiples of clinical exposure; therefore, these hematological findings are not considered relevant to clinical use and should not cause an overlapping toxicity with DRV that produced minimal decreases (<10%) in RBC parameters. In COBI repeat dose toxicity rat studies, the slight hepatocellular hypertrophy and follicular cell hyperplasia/hypertrophy in the thyroid, and the one follicular cell carcinoma in the thyroid (one high-dose male animal in the 26-week study) are considered rodent-specific changes that are commonly seen with microsomal enzyme inducers. Given the species specificity of the effects, these findings are not of concern for humans. Minimal-to-mild increases in liver enzyme activities (ALT, bilirubin, and ALP) were observed in dogs after 4 weeks of dosing, and hepatocyte vacuolation and/or hypertrophy were observed in the liver of dogs treated with COBI in 4-week and 39-week studies. These findings do not indicate a safety concern for D/C/F/TAF FDC.

For COBI, decreased IgG levels were noted in female rats at dose levels (≥50 mg/kg/day) above the NOAEL for systemic toxicity in long term studies (30 mg/kg/day). However, in standard toxicity studies with COBI in mice (up to 13 weeks dosing), rats (up to 26 weeks dosing) and dogs (up to 39 weeks dosing), and at higher dose levels and exposures, no signs of immune function changes have been observed. Darunavir, FTC and TAF have not shown any potential for changes in the immune function, so these findings do not indicate a safety concern for D/C/F/TAF FDC.

Given the favorable toxicity profile of FTC and no evidence of overlapping toxicity, combinations with other agents are unlikely to induce new toxicities caused by FTC or to exacerbate the known toxicity of other agents (ie, DRV, COBI, and TAF).

In mice treated with TAF for up to 13 weeks, minimal to slight neutrophil infiltrates and minimal to moderate degeneration/regeneration of olfactory epithelium occurred in the nasal turbinates of males and females at ≥10 mg/kg/day. Because these changes were not observed in rats, dogs or monkeys or for longer duration of administration, the risk of nasal inflammation in humans is considered to be very low.

For TAF, kidney (renal tubular karyomegaly and tubular degeneration) and bone (atrophy of metaphyseal cancellous bone) were the primary target organs in rats and dogs. Tenofovir, the active metabolite of TAF, is associated with renal pathology (tubular degeneration) after high subcutaneous doses. Cobicistat was associated with urinalysis and urine chemistry changes (increased urine volume; decreased urine specific gravity; increased electrolyte excretions) at high doses in the 28-day and 26-week rat studies and in the 39-week dog study. These changes showed no progression after long-term dosing, were reversible, were not associated with remarkable clinical chemistry changes, including serum creatinine, and BUN, and were without morphological evidence of kidney damage. Cobicistat is associated with weak inhibition of human renal transporters OCT2, MRP4, and MATE2-K, and is a more potent inhibitor of

OCTN1 and MATE1, with similar potencies being found for RTV. In clinical studies, COBI has been showed to decrease CrCl due to inhibition of tubular secretion of creatinine without affecting glomerular function. In addition, there is no apparent pathological change in the kidney due to COBI and the routes of excretion differ for TFV and COBI, and COBI would not be expected to inhibit the renal transporters of TFV at clinically relevant concentrations. The potential of the 2 renally excreted compounds (FTC and TAF) to interact has not been tested but the combination of FTC and TDF did not exacerbate the renal toxicity of TDF when tested in a 4-week dog study. While the combination of D/C/F/TAF is not anticipated to have an effect on kidney function, monitoring of creatinine along with other standard clinical pathology tests is performed in clinical trials of the D/C/F/TAF FDC.

Darunavir, COBI, and FTC have not shown any potential for bone toxicity in chronic rat and dog toxicity studies; thus, exacerbation of any TAF effects on bone is not expected with the D/C/F/TAF FDC.

For TAF, at the highest dose tested (18/12 mg/kg/day) in dogs, a minimal infiltration of histiocytes was present in some organs (eye (choroid plexus, ciliary body), lung, and spleen) in some animals. In-life ophthalmologic examinations were normal. The histiocytic infiltration observed in multiple organs was most likely an indirect drug effect due to general debilitation and was not observed in other repeat dose toxicity studies. There were no drug-related effects on ophthalmic exams or microscopic exams of ocular tissue observed in repeat dose toxicity studies in mice (up to 13 weeks), rats (up to 26 weeks), and nonhuman primates (4 weeks) or in the 4-week dog toxicology study. The minimal to slight infiltration of mononuclear cells in the ocular posterior uvea occurred only in dogs administered the highest dose of TAF where compound-related toxicities occurred on clinical condition, weight gain, serum chemistry, BMD, and other organ histopathology. Distribution of ^{14}C-TAF to eyes has been assessed in mice, rats, and dogs. Melanin binding has specifically been assessed by comparing distribution in pigmented and non-pigmented mice (C57 black and CD-1, respectively) and rats (Long Evans and Sprague-Dawley, respectively). ^{14}C-TAF-related radioactivity distributed poorly to the eyes of rats and dogs (C_{max} in eyes <8% that observed in plasma). Transient exposure to low levels of ^{14}C-TAF-related radioactivity was observed in the eyes of rats decreasing to undetectable levels at 8 hours postdose. No difference in distribution was observed between Sprague-Dawley and Long Evans rats, including in the skin and eyes, suggesting no binding to melanin. The distribution of ^{14}C-TAF to eyes in mice was higher than other species studied (C_{max} in eyes 15%-20% that observed in plasma). More persistent exposures in eye lens, eye uveal tract, and eyes were observed in C57 black mice compared to CD-1 mice. However, no difference in distribution between pigmented and nonpigmented skin was observed illustrating that ^{14}C-TAF-related radioactivity was not selectively associated with melanin-containing tissues. The posterior uveitis in dogs administered the highest dose of TAF occurred at 15- and 40-fold higher exposure to TAF and TFV, respectively, than that observed in human subjects administered a 10-mg dose of TAF and does not correlate with the tissue distribution where TAF was found to poorly penetrate across the blood brain and blood retinal barrier in dogs. Because TAF has poor penetration across the blood brain and blood retinal barrier in dogs it is unlikely that TAF directly caused the observed histiocytic infiltration in the posterior uvea. Based on the

evidence from tissue distribution and toxicology studies, the risk of posterior uveitis in humans is very low.

None of the 4 components had positive findings in genotoxicity studies. The combination of the 4 components is not expected to have an altered genotoxicity profile as compared with that of the individual agents.

Carcinogenicity studies with DRV showed an increase in the incidence of hepatocellular adenomas and carcinomas in mice and rats and thyroid follicular cell adenomas in male rats, at exposure levels below those observed in humans at the recommended therapeutic doses. These findings are considered to be of limited to no relevance to humans based on mechanistic and epidemiological data. Long-term carcinogenicity studies with COBI in rats and mice did not reveal effects relevant for humans. Long-term carcinogenicity studies of FTC in rats and mice did not show any evidence of a carcinogenic effect. Because there is a lower TAF exposure in rats and mice after TAF compared to TDF, carcinogenicity studies were only conducted with TDF. After TDF administration, female mice showed a low incidence of duodenal tumors at the highest dose of 600 mg/kg/day. Rats did not show any carcinogenic potential in the long-term study. Although data is not available for all 4 compounds, it is considered unlikely that combination dosing would change these profiles based on the TAF chronic data and TDF carcinogenicity data.

No significant adverse effects have been observed in developmental toxicity studies with DRV, COBI, FTC, or TAF. No significant adverse effects have been observed in fertility toxicity studies with DRV, COBI, or FTC. The combination of the 4 components is not expected to have an altered reproductive toxicity profile based on the TAF chronic data and TDF reproductive toxicity data.

Overall, DRV safety pharmacology studies did not detect any significant nonclinical safety signals. Although COBI showed the potential to decrease LV function and prolong the PR interval in isolated rabbit hearts, the TQT study, and ECGs and echocardiograms conducted during clinical development did not reveal clinically-significant changes in these parameters. In the 39-week dog study, TAF had no discernible electrocardiographic effect at the low dose of 2 mg/kg/day. There was some evidence at 6 and 18/12 mg/kg/day for an effect to slightly prolong PR intervals (~13-24%) which was associated with significant decreases in T_3 [27, 53]. After the 13-week recovery period, serum T_3 values returned to levels similar to the control group animals at the end of the study. The thorough QT studies (NDA21976/TMC114-C153, NDA203094/GS-US-216-0107 and Genvoya® NDA207561/GS-US-120-0107) were interpreted as negative according to the E14 ICH Guidelines since the results demonstrated that administration of respectively DRV/rtv 1600/100 mg or 800/100 mg for 7 days, COBI doses of 250 mg or 400 mg, and TAF doses of 25 or 125 mg are not associated with a clinically relevant increase in QT interval. These results do not indicate a safety concern for the D/C/F/TAF FDC.

There was no evidence of toxicity to mitochondrial toxicity in vitro or in vivo for FTC and TAF. There is no safety concern for D/C/F/TAF FDC.

Based on the nonclinical program and clinical experience to date with all four compounds, no additive or synergistic toxicology/safety effects are expected for the D/C/F/TAF FDC beyond the expected PK boosting of DRV by COBI. In view of the established nonclinical profile for each of these compounds, new nonclinical studies to support the D/C/F/TAF FDC have not been conducted. The absence of nonclinical safety studies with the combination is in accordance with the FDA Guidance for Industry on the Nonclinical Safety Evaluation of Drug or Biologic Combinations (US Department of Health and Human Services, FDA, CDER, [redacted]).

In conclusion, the comprehensive preclinical programs of the individual agents DRV, COBI, FTC and TAF support the use of the D/C/F/TAF FDC tablet as proposed in the Prescribing Information in Mod1.14.1.

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