Question and Answer for the “Guideline on drug interaction for drug development and appropriate provision of information”

<Overall>

Q1. How should pharmacodynamic drug interactions be evaluated concretely?

A1. For pharmacodynamic drug interactions, there are no indices that can be used universally such as drug concentration, and therefore it is difficult to concretely describe general points to consider or what to determine for the evaluation. Basically, what to evaluate should be scrutinized and the necessity and implementation method of pharmacodynamic drug interaction studies should be considered according to the pharmacological mechanism of action or the expected administration method, clinical application, concomitant drugs, etc. with reference to the views described in section “1.3 Principles of drug interaction studies” of “Guideline on drug interaction for drug development and appropriate provision of information”.

<Metabolism>

Q2. What are the points to consider when calculating a contribution ratio (CR)?

A2. When estimating the contribution ratio of P450 isoenzymes from *in vitro* metabolism studies, it is generally studied by a test system using human liver microsomes, and the validity of the test system is usually confirmed by evaluating reaction time dependency and microsomal protein amount dependency, etc., using the rate of metabolite production as an indicator. Evaluation of CR by fm (fraction metabolized by the enzyme) by *in vitro* metabolism studies using human liver microsomes can be directly applied to oral drugs that are metabolized less in the small intestine and whose biliary and urinary excretion clearance as well as non-P450 metabolic clearance in the liver are negligible. Also, strict CR assessment is limited to cases where the degree of drug interaction can be simply calculated from the primary metabolic reaction. When contribution of membrane transport in the liver or extrahepatic loss is significant, fm may overestimate CR, so it is necessary to judge carefully the evaluation of CR. When the contribution ratio of P450isoenzymes differs depending on conditions of studies such as concentration of test drug used in *in vitro* metabolism test system, it is necessary to evaluate in consideration of *in vivo* conditions.

In the case of intravenous administration (injection drug), it is necessary to evaluate CR against not CL / F but whole body clearance (CL_{tot}) .

Q3. What are points to consider when identifying a drug-metabolizing enzyme in an *in vitro* metabolism studies?

A3. When conducting an *in vitro* metabolism studies, an experimental method, test system, appropriate substrate and interacting drug, and their concentrations which reflect the *in vivo* metabolic profile should be selected. Usually, depending on the type of the enzyme, an appropriate test system is selected from human liver and small intestinal microsomes, S9 fractions, human hepatocytes, and human enzyme expression systems, etc. P450s and UGTs exist in all of the above-mentioned systems excluding expression systems (Usually, only one type of enzyme is expressed at a high level in expression systems). Enzymes that exist in cytozols such as

sulfotransferases, glutathione transferases, aldehyde dehydrogenases, alcohol dehydrogenases, etc. are included in S9 fractions and hepatocytes. In hepatocytes, transporters are also expressed. When the results of studies are interpreted, the characteristics of *in vitro* test systems used should be sufficiently taken into consideration.

Usually, an *in vitro* metabolism studies are conducted with the use of a therapeutically relevant test drug concentration under linear conditions if possible. In multienzyme systems, it is possible to evaluate the contribution of each enzyme for the metabolism of a test drug by adding the selective inhibitor (See Table 1-2 in section 11.3 of "Guideline on drug interaction for drug development and appropriate provision of information") for each enzyme. If the specificity for an inhibitor is not high enough, it is recommended to use an *in vitro* test system in which metabolic enzymes other than particular one are not expressed. If an antibody is available whose specificity has been well-supported, it may be used as a substitute for an inhibitor. To specify major enzymes responsible for the metabolism *in vitro*, it is recommended to perform evaluations in multiple *in vitro* test systems and compare the results. In a correlative study comparing the metabolism of a test drug with the activity of a particular enzyme (metabolism of an index substrate) by using liver microsomes, etc. prepared from multiple individuals for the purpose of identifying isoenzyme which greatly contributes to the metabolism, the intensity of activity of various isoenzymes may be mutually correlated among individuals in some cases. If a correlation study is conducted out of necessity in such cases where no highly selective enzyme inhibitor is available, it is necessary to combine it with other methods for evaluation. One of available methods for evaluating the contribution ratio is RAF (relative activity factor) method where the metabolic activity by microsomes prepared from the expression cell systems for each P450 isoenzyme is corrected for its content of each P450 isoenzyme in the liver. But in general, the validation of the RAF method requires sufficient verification, and therefore it is necessary to combine it with other methods in a similar way.

In *in vitro* metabolism studies, the metabolic activity is determined as the elimination rate of the test drug or the formation rate of metabolites. When the activity of an enzyme which catalyzes a particular metabolic pathway is evaluated, it is recommended to investigate the dependence on the reaction time and microsomal protein content etc. based on the formation rate of metabolites rather than reductions in the test drug or index drug. On the other hand, when the purpose is to understand the contribution of the metabolic pathway in the overall elimination of the test drug, it is important to evaluate the contribution based on the elimination rate of the test drug.

Q4.	Please show test methods (dilution method, IC_{50} shift method, etc.) and cases of time dependent-inhibition (TDI).

A4. The IC_{50} shift method and dilution method are often used as test methods of TDI. For both of them, human liver microsomes are widely used as enzyme sources. The IC_{50} shift method is generally a method to investigate the presence or absence of changes in IC_{50} by pre-incubating with a test drug for about 30 minutes in the presence of NADPH. If a reduction in IC_{50} is observed following pre-incubation, the test drug will be judged to possibly show TDI. On the other hand, the dilution method is a method to evaluate TDI by investigating the inhibitory activity under the condition in which influences of reversible inhibition are

suppressed as much as possible by diluting the reaction mixture 10-fold or more after pre-incubation with the test drug in the presence of NADPH. In both methods, a concentration at which the metabolism of substrates is saturated (4-fold or more of K_m) is often used to make it easier to detect TDI even when the test drug or metabolites show strong reversible inhibition. The dilution method uses multiple pre-incubation time and test drug concentration conditions, thereby enabling the calculation of TDI parameters as maximum inactivation rate constant (k_{inact}) and concentration of the inhibitor that yields 50% of the maximum inactivation rate (K_I) to be used for the prediction of drug interactions (Apparent inactivation rate constant (k_{obs}) is obtained by linear regression from the negative slope in a natural logarithm plot of residual metabolic activity against the pre-incubation time, and k_{inact} and K_I are obtained by non-linear regression from a plot of k_{obs} against each test drug concentration). Although there are literature reports on the degradation rate constant (k_{deg}) of each P450 isoenzyme[1)], when referring to the reported value, , it is recommended to conduct the sensitivity analysis taking into consideration the range of the reported value and clarify the influence of the variability of k_{deg} on the estimation result. In addition, it is noted that enzymes present in both the intestine and the liver like CYP3A differ in k_{deg} depending on each tissue[2)].

A typical example of TDI is known as TDI of CYP3A by drugs such as ritonavir and saquinavir among HIV protease inhibitors, erythromycin and clarithromycin among macrolide antibiotics, and verapamil and diltiazem among calcium channel inhibitors[3)], and as TDI of CYP2D6 by paroxetine[4)]. In a similar way to the case of inducers, the effect of TDI reaches the maximum at the time point where enzymes subjected to the inhibition reach a new steady state level. This is dependent on the k_{deg} and k_{inact} of enzymes, but the inhibition can be intensified with time following repeated administration of an inhibitor and can often persist for a long period after discontinuation of administration of the inhibitor. For example, the inhibition of the CYP3A activity in humans, when erythromycin at 800 mg per day was repeatedly administered, reached the maximum after 4 days of administration[5)].

1) Yang J, Liao M, Shou M, Jamei M, Yeo KR, Tucker GT, Rostami-Hodjegan A.: Cytochrome P450 turnover: regulation of synthesis and degradation, methods for determining rates, and implications for the prediction of drug interactions. Curr Drug Metab. 2008;9:384-93.
2) Obach RS, Walsky RL, Venkatakrishnan K.: Mechanism-based inactivation of human cytochrome p450 enzymes and the prediction of drug-drug interactions. Drug Metab Dispos. 2007;35:246-55.
3) Zhao P, Lee CA, Kunze KL.: Sequential metabolism is responsible for diltiazem-induced time-dependent loss of CYP3A. Drug Metab Dispos. 2007;35:704-12.
4) Bertelsen KM, Venkatakrishnan K, Von Moltke LL, Obach RS, Greenblatt DJ.: Apparent mechanism-based inhibition of human CYP2D6 in vitro by paroxetine: comparison with fluoxetine and quinidine. Drug Metab Dispos. 2003;31:289-93.
5) Okudaira T, Kotegawa T, Imai H, Tsutsumi K, Nakano S, Ohashi K.: Effect of the treatment period with erythromycin on cytochrome P450 3A activity in humans. J Clin Pharmacol. 2007;47:871-76.

Q5. What are points to note for enzyme induction tests using hepatocytes?

A5. Since cultured human hepatocytes have great interindividual variability or lot-to-lot variation, it is desirable to use hepatocytes derived from 3 or more donors. Also, if the cell viability at the start of culture is clearly lower than 80% or the cell viability at the end of culture markedly decreases, a test should be conducted with the use of hepatocytes derived from new donors. In this test system, the exposure of the test drug is usually continued by changing the culture media containing the drug once a day. Usually, the necessity of a clinical study is judge based on the results with hepatocytes that show the most prominent inducing action. In the meanwhile, it should be confirmed that the cytotoxicity has not influenced the enzyme induction by appropriately evaluating the cell morphology or cell viability before culture and at the end of the culture period. If the toxicity or a reduction in viability is observed, its influence on the test results should be discussed. If a marked reduction in the drug concentration is expected due to the metabolism or degradation of the test drug under culture conditions or protein-binding, etc. in the culture media, it is recommended to understand the actual drug concentration through measurement of the test drug concentration or protein-binding rate in the culture media and increase the frequency of changing the culture media as necessary.

Q6. What are points to consider when making a judgment according to the cutoff value in enzyme induction tests?

A6. It is possible to determine an own cutoff value to make a judgment on the necessity of a clinical study for an enzyme induction evaluation, but in doing so, judgment should be made based on the result of use of inducers (positive control) and non-inducers (negative control) that have a sufficient amount of clinical evidence[6]. If the result with hepatocytes derived from at least one donor exceeds the pre-defined reference value, the drug is considered as an inducer and an additional evaluation should be performed. In the enzyme induction test, if it is judged that the conclusion can not be deduced in such cases as when the the test drug in the *in vitro* test can not be set to a high concentration due to its poor solubility or cytotoxicity, and it is difficult to calculate EC_{50} and Emax, the enzyme induction should be examined by clinical drug interaction studies if necessary.

6) Fahmi OA, Kish M, Boldt S, Obach RS.: Cytochrome P450 3A4 mRNA is a more reliable marker than CYP3A4 activity for detecting pregnane X receptor-activated induction of drug metabolizing enzymes. Drug Metab Dispos. 2010;38:1605-11.

Q7. Please indicate the jugdement criteria for the down-regulation of drug-metabolizing enzymes.

A7. When mRNA decreases versus the control group by 50% or more in a concentration-dependent manner and the decrease is considered not attributable to cytotoxicity in an *in vitro* enzyme induction test, the down-regulation of expression level of enzymes is generally suggested. As an example of down-regulation caused by drugs, there is a report that the clearance of phenytoin or warfarin decreased possibly because fluoropyrimidine drugs reduced the activity of CYP2C9, but the detailed mechanisms are unknown at present[7]. Under present circumstances, because knowledge about down regulation and expression mechanism caused by drugs is limited, it is recommended to examine in clinical drug interaction studies when concentration-dependent down regulation is observed *in vitro*.

7) Gilbar PJ, Brodribb TR.: Phenytoin and fluorouracil interaction. Ann Pharmacother. 2001;35:1367-70.

Q8. The determination criteria for metabolites to be evaluated include "Metabolites accounting for 10% or more of the total AUC of drug-related substances." How are the total AUC of drug-related substances and the metabolites accounting for 10% or more of the value calculated?

A8. "The total AUC of drug-related substances" means the sum of AUC of unchanged drug and all metabolites. It is possible to calculate the total AUC of drug-related substances based on the blood concentration profile of the total radioactivity obtained from a mass balance study (single-dose) using a radiolabeled materials in humans. Also, using the unlabeled drugs, the sum of the AUCs of unchanged and measurable metabolites can be used as a substitute for the total AUC of drug related substances. In this case, if the AUC of a particular metabolite is less than 10% of the sum of the AUCs of unchanged and other measurable metabolites, then that metabolite is considered not to exceed 10% of the total AUC of the drug-related substance. However, with the method using unlabeled drugs, it is difficult to obtain the AUC of all metabolites particularly for drugs having many metabolites.

Q9. It is described that if the contribution of main P450 isoenzymes is low, other P450 isoenzymes (e.g. CYP2A6, 2E1, 2J2, 4F2) should also be examined. Please exemplify substrate marker reactions and inhibitors of these isoenzymes for conducting an *in vitro* test.

A9. For CYP2A6 and 2E1, the following examples are known as substrate marker reactions and inhibitors in *in vitro* tests[8-13] . As for other isoenzymes, refer to latest published articles as there are few cases. .

Enzyme	Marker reaction	Inhibitor
CYP2A6	Coumarin 7-hydroxylation	Methoxsalen (8-Methoxypsoralen), Tranylcypromine
CYP2E1	Chlorzoxazone 6-hydroxylation	Diethyldithiocarbamate, Disulfiram, Tranylcypromine, Clomethiazole

8) Yuan R, Madani S, Wei X, Reynolds K, Huang S-M.: Evaluation of cytochrome P450 probe substrates commonly used by the pharmaceutical industry to study in vitro drug interactions. Drug Metab Dispos. 2002;30:1311-9.

9) Walsky RL, Obach RS.: Validated assays for human cytochrome P450 activities. Drug Metab Dispos. 2004;32:647-60.

10) Grimm SW, Einolf HJ, Hall SD, He K, Lim H-K, Ling KJ, Lu C, Nomeir AA, Seibert E, Skordos KW, Tonn GR, Van Horn R, Wang RW, Wong YN, Yang TJ, Obach RS.: The conduct of in vitro studies to address time-dependent inhibition of drug-metabolizing enzymes: a perspective of the Pharmaceutical Research and Manufacturers of America. Drug Metab Dispos. 2009;37:1355-70.

11) Fontana E, Dansette PM, Poli SM.: Cytochrome P450 enzymes mechanism based inhibitors: Common sub-

structures and reactivity. Curr Drug Metab. 2005;6:413-54.

12) Guengerich FP., Kim DH., Iwasaki M.: Role of human cytochrome P-450 IIEl in the oxidation of many low molecular weight cancer suspects. Chem. Res. Toxicol. 1991;4:168-79

13) Bjornsson TD, Callaghan JT, Einolf HJ, Fischer V, Gan L, Grimm S, Kao J, King SP, Miwa G, Ni L, Kumar G, McLeod J, Obach RS, Roberts S, Roe A, Shah A, Snikeris F, Sullivan JT, Tweedie D, Vega JM, Walsh J, Wrighton SA.: The conduct of in vitro and in vivo drug-drug interaction studies, A PhRMA perspective. J Clin Pharmacol. 2003;43:443-469.

Q10. What are points to consider when estimating the contribution ratio of UGT isoenzymes? Also, please exemplify concrete *in vitro* test systems and specific substrates.

A10. Because there have been no established standard investigation methods to estimate the contribution ratio of UGT isoenzymes and it may be difficult to estimate the contribution ratio, it is important to perform a multifaceted analysis while referring to latest published articles, etc. Take into consideration the two factors: The UGT activity is easily influenced by experimental conditions and the level of the UGT activity in extrahepatic tissues is relatively high. As a general method to estimate the contribution ratio of UGT isoenzymes, the method to estimate that of P450 isoenzymes can serve as a reference, but pay attention to the low substrate specificity among UGTisoenzymes. Examples of investigation methods include identifying the UGT isoenzymes that have the conjugation activity of a test drug by using expression systems for major isoenzymes (UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B7, 2B15, etc.) and subsequently combining the evaluation such as inhibition studies using human liver microsomes with available inhibitors (many of them are substrates with high selectivity or affinity for these isoenzymes) and correlation analysis where the conjugation activity with liver microsomes from multiple individuals are compared with the conjugation activity of substrates specific for each isoenzyme. It may be also useful to investigate similarities in enzyme kinetic parameters or inhibition constant between liver microsomes and UGT expression systems. Known substrates specific for each isoenzymes in humans include bilirubin or β-estradiol for UGT1A1, trifluoperazine for UGT1A4, propofol for UGT1A9, and morphine or zidovudine for UGT2B7[14)].

14) Miners JO, Mackenzie PI, Knights KM.: The prediction of drug-glucuronidation parameters in humans: UDP-glucuronosyltransferase enzymeselective substrate and inhibitor probes for reaction phenotyping and in vitro–in vivo extrapolation of drug clearance and drug-drug interaction potential. Drug Metab Rev. 2010;42:196-208.

Q11. Please show actual cases of interactions with biotechnological/biological products (Therapeutic proteins).

A11. The following examples as an interaction between biotechnological/biological products and drugs are reported. Cytokines such as IFN α -2b are thought to cause a decrease in the enzymatic activity of some P450 isoenzymes, thereby increasing the blood concentration of the corresponding P450 substrates[15)]. The decreased clearance of methotrexate is considered due to the reduction in the amount of antibody formed against the combined biotechnological/biological products by the immunosuppressive action of methotrexate[16)].

15) Islam M, Frye RF, Richards TJ, Sbeitan I, Donnelly SS, Glue P, Agarwala SS, Kirkwood JM.: Differential effect of IFN α -2b on the cytochrome P450 enzyme system: a potential basis of IFN toxicity and its modulation by other drugs. Clin Cancer Res. 2002;8:2480-7.
16) Seitz K, Zhou H.: Pharmacokinetic drug-drug interaction potentials for therapeutic monoclonal antibodies: reality check. J Clin Pharmacol. 2007;47:1104-18.

<Transporters>

Q12. About the evaluation of drug interactions via transporters:
[1] What are points to consider in a transport study using membrane vesicles?
[2] Should the inhibition of transporters by metabolites be also investigated?

A12.

[1] In a transport study using membrane vesicles, highly lipophilic drugs particularly tend to have an extensive non-specific adsorption/distribution of an investigational drug to the membrane, and consequently the transport via transporters may not be clearly observed. On the other hand, if the substrate of an efflux transporter is highly hydrophilic, the function of a transporter may be sufficiently observed. In this case, it is possible to perform a transport study using membrane vesicles. The method that observes the activity of ATPase as an alternative index for the transport (ATPase assay) which is a method known to evaluate the transport by ABC (ATP binding cassette) transporters may bring results different from the transport activity of transporters[17], and therefore the method should not be used in principle. IC_{50} value which is used in the decision tree for P-gp or BCRP is a value defined on the basis of the concentration of inhibitors in the medium for Caco-2 cells. It is fundamentally different from the K_i value defined on the basis of the concentration of unbound inhibitors inside the cells which is obtained from an inhibition experiment using membrane vesicles[18]. For this reason, when making a determination with the use of the decision tree for P-gp or BCRP (Fig. 2-3 of Section 11.2), it is not appropriate to directly use the K_i obtained from an inhibition experiment using membrane vesicles. Therefore, when the possibility of the investigational drug serving as the inhibitor of P-gp and BCRP is examined, it is desirable to conduct bidirectional transcellular transport studies using Caco-2 cells or other cell lines over-expressing particular transporters for in vitro experimental system, for the comparison of experimentally-obtained K_i value with cutoff value.

17) Adachi Y, Suzuki H, Sugiyama Y.: Comparative studies on in vitro methods for evaluating in vivo function of MDR1 P-glycoprotein. Pharm Res. 2001;18:1660-8.
18) Tachibana T, Kitamura S, Kato M, Mitsui T, Shirasaka Y, Yamashita S, Sugiyama Y.: Model analysis of the concentration-dependent permeability of P-gp substrates. Pharm Res. 2010;27:442-6.

[2] Cases where a metabolite caused clinically significant transporter inhibition are very limited[19,20], thus it is difficult to standardize the selection method of metabolites for which the transporter inhibition potency should be evaluated, but the possibility of drug interactions with the metabolites of the investigational drug should also

be studied if necessary, when the blood concentration of the metabolites is high, metabolites that may be harmful are produced, or metabolites with clinically significant pharmacological activity are produced.

19) Gertz M, Cartwright CM, Hobbs MJ, Kenworthy KE, Rowland M, Houston JB, Galetin A.: Cyclosporine inhibition of hepatic and intestinal CYP3A4, uptake and efflux transporters: application of PBPK modeling in the assessment of drug-drug interaction potential. Pharm Res. 2013;30:761-80.

20) Shitara Y, Hirano M, Sato H, Sugiyama Y.: Gemfibrozil and its glucuronide inhibit the organic anion transporting polypeptide 2 (OATP2/OATP1B1:SLC21A6)-mediated hepatic uptake and CYP2C8-mediated metabolism of cerivastatin: analysis of the mechanism of the clinically relevant drug-drug interaction between cerivastatin and gemfibrozil. J Pharmacol Exp Ther. 2004;311:228-36.

Q13. Inhibition of MATE1 and MATE2-K expressed on the luminal membrane of renal tubular epithelial cells is unlikely to be reflected in the blood concentration, but what are points to consider when conducting a clinical drug interaction study to investigate MATE1 and MATE2-K?

A13. In a clinical drug interaction study, it is desirable to also evaluate the renal clearance by measuring the cumulative amount of unchanged drug excreted in the urine in addition to the blood concentration, because MATE1 and MATE2-K are transporters involved in renal excretion. Inhibition of MATE1 and MATE2-K may cause elevations in the concentration in the kidneys without changes in the blood concentration or renal clearance, and therefore changes in clinical laboratory test results on renal function (BUN, cystatin C, creatinine, etc.) and findings about the safety can serve as reference for examining interactions in addition to the data of clinical drug interaction studies.

<Clinical drug interaction studies >

Q14. When an evaluation is performed through modeling and simulation at the time of determining the necessity of clinical drug interaction studies, what data should be presented in approval application to explain the appropriateness of the evaluation?

A14. The modeling and simulation performed must be objectively reproducible, and it should be described the explanation of the appropriateness of the model structure, the setting rationale for physiological parameters and drug dependent parameters and its accuracy, outputs of analysis, information of the reliability of the parameters obtained, and the results of sensitivity analysis. It should be disclosed the final model formula and the data and parameters used. Furthermore, based on the relevant regulatory documents regarding practical matters of the submission by electronic data at the time of application for approval, it should be considered to provide them by electronic medium. The information of the software used should be provided. It is necessary to specify the predefined model if used, and details of any changes in the model or the settings if exist.

Q15. What are points to consider when applying mechanistic static pharmacokinetic (MSPK) models?

A15. When clinical information of the test drug is poor, such as early in drug development, the risk of interaction is examined sensitively by modeling and simulation using the MSPK model with conservatively set

concentration of the test drug. In that case, it is allowed that there is a tendency to overestimate its influence. When the maximum interaction is estimated for a particular drug-metabolizing enzyme if a test drug is an interacting drug, set fm to be 1 in Formula 3 in Figure 1-2 Footnote g) in section 11.2 of "Drug interaction guideline for drug development and labeling recommendations". Also, if there is extrahepatic clearance for affected drugs such as urinary excretion, this should be taken into consideration theoretically when calculating AUCR, but in Formula 3, it is assumed that there is no such contribution to estimate the maximum interaction. Regarding the test drug concentration in a pharmacokinetic model such as MSPK model, the unbound blood concentration in the portal vein and the maximum concentration in the vicinity of the gastrointestinal epithelial cells are often used in consideration of risks as concentrations in regions where enzymes to be inhibited or induced are mainly present (in hepatocytes or gastrointestinal epithelial cells). $[I]_h$ or $[I]_{u,inlet,\ max}$ refers to unbound maximum blood concentration of inhibitors or inducer (at entrance to the liver). It can be conservatively estimated as $[I]_h = f_{u,b} \times ([I]_{max,b} + F_a \times F_g \times ka \times Dose/Q_H)$[21]. Here, F_a refers to the gastrointestinal absorption ratio or to be precise, the proportion of drugs reaching from the gastrointestinal lumens to gastrointestinal epithelial cells; F_g refers to the proportion of drugs reaching to the portal blood after absorption in the gastrointestinal epithelial cells; ka refers to the absorption rate constant; Q_H refers to the total hepatic blood flow (e.g. 97 L/hr/70 kg)[21]; $f_{u,b}$ refers to the unbound ratio in the blood; and $[I]_{max,b}$ refers to the maximum total blood concentration of inhibitors in the steady state (unbound + bound). If the protein binding ratio in the blood is high (99% or higher) and the reliability of the measured value is low, it is generally accepted as $f_{u,b} = 0.01$. There is a report of a method to estimate $[I]_g$ from $[I]_g = F_a \times ka \times Dose/Q_{en,}$ with the use of an hypothetical blood flow (Q_{en}, 18L/hr/70 kg)[23] to the gastrointestinal epithelial cells[24]. It is desirable to actually measure ka, but the maximary estimated value may be set to be 0.1/min. Regarding the method to estimate ka and F_g which is used, the validity for the method needs to be shown. A sensitivity analysis should be performed as necessary.

The parameters representating induction in the formula (B_h and B_g) should be evaluated after the lot of hepatocytes meet the criteria of validation. In the validation, for the target lot of hepatocytes to be used as an *in vitro* test system, measure the induction parameters (EC_{50} and E_{max}) of multiple control inducers with different induction potencies, and predict the *in vivo* clearance changes of the index drug (for example, midazolam). Compare the predicted induction potential and clearance changes of the index drug in clinical settings, and calculate the d value. Calculate AUCR based on the d value and the measured values of EC_{50} and E_{max} of the test drug. In this analysis, it is recommended to conservatively select parameters to be used.

21) Ito K, Chiba K, Horikawa M, Ishigami M, Mizuno N, Aoki J, Gotoh Y, Iwatsubo T, Kanamitsu S, Kato M, Kawahara I, Niinuma K, Nishino A, Sato N, Tsukamoto Y, Ueda K, Itoh T, Sugiyama Y.: Which concentration of the inhibitor should be used to predict in vivo drug interactions from in vitro data? AAPS PharmSci. 2002;4:53-60.

22) Yang J, Jamei M, Yeo KR, Rostami-Hodjegan A, Tucker GT.: Misuse of the well-stirred model of hepatic drug clearance. Drug Metab Dispos. 2007;35:501-2.

23) Yang J, Jamei M, Yeo KR, Tucker GT, Rostami-Hodjegan A: Prediction of intestinal first-pass drug

metabolism. Curr Drug Metab. 2007;8:676-84.

24) Rostami-Hodjegan A, Tucker GT.: ‘In silico' simulations to assess the ‘in vivo' consequences of ‘in vitro' metabolic drug-drug interactions. Drug Discov Today: Technol. 2004;1:441‐8.

Q16. What are points to consider when applying physiological pharmacokinetic (PBPK) models?

A16. It should be noted that the application of PBPK models are useful when the time course of the drug concentration of the test drug is known, so it is often the case that its its application is generally limited in the early stages of development in general. In cases when PBPK model analysis is applied for examination of the necessity of conducting additional clinical drug interaction studies with other concomitant medications after conducting one or some clinical drug interaction studies have been conducted, or for description of alerts in the package insert, the model should be validated based on clinical data. For those cases, it is important that the clearance pathway is quantitatively described correctly by the model. It may be validated by confirming that the model can predict clearance changes with clinically acceptable accuracy as for interaction with a strong inhibitor or a substrate susceptible to interaction, or for changes of pharmacokinetics due to polymorphism of drug metabolizing enzymes.

Q17. In regards to conducting clinical drug interaction studies with inducers, please explain the following points:

[1] It is described that the execution of a clinical drug interaction study may be judged by simulations based on results of the clinical drug interaction study with an inhibitor. What kind of evaluation should be considered in reality?

[2] Although it is desirable to use a strong inducer in choosing the inducer used in the clinical drug interaction study, it is stated that attention should be payed to the safety of the subjects maximaumly. Exposure of affected drugs reduce by concomitant use of the strong inducer drug, so unlike inhibitors, safety concerns do not increase. Why is there a need to use moderate or lower inducer drugs?

A17.

[1] Using the PBPK model constructed on the basis of existing findings including the results of clinical drug interaction studies with inhibitors, if it is possible to give a good explanation of the influence of inducing drugs on the pharmacokinetics of typical or interacting substrates, it is sometimes possible to consider the degree of drug interaction when inducing drug is used in combination by applying the model to the test drug.

[2] Since there are cases where safety due to an increase in metabolites should also be taken into consideration, it is described from the viewpoint of securing the safety of the subjects in clinical trials.

Q18. The timing of the clinical drug interaction study and the dietary conditions

[1] Isn’t it recommended to conduct a clinical drug interaction study until clinically recommended dosage or regimen (including formulations) are determined? Also, if the study is conducted before determination of dosage or regimen, is it possible to use study results obtained at different dosage or regimen for application?

[2] Upon execution of the clinical drug interaction study, what are points to consider for dietary conditions?

A18.

[1] If clinically recommended dose/regimen (including change to sustained release formulation etc.) are changed after conducting the clinical drug interaction study, drug interaction study with the changed dose/regimen is not always necessary. However, it is important to explain the degree of drug interaction in the changed dose/regimen by considering the influence of drug interaction using such as the PBPK model constructed on the basis of initial clinical drug interaction study.

[2] In the drug interaction study it is acceptable to conduct under either the fasting or postprandial conditions. However, for a case when the dietary condition for the most suitable absorption is different from the investigational drug and the concomitant drug, the dietary condition should be selected considering the characteristics of each drug such as poor solubility in order to allow reasonable interpretation of outcomes from the clinical drug interaction study.

Q19. What are points to consider for duration and timing of administration of drugs in clinical drug-drug interaction studies?

A19. If a drug is an inhibitor as well as an inducer of drug-metabolizing enzyme(s) such as ritonavir which is an inhibitor of CYP3A and also an inducer of CYP2C9 and some other enzymes, interactions observed may differ depending on the time of co-administration[24,25]. In such a case, it is recommended to set a sufficient administration period so that the expression level of the drug-metabolizing enzymes becomes the new steady state, and also to conduct a clinical drug-drug interaction study where the administration timings of the test drug and concomitant drug are changed as necessary and examine its influences.

Rifampicin is known as a strong inducer of drug-metabolizing enzymes including CYP3A, but is also an inhibitor of transporters including OATP1B1[26,27]. If a co-administration study is conducted for the purpose of investigating the inhibiting action of rifampicin on transporters, it is most appropriate that sampling for measuring the concentration of the test drug as an interacted drug be performed immediately after single-dose administration of rifampicin. On the other hand, if the purpose is to clearly show the influences of rifampicin as a strong enzyme inducer, the enzyme-inducing action may be underestimated due to the OATP1B1-inhibiting action of rifampicin, and therefore it is appropriate to measure the concentration of the test drug the next day after the last dose of rifampicin.

25) Foisy MM, Yakiwchuk EM, Hughes CA.: Induction effects of ritonavir: implications for drug interactions. Ann Pharmacother. 2008;42:1048-59.

26) Kirby BJ, Collier AC, Kharasch ED, Dixit V, Desai P, Whittington D, Thummel KE, Unadkat JD.: Complex drug interactions of HIV protease inhibitors 2: in vivo induction and in vitro to in vivo correlation of induction of cytochrome P450 1A2, 2B6, and 2C9 by ritonavir or nelfinavir. Drug Metab Dispos. 2011;39:2329-37.

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28) Reitman ML, Chu X, Cai X, Yabut J, Venkatasubramanian R, Zajic S, Stone JA, Ding Y, Witter R, Gibson C, Roupe K, Evers R, Wagner JA, Stoch A.: Rifampin's acute inhibitory and chronic inductive drug interactions: experimental and model-based approaches to drug-drug interaction trial design. Clin Pharmacol Ther. 2011;89:234-42.

Q20. What are points to note when selecting substrates for drug-metabolizing enzymes?

A20. If drugs to be combined with an investigational drug include a substrate with a narrow therapeutic range, there may be a serious safety concern even if increases in C_{max} or AUC are not large when combining with a P450 inhibitor. Typical examples of substrates with a narrow therapeutic range include warfarin, drugs that may cause torsade de pointes, almost all cytotoxic antineoplastic drugs, and aminoglycoside antibiotics. If it is assumed that an investigational drug will be combined with any of these substrates with a narrow therapeutic range, the necessity of a clinical drug-drug interaction study, the dose level and administration period of the substrate when a study is conducted should be considered from a safety standpoint.

Some of the index drugs that are used in clinical drug-drug interaction studies are a substrate of two or more molecular species of P450 or transporters. Pay attention to the fact that they are not a specific substrate. For example, omeprazole is a substrate of CYP2C19 but is also metabolized by CYP3A. If omeprazole is used as a substrate to evaluate the inhibition (induction) of CYP2C19, it is recommended to measure the levels of metabolites (hydroxy omeprazole via CYP2C19 and omeprazole sulfone via CYP3A) with the unchanged drug[28]. Also, repaglinide is used as an index drug for CYP2C8, but is also a substrate of OATP1B1, and therefore attention needs to be paid to the interpretation of results of interaction studies with drugs which inhibit the same transporter.

29) Michaud V, Ogburn E, Thong N, Aregbe AO, Quigg TC, Flockhart DA, Desta Z.: Induction of CYP2C19 and CYP3A activity following repeated administration of efavirenz in healthy volunteers. Clin Pharmacol Ther. 2012;91:475-82.

Q21. What points to consider for substrates to be used in cocktail substrate clinical studies.

A21. In general, cocktail substrate clinical studies are conducted to investigate actions shown *in vitro* in a similar way to general clinical drug interaction studies, but may be conducted for the purpose of evaluating the inhibition potency and induction potency of a wide variety of metabolites for drug-metabolizing enzymes (and transporters).

For substrates to be used in studies, it is necessary that their specificity has been clinically proven in a drug interaction study using a selective inhibitor against a particular drug-metabolizing enzyme (and transporter), or a pharmacogenetic study, etc. It is desirable that the validity of dose levels in the cocktail substrate clinical study be shown with no interactions among the substrates in humans, but if the C_{max} in the circulating blood or the estimated concentrations in the gastrointestinal tract are sufficiently lower compared to the K_m value for the drug-metabolizing enzyme (and transporter) to be evaluated, it can be considered that there are no

interactions among substrates.

If drug interactions are found in a cocktail substrate clinical study, it is necessary to conduct a usual clinical drug interaction study to quantitatively confirm the influences of combination of many substrates, the warranty of linearity, etc.

Q22. What are points to consider for evaluating clinical drug interaction studies in consideration of genetic polymorphisms?

A22. If isozymes which have defective activity due to a genetic polymorphism (e.g. CYP2C19 and CYP2D6)are substantially related to metabolic pathways, take into consideration that the contribution ratio may be substantially different in particular populations such as activity-deficient individuals. In cases where the degree of influence on drug interaction by gene polymorphism is expected to be large and there is a possibility of clinical problem, it is useful to add clinical drug interaction study considering gene polymorphism. With regard to the method of investigating drug interactions considering genetic polymorphisms, although specific requirements are not sought, in a trial design in which a genotype is specified and subjects are incorporated and stratified, it is easy to analyze the influence of the genotype on the pharmacokinetics of the investigational drug. Choose the appropriate examination method with reference to the latest published literature etc. When carrying out clinical drug interaction study considering genetic polymorphism, it is expected that blood concentration of drug in the metabolic deficient person will be high, considering the safety of subjects to the utmost. It is also useful to examine the possibility of affecting drug interaction by modeling and simulation.

< Information and alerts on drug interaction in package inserts >

Q23. Please show case examples of how to describe the “Interactions” section when calling attention to pharmacokinetic interactions via CYP3A in a package insert.

A23. Regarding the description of ‘‘Drugs’’ for a precaution for co-administration, it should be put an expression to categorize the item requiring a precaution for concomitant use such as “strong CYP3A inhibitor” and “drug metabolized by CYP3A,” and also should be put the representative nonproprietary name within such a category as an example (Refer to the case examples below). It should be categorized them appropriately to enable professionals involved in patient care in actual medical practice to be aware of that nonproprietary names of drugs are only representative examples and there are also other drugs requiring a precaution for concomitant use. Regarding how to categorize, if clinical symptoms and measures are the same, multiple categories of strength may be described collectively.

Regarding the description of ‘‘Drugs’’ for a contraindication for co-administration, it should be described the nonproprietary name and representative brand name of the drug as a contraindication for concomitant use, without the above-mentioned category (Refer to the case examples below).

<u><Case Example 1 (A drug inhibiting CYP3A)></u>

CONTRAINDICATIONS (Do not use in the following patients.)

Patients being treated with the following drugs: ○○○, △△△

INTERACTIONS

This drug is a strong inhibitor of CYP3A.

Contraindications For Co-Administration (Do not co-administer with the following drugs.)

Drugs	Signs, Symptoms, and Treatment	Mechanism and Risk Factors
○○○ (Representative brand name of ○○○) △△△ (Representative brand name of △△△)	-*Omitted*-	Metabolism of these drugs is inhibited by this drug that is a strong inhibitor of CYP3A.

Precautions For Co-Administration (Be careful about concomitant use)

Drugs	Signs, Symptoms, and Treatment	Mechanism and Risk Factors
Drug metabolized by CYP3A ●●● □□□ XXXX etc.	-*Omitted*-	Metabolism of these drugs is inhibited by this drug that is a strong inhibitor of CYP3A.

<Case Example 2-1 (A drug metabolized by CYP3A)>

CONTRAINDICATIONS (Do not use in the following patients.)

Patients being treated with ○○○

INTERACTIONS

This drug is mainly metabolized by CYP3A.

Contraindications For Co-Administration (Do not co-administer with the following drugs.)

Drugs	Signs, Symptoms, and Treatment	Mechanism and Risk Factors
○○○ (Representative brand name of ○○○)	-*Omitted*-	Metabolism of this drug is inhibited by ○○○ that is a strong inhibitor of CYP3A.

Precautions For Co-Administration (Be careful about concomitant use)

Drugs	Signs, Symptoms, and Treatment	Mechanism and Risk Factors
Strong CYP3A inhibitor ●●● □□□ XXXX etc.	-*Omitted*-	Metabolism of this drug is inhibited by these drugs that are inhibitors of CYP3A.

Moderate CYP3A inhibitor △△△ ■■■ □□□ etc.	*-Omitted-*	Metabolism of this drug is inhibited by these drugs that are inhibitors of CYP3A.
Strong CYP3A inducer ▲▲▲ ◇◇◇ ★★★ etc.	*-Omitted-*	Metabolism of this drug is accelerated by these drugs that are inducers of CYP3A.

<Case Example 2-2 (A drug mainly metabolized by CYP3A and partially metabolized by CYP2D6)>

CONTRAINDICATIONS (Do not use in the following patients.)

Patients being treated with ○○○

INTERACTIONS

This drug is mainly metabolized by CYP3A, and partially metabolized by CYP2D6.

Contraindications For Co-Administration (Do not co-administer with the following drugs.)

Drugs	Signs, Symptoms, and Treatment	Mechanism and Risk Factors
○○○ (Representative brand name of ○○○)	*-Omitted-*	Metabolism of this drug is inhibited by ○○○ that is a strong inhibitor of CYP3A.

Precautions For Co-Administration (Be careful about concomitant use)

Drugs	Signs, Symptoms, and Treatment	Mechanism and Risk Factors
Strong or moderate CYP3A inhibitor ●●● □□□ XXXX etc.	*-Omitted-*	Metabolism of this drug is inhibited by these drugs that are inhibitors of CYP3A.
CYP2D6 inhibitor ■■■ etc.	*-Omitted-*	Metabolism of this drug is inhibited by these drugs that are strong inhibitors of CYP2D6.
Strong CYP3A inducer ▲▲▲ ◇◇◇	*-Omitted-*	Metabolism of this drug is accelerated by these drugs that are inducers of CYP3A.

★★★ etc.		

Q24. Please show case examples of how to describe the "Interactions" section when calling attention to any type of pharmacokinetic interactions other than the interaction via CYP3A enzyme in a package insert.

A24. Regarding the description of "Drugs" for a precaution for concomitant use, when it is necessary to express the drugs requiring a precaution for concomitant use as a category such as "CYP2D6 inhibitor" and "drug metabolized by CYP1A2", etc. and there is an expression which can be appropriately categorized, put the expression, and then also should put the representative nonproprietary name within the category as an example. Put the category of strength of inhibition or induction in "Mechanism and Risk Factors" only when the description is particularly necessary (Refer to the case examples below). Regarding the description of "Drugs" for a contraindication for concomitant use, it should be described the nonproprietary name and representative brand name of the drug as a contraindication for concomitant use, without the category, similarly to pharmacokinetic interactions via CYP3A.

<Case Example 3 (A drug metabolized by CYP2D6 and inhibiting CYP1A2)>

INTERACTIONS

This drug is mainly metabolized by CYP2D6, and inhibits CYP1A2.

Precautions For Co-Administration (Be careful about concomitant use)

Drugs	Signs, Symptoms, and Treatment	Mechanism and Risk Factors
CYP2D6 inhibitor ●●● □□□ etc.	*-Omitted-*	Metabolism of this drug is inhibited by these drugs that are strong inhibitors of CYP2D6.
Drug metabolized by CYP1A2 ■■■ etc.	*-Omitted-*	Metabolism of drugs that are metabolized by CYP1A2 is inhibited by this drug that is a inhibitor of CYP1A2.

<Case Example 4 (A drug metabolized by CYP2B6 and CYP2C8)>

INTERACTIONS

This drug is metabolized by CYP2B6 and CYP2C8.

Precautions For Co-Administration (Be careful about concomitant use)

Drugs	Signs, Symptoms, and Treatment	Mechanism and Risk Factors
□□□	*-Omitted-*	Metabolism of this drug is inhibited by ☆☆☆ that is a

		inhibitor of CYP2B6 and CYP2C8.

<Case Example 5 (A drug inhibiting P-gp and metabolized by UGT1A1)>

INTERACTIONS

This drug is metabolized by UGT1A1, and has a P-gp-inhibiting action.

Precautions For Co-Administration (Be careful about concomitant use)

Drugs	Signs, Symptoms, and Treatment	Mechanism and Risk Factors
drug excreted by P-gp ★★★ etc.	-*Omitted*-	Because this drug inhibits P-gp.
□□□	-*Omitted*-	Metabolism of this drug is inhibited by □□□ that is a inhibitor of UGT1A1.

Q25. When an approximate estimate of contribution is described at the beginning of the section of the "INTERACTIONS" section in a package insert, how should it be described?

A25. It should be described such as "mainly metabolized by CYP○○ and partially metabolized by CYP▲▲," taking into account the *in vivo* contribution ratios of pathways that cause drug interactions (For example, calculate it considering Contribution Ratio, CR), etc. Regarding specific contribution ratios, etc., it is desirable to collectively provide the information in the section of "PHARMACOKINETICS," etc.