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「医薬品開発と適正な情報提供のための薬物相互作用ガイドライン」等の英文版の送付について

　標記について、別添１及び２のとおり取りまとめましたので、貴管下関係業者に対して周知方お願いします。

別添１　Guideline on drug interaction for drug development and appropriate provision of information

別添２　Question and Answer for the "Guideline on drug interaction for drug development and appropriate provision of information"

Guideline on drug interaction for drug development and appropriate provision of information

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1. Introduction

1.1 Background and objectives

It is common in clinical practice for several drugs to be prescribed concurrently to achieve a therapeutic goal, and under such circumstances, particular attention must be paid to possible interactions among the drugs. Drug interactions may cause serious adverse reactions or attenuation of the therapeutic effect. Therefore, it is necessary to appropriately evaluate the characteristics and severities of possible drug interactions, and to deal with such interactions so as to prevent any potential risk.

Evaluation of drug interactions during the drug development process requires stepwise accumulation of basic study data and accurate judgment depending on the situation. Thus, planned and systematic investigations are important. The purpose of this guideline is to provide general methods, criteria for judgment, and a general guide for interpretation of the study results and provision of information concerning nonclinical studies aimed at predicting drug interaction potentials and judging the need for implementation of clinical studies; it is also aimed at providing information concerning clinical studies carried out to confirm the presence or absence and degree of drug interactions in humans. If the possibility of drug interactions that may become major clinical problems is judged in the early development phase based on these guidelines, more efficient development of drugs is expected to become possible. In addition, adequate provision of information obtained during the drug development process to clinical practice may avoid the occurrence of adverse reactions based on drug interactions and/or decrease in the efficacy of drug therapy. These actions may be expected to lead to an optimized risk-benefit balance of drugs, eventually promoting the proper use of drugs.

This guideline presents general procedures that are considered to be scientifically valid at the present moment. However, because the physical and chemical properties, pharmacological actions, pharmacokinetics, and clinical usage vary among individual drugs, the methods of evaluation of drug interactions also vary among investigational drugs. Therefore, while implementing drug interaction studies, it is necessary to select the appropriate methods of study according to the properties of the drug in question, based on the principles described in these guidelines. In case of need, new methods of study and means of providing information derived from advances in academic studies and scientific technologies may also be evaluated and adopted proactively.

1.2 Scope

This guideline presents the principles and methods of drug interaction studies during the development of new drugs for appropriate provision of information on drug interaction studies and their results. This guideline applies to *in vitro* studies that are carried out in the early phase of drug development using

human tissue-derived specimen and expression systems of human drug metabolizing enzymes and transporters to predict drug interactions in humans and to judge the need to implement clinical studies. This guideline also applies to clinical drug interaction studies that are conducted as needed during drug development, drug interaction studies carried out as necessary after marketing, and provision of information about the results of such studies in the package inserts.

Drug interactions can occur in any route of administration. Although these guidelines mainly provide an outline of drug interactions following oral administration, other routes of administration are also dealt with as appropriate. Drug interactions associated with routes of administration other than the oral route should be studied with reference to this guideline, bearing in mind the fact that the degree of drug interactions would vary among different routes of administration.

This guideline defines drug interactions as interactions among the drugs administered concomitantly that may affect the beneficial effects, adverse effects, or pharmacokinetics of the drugs (including, biotechnological and biological products) and between the drug under study and foods, beverages, or nonessential grocery items (e.g., tobacco, alcohol, nutritional supplements).

Drug interactions are broadly classified into pharmacokinetic drug interactions and pharmacodynamic drug interactions, according to the mechanism of occurrence. The pharmacokinetic drug interactions are caused by changes in the blood concentrations or tissue distribution of the drugs or their metabolites resulting from interactions occurring in their absorption, distribution, metabolism, or excretion. The pharmacodynamic drug interactions result from overlapping or cancellation of pharmacological actions, or changes in the drug sensitivity due to concomitantly administered drugs. It is difficult for the present guideline to provide general procedures relevant to pharmacodynamic drug interactions. It is necessary to appropriately determine whether studies of pharmacodynamic drug interactions must be implemented according to the pharmacological actions of drugs and anticipated clinical indications. In this guideline, descriptions are focused on pharmacokinetic drug interactions mediated by general drug metabolizing enzymes or transporters. However, it should also be borne in mind that some drugs strongly inhibit enzymes other than the general drug metabolizing enzymes shown in this guideline, as in the case of harmful effects caused by concomitant use of sorivudine and fluorouracil-based anticancer drug, exerting inhibitory influences on the metabolic disposition of concomitantly used drugs that are metabolized by the enzymes other than general drug metabolizing enzymes, resulting in pharmacokinetic drug interactions.

1.3 Principles of drug interaction studies

Drug interactions between a drug under development (investigational drug) and approved drugs that may be used with the investigational drug should be studied from the two aspects, i.e., the case where the investigational drug is the "affected drug" (affected by concomitant drugs) and the case where the investigational drug is the "interacting drug" (drug affecting concomitant drugs). In general, to predict the clinical influences of drug interactions, it is necessary to quantitatively determine to what degree the interacting drug affects the activity of the major clearance pathway of the affected drug. Towards this objective, *in vitro* studies using human tissue-derived specimen and expression systems of drug metabolizing enzymes and transporters should be conducted to investigate the basic factors contributing to drug interactions and the possibility of drug interactions occurring in the clinical practice. Then clinical drug interaction studies should be performed to confirm the degree of the interaction. Finally, it is important to identify the interactions that should be avoided in the clinical practice that require special attention from among various combinations of drugs, based on the results of the clinical drug interaction studies and taking into account the effects on drug therapy. The information thus obtained should be appropriately provided to healthcare professionals.

Drug interaction studies are planned and implemented on the basis of the mechanism of drug interactions expected from preliminarily obtained physiological, chemical, pharmacological properties and pharmacokinetic properties of the investigational drugs. The results of *in vitro* studies and clinical drug interaction studies using strong inhibitors, etc., of drug metabolizing enzymes or transporters are useful for predicting interactions with other drugs that may be used concomitantly. The possibility of drug interactions with the metabolites of the investigational drug should also be studied if necessary, when the blood concentration of the unchanged drug is low, in contrast to high concentrations of metabolites, when metabolites that may be harmful are produced, or when metabolites with clinically significant pharmacological activity are produced. When the investigational drug is developed for the condition of using concomitantly with other drugs, as in the case of development of combination products or combination therapy, clinical drug interaction studies should be conducted in combination with investigational drugs and corresponding concomitant drugs, in principle.

Drug interaction studies in the drug development process should be carried out in a stepwise manner based on the phase of development. *In vitro* studies evaluating the possibility for the effects of concomitant drugs on the investigational drug and the effects of the investigational drug on concomitant drugs should be carried out before the implementation of studies in a large number of patients or long-term administration (usually before the initiation of phase III studies). Usually, prior to the initiation of phase I studies, the information of plasma (serum) protein binding and drug metabolism of the investigational drug should be obtained based on *in vitro* studies. In addition, it is desirable that the

results of clinical drug interaction studies and mass balance studies should be obtained before the initiation of phase III studies. Information obtained stepwise from results of nonclinical or clinical studies according to the aforementioned policies should be provided appropriately at the time of implementing later phase clinical studies by providing appropriate descriptions in investigational brochures.

At each stage of drug development, modeling and simulations using such models as the physiologically-based pharmacokinetic (PBPK) model may be useful for predicting the possibility of drug interactions and obtaining the information required for the necessity of implementation of clinical studies or their designs. In modeling and simulations, a full understanding of the model used and the simulation applied and verification of the reliability of modeling and simulation results are necessary according to the study purpose. In the case of using simulation results at the time of New Drug Application (NDA), it is necessary to justify the assumption concerning the setting of the model and information about the process of model building, and to show the validity of the modeling and simulation results from the physiological, medical and pharmaceutical viewpoints, as well as from the statistical viewpoint.

When the mechanism of the major drug interactions observed between the investigational drug and concomitant drugs in the clinical practice is inconclusive, it is recommended to elucidate the mechanism that produces such drug interactions by additional studies.

Clinical drug interaction studies should be conducted in compliance with Good Clinical Practice (Standards for the Conduct of Clinical Trials of Medical Products) (GCP), and pharmacokinetic drug interactions should be evaluated in accordance with "Clinical Pharmacokinetic Studies on Pharmaceuticals." (Notification No. 796 of the Evaluation and Licensing Division, PMSB, dated June 1, 2001)

2. Drug interactions in absorption

Drug interactions of concern involving the process of absorption from the gastrointestinal (GI) tract are mainly associated with investigational drugs that are administered orally. However, drug interactions of the same type in absorption should also be considered for drugs administered that may be absorbed from the GI tract after drug administration including by inhalation, nasal, or the buccal transmucosal route, etc.

Not only concomitant drugs, but also components of food and beverage may exert significant influences on the process of drug absorption. Many of these influences can be predicted qualitatively based on full understanding of physical and chemical properties and pharmacological actions of the drugs and formulations. The applicability of the following items 2.1-2.2 should be primarily considered. In

case pharmacokinetic changes not predictable from these items are observed, the causes of the changes should be investigated, including the possibility of drug interactions with drug metabolizing enzymes or transporters mentioned below, if necessary.

The influences of food on the drug absorption process should be examined using the final formulation of the drug, because the influences vary among different formulations. For the definition of the final formulation, "Clinical Pharmacokinetic Studies of Pharmaceuticals" (Notification No. 796 of the Evaluation and Licensing Division, PMSB, dated June 1, 2001) should be consulted.

2.1 Effects on gastrointestinal pH, complex/chelate formation, and solubility

2.1.1 Effects of concomitant drugs on the investigational drug

If the investigational drug has pH-dependent solubility, the need for implementation of clinical drug interaction studies with concomitant drugs that cause changes in the gastric pH (proton pump inhibitors, H_2 receptor antagonists, antacids, etc.) on the GI absorption should be considered.

In addition, because formation of complexes・chelates, micelles may occur as a result of the influence of concomitant drugs and components of food and beverage (e.g., calcium) that decrease or increase the GI absorption of the investigational drug, the possibility of a complex formation should be evaluated *in vitro* if necessary, based on the physical and chemical properties of the drug. Furthermore, if the possibility that the formation of complexes becoming a clinical concern is suggested by the physical and chemical properties and *in vitro* data, the need for implementation of clinical drug interaction studies with foods or/and beverages should be considered. In the case of pediatric drugs, the characteristics of the foods, such as ingestion of milk in neonates and infants should also be taken into consideration.

It is recommended that influence of meals is examined under the conditions that are most probable to cause drug interaction. For example, it should be borne in mind that drugs that are highly lipid-soluble and low solubility in the GI tract may show increased gastrointestinal absorption due to enhanced solubility in the GI tract caused by increased bile secretion after high-fat meal.

2.1.2 Effects of the investigational drug on concomitant drugs

If the investigational drug changes gastric pH, the need for clinical drug interaction studies should be considered after predicting the influences on the GI absorption of other drugs that have pH dependency. According to the chemical structure of the investigational drug, the possibility of other mechanisms, such as inhibition of absorption with complex formation, should also be considered.

2.2 Effects on gastrointestinal motility

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2.2.1 Effects of concomitant drugs on the investigational drug

Concomitant drugs that influence the gastric emptying rate (propantheline, metoclopramide, etc.) may change the rate of absorption of the investigational drug from the GI tract by affecting the dissolution rate of drug formulation and passing into the small intestine. In addition, ingestion of food and beverage delays absorption in the small intestine due to a delay in the gastric emptying rate in some cases. Among these, if pharmacokinetic changes associated with change in the area under the blood concentration time-curve (AUC) will be observed, attention should be paid to the possible influences on the metabolism of the investigational drug.

2.2.2 Effects of the investigational drug on concomitant drugs

The investigational drug that influences gastric emptying or intestinal motility could also affect the pharmacokinetics of other concomitant drugs. In this case, the possibility of occurrence of drug interactions of clinical concern should be considered, and if necessary, clinical drug interaction studies should be conducted using the appropriate marker drugs (e.g., acetaminophen as an index of the effect on gastric emptying). It should be borne in mind that such influences of the investigational drug on gastric emptying or intestinal motility can occur even if the investigational drug is administered by the parenteral route.

2.3 Drug interaction mediated by transporters in the gastrointestinal tract

Drugs that are absorbed by uptake transporters expressed on the luminal membrane of the intestinal epithelial cells may interact with other drugs or components of food and beverages that are subject to absorption by the same transporters, resulting in decreased absorption. Efflux transporters are expressed on the luminal membrane of intestinal epithelial cells, and some drugs taken up by epithelial cells from the intestinal lumen are sometimes pushed back to the luminal side of the gastrointestinal (GI) tract by efflux transporters before reaching the basal side (portal side). In some cases, certain drug interactions cause increased drug absorption through inhibition of these efflux transporters in the GI tract. In addition, some drugs may induce the expression of efflux transporters (P-glycoprotein [P-gp]) in the GI tract and decrease the absorption of other drugs.

P-gp and breast cancer resistance protein (BCRP) expressed on the luminal membrane of intestinal epithelial cells reduce GI absorption of substrates as efflux transporters. Meanwhile, concomitant use of the substrate of P-gp or BCRP and inhibitors may increase substrate absorption. Because of this, in principle, *in vitro* studies evaluating the possibility of serving as the substrate of P-gp or BCRP and

inhibitory effect of the investigational drug on P-gp or BCRP should be evaluated (see Section 6.2 and Figs. 2-1 to -3 of Section 11.2 for the procedure).

2.4 Drug interaction mediated by drug metabolizing enzymes in the gastrointestinal tract

CYP3A (CYP3A4 and CYP3A5), a molecular species of cytochrome P450 (P450), is abundantly expressed in the GI tract, particularly in the mucosa of the small intestine. In case of the investigational drug undergoing significant first-pass metabolism by CYP3A in the small intestine, concomitant drugs that inhibit CYP3A increase the bioavailability of the investigational drug. On the other hand, in case of CYP3A in the small intestine as well as in the liver being induced by concomitant drugs that induce CYP3A, the blood concentration of the investigational drug decreases. Therefore, the drug interactions in the small intestine should be evaluated if necessary, taking into account the degree of first-pass metabolism of the investigational drug (see Section 4 for the procedure and points to consider). On the other hand, in case of the investigational drug inhibiting CYP3A, the drug interaction should be investigated from the viewpoint of metabolism inhibition in the small intestine.

Influences of food and beverage components that inhibit CYP3A should also be taken into consideration. For example, grapefruit juice contains a substance that strongly inhibits CYP3A. It should be borne in mind that the bioavailability of oral drugs that are metabolized mainly by CYP3A could be increased when they were taken with grapefruit juice or after taking grapefruit juice.

Because the substrates of CYP3A are often also the substrates of P-gp, drug interactions should be evaluated bearing in mind the risk of interactions due to inhibition or induction of CYP3A and P-gp.

3. Drug interactions in tissue distribution

Many drugs bind to plasma proteins and proteins and/or other components in tissues. Since drugs are available in an unbound form for transport between the plasma and tissue, changes in the unbound fraction due to displacement of binding may lead to drug interactions. In the case of some drugs, transporters are involved in their distribution in tissues.

3.1 Plasma protein binding

Although the major protein to which drugs bind in the plasma is albumin, some drugs also bind to α_1-acid glycoprotein, lipoprotein, etc. If the investigational drug shows high binding ratios to plasma proteins (90% or higher) *in vitro*, it is necessary to identify the species of binding protein and the degree of binding.

One of the causes of changed distribution of the investigational drug due to drug interactions is displacement of the drug bound to plasma protein. In the presence of concomitant drugs that strongly bind to plasma protein, the investigational drug dissociate from the binding protein, resulting in an increase in unbound fraction of the investigational drug in plasma. Although, displacement of the investigational drug does not cause clinically significant changes in most cases because changes in unbound concentrations in plasma are small, significant drug interactions may occur between the investigational drug and concomitant drugs that strongly bind to plasma proteins in the cases of the investigational drug which has a plasma protein binding ratio of about 99% or higher, a narrow therapeutic range, and fulfills any one of the following conditions.

1) The investigational drug shows a small distribution volume. In this case, clearance of the drug and route of administration of the investigational drug are irrelevant.

2) The investigational drug is eliminated mainly via the liver with a high hepatic clearance, and is administered intravenously.

3) The investigational drug is eliminated mainly via the kidney, and its renal clearance is high. In this case, the route of administration is irrelevant.

On the other hand, attention must be paid that drugs that change pharmacokinetics of concomitant drugs through displacement of protein binding be limited to drugs that have at least similar level of plasma concentration compared to binding protein concentration.

3.2 Tissue distribution

In addition to drug interactions due to changes in binding to specific tissue components, we should bear in mind the possibility that the tissue distribution of the investigational drug might vary in response to inhibition or induction of uptake or efflux transporters expressed in each tissue.

3.2.1 Binding to specific tissue components

Some drugs specifically bind to receptors, proteins, lipid, etc., in tissues, and may be associated with drug interactions due to changes in the tissue concentration of the unbound form of the drug as a result of competition in binding.

3.2.2 Involvement of transporters in uptake and efflux in tissue

Uptake or efflux transporters are expressed in drug elimination organs such as the liver and kidney and barrier tissue in the brain, placenta, retina, etc. and are involved in the distribution of the drug in each tissue. When drug interactions involving the active transport process via transporters take place, the

unbound concentration of the drug in the tissue in question may be affected (the concentration is decreased by inhibition of uptake, and increased by inhibition of efflux), resulting in some changes in the pharmacological/adverse effects in the tissues.

Drug interactions involving tissue distribution do not necessarily reflect in changes in the plasma concentrations of the drugs. In particular, when drug interactions involving the active transport process via transporters take place only in the tissues with a small distribution volume relative to the distribution volume of the whole body, changes in the drug concentration in the tissue in question do not reflect in changes in the plasma concentrations of the drugs, necessitating careful attention be paid. On the other hand, when drug interactions occur in major distribution and excretion organs such as the liver and kidney, they may affect the distribution volume and systemic clearance of the drug, causing changes in the plasma concentrations of the drugs (See 5.1 and 5.2).

4. Drug interactions in drug metabolism

In drug interaction studies involving drug metabolism, it is important to identify the metabolic pathway associated with the interactions. When the investigational drug is the "affected drug", the importance of the metabolic pathway in the overall elimination pathway should be quantitatively determined. When the investigational drug is the "interacting drug", the effects on the metabolic activity should be evaluated considering the mechanism of the interactions such as inhibition and induction . Many of the drug interactions involving drug metabolism are related to oxidative metabolism, particularly by P450. Enzymes other than P450 such as UDP glucuronosyl transferase (UGT) are also known to be involved in drug interactions.

This section mainly discusses the possibility of drug interaction mediated by major primary P450 isoenzymes, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A (CYP3A4 and CYP3A5). For specific procedures related to interaction studies, see Section 4.1 to investigate whether the investigational drug can be metabolized by these isoenzymes and Sections 4.2 and 4.3 to investigate the possibility of inhibition and induction. Also, representative marker or index drugs that are recommended to be used when conducting *in vitro* metabolism studies and clinical drug interaction studies are also presented (see Tables 1-1 to 1-6 of Section 11.3).

When contributions of the major isoenzymes of P450 in the metabolism of the investigational drug is small, the possibility of drug interaction mediated by other isoenzymes of P450 (e.g., CYP2A6, CYP2E1, CYP2J2, CYP4F2) or enzymes other than P450 should be examined (see Section 4.4). Possibility of drug interaction caused by major metabolites of the investigational drug should also be evaluated in the same manner.

In drug metabolism, a single enzyme is frequently involved in the elimination of many drugs. In particular, the most important drug metabolizing enzyme, CYP3A, has a wide substrate specificity, and a very large number of drugs are the substrates for this enzyme. Therefore, it is difficult to carry out exhaustive clinical studies. Use of modeling and simulation based on the results of a relatively limited number of clinical drug interaction studies, with due consideration of the reliability, may be helpful.

4.1 Evaluation of the possibility of the investigational drug as an affected drug (see Figs. 1-1 of Section 11.2)

To investigate the possibility of the effects of an orally administered investigational drug being affected by other drugs, and to quantitatively evaluate the degree of contribution of drug interactions, an important factor is the *in vivo* contribution ratio (CR) of the pathway involved in the drug interactions to the clearance (CL/F) of the investigational drug after oral administration. If the major elimination pathway of the investigational drug is metabolism, drug metabolizing enzymes contributing highly should be identified and the degree of the contribution should be clarified as much as possible. When estimating the CR from *in vitro* metabolism studies, in general the fraction metabolized (fm) by the enzyme in question in human liver microsomes, etc., is used in substitution. When *in vivo* contribution ratio (maximum presumed values) in each (major) elimination pathway was calculated from the results of *in vitro* metabolism studies and clinical pharmacokinetic studies (e.g., mass balance studies, intravenous administration studies, etc.) and the CR of the elimination pathway controlled by a certain drug metabolizing enzyme to the overall elimination of the investigational drug is estimated to be 25% or more, implementation of clinical drug interaction studies using drugs that affect the enzyme in question (clinical index drugs: see Tables 1-5 and 1-6 of Section 11.3) should be considered. Even in the case of an orally administered drug in the clinical indication, implementation of intravenous administration study of the investigational drug make it possible to evaluate the contribution of hepatic metabolism and renal excretion to the total clearance of the drug.

In the implementation of clinical drug interaction studies, a strong inhibitor (see section 7.6 and Table 1-5 of Section 11.3) should be used first as much as possible to evaluate the degree of changes in the pharmacokinetics of the investigational drug. When the study results are judged to be negative or when the degree of interactions are minimal, the contribution of the enzyme in question to the overall elimination of the investigational drug is likely to be small, and thus there is no need to implement additional clinical drug interaction studies in most cases. On the other hand, when the results of the interaction study using a strong inhibitor have suggested that the investigational drug is affected by drug interactions that may require dose adjustment, the effects of other inhibitors in the same metabolic

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pathway should be evaluated in clinical drug interaction studies if necessary, taking into consideration the possibility that they are used concomitantly in clinical practice, or should be evaluated on the basis of data on cases of concomitant use in usual clinical studies. Clinical drug interaction studies with inducers are required when the risk of clinically significant drug interactions is inferred by modeling and simulations (e.g., if the validity of the PBPK model is confirmed and the results of the clinical studies can be described consistently by the model) or other procedures based on the results of clinical drug interaction studies with inhibitors.

4.2 Evaluation of the possibility of the investigational drug inhibiting drug metabolizing enzymes (see Figs. 1-2 of Section 11.2)

In vitro metabolism studies should be carried out to determine whether the investigational drug exerts an inhibitory effect on P450.

To determine whether or not clinical drug interaction studies should be conducted to examine the possibility of the investigational drug acting as an inhibitor, the ratio of intrinsic clearance value of the substrate (R value) under the presence and absence of the investigational drug for specific enzyme reaction (R value) should be calculated and compared with the cutoff values. If a value is over this criterion in the evaluation of the investigational drug, clinical studies should be conducted using a substrate that is susceptible to pharmacokinetic drug interactions (see Section 7.8 and Table 1-4 of Section 11.3). In addition to evaluation by the cutoff values, investigation using models are useful, such as the mechanistic static pharmacokinetics (MSPK) model, PBPK model, etc.

It is desirable to investigate the enzyme inhibition effects of major metabolites in addition to those of the unchanged drug. Target metabolites should be chosen based on the evaluation from the viewpoints of systemic exposure and chemical structure compared with the unchanged drug. When it is shown that a drug interactions observed in *in vivo* studies is attributable to a specific metabolite, implementation of *in vitro* enzyme inhibition studies using the metabolite would be helpful for designing clinical drug interaction studies and interpreting their results. Determination of the blood concentrations of the metabolite that is possibly related to the drug interaction is also recommended in clinical drug interaction studies.

4.3 Evaluation of the possibility of the investigational drug inducing drug metabolizing enzymes (see Figs. 1-3 of Section 11.2)

The investigational drug can cause induction or down-regulation of the drug metabolizing enzyme via influences on the nuclear receptors or other regulation pathways of P450 expression. Therefore, the

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possibility of drug interactions should be investigated. In general, the need for clinical drug interaction studies is examined based on the results of *in vitro* metabolism studies. Induction may be evaluated directly by clinical drug interaction studies in some cases.

Whether to implement clinical drug interaction studies to evaluate the possibility of the investigational drug acting as an inducer should be determined by the cutoff values based on *in vitro* data, etc. In addition to evaluation by the cutoff values, the MSPK model and PBPK model are also useful for evaluation.

4.4 Drug interactions mediated by enzymes other than cytochrome P450

Phase I enzymes (enzymes involved in oxidation, reduction, hydrolysis, ring closure and ring-cleavage reactions) other than P450 that are involved in drug metabolism include monoamine oxidase (MAO), flavin monooxygenase (FMO), xanthine oxidase (XO), aldehyde oxidase (AO), alcohol dehydrogenase, and aldehyde dehydrogenase. When the investigational drug is the substrate of these phase I enzymes other than P450 and their contribution to the clearance is large, identification of the enzymes/isoenzymes involved and determination of the degree of contribution are also recommended. The possibility of the investigational drug working as a substrate for these drug metabolizing enzymes may be evaluable based on the previous available findings in the same class of drugs or compounds with a similar structure in some cases.

As for phase II enzymes, if the investigational drug is mainly metabolized by UGT, the degree of contribution of the isoenzymes mainly involved in its elimination should be investigated (see Figure 1-1 of Section 11.2). In this case, examination of the inhibitory effect of the investigational drug is recommended not only on the isoenzymes of UGT mainly involved in its metabolism but also on isoenzymes known to be involved in metabolism of a relatively large number of medical products (e.g., UGT1A1 and UGT2B7) (see Figure 1-2 of Section 11.2).

Bearing in mind the examples of serious adverse effects observed in the concomitant use of sorivudine and fluorouracil-based anticancer drugs, if the degree of contribution of the enzymes other than general drug metabolizing enzymes to the major metabolic pathways of the drugs that are co-administered with the investigational drug is large, the inhibitory effects of the investigational drug and its metabolites on the corresponding enzymes should be examined. The need to implement clinical drug interaction studies based on the results of the above studies can be evaluate in accordance with the case for P450. In that case, the feasibility of clinical drug interaction studies should be judged by the presence/absence of known inhibitors and inducers.

4.5 Drug interactions with biotechnological/biological products (Therapeutic proteins)

In general, biological products are eliminated through internalization and degradation in lysosomes of the target cells, after binding with specific receptors on the cell surface. Therefore, the possibility of pharmacokinetic drug interactions between biological products and concomitant drugs is considered to be limited.

If the investigational drug is a cytokine or a cytokine modifier, the need for implementation of clinical drug interaction studies to evaluate the effects of the investigational drug on P450 or transporter should be considered, from the viewpoint of the efficacy and safety of the investigational drug and concomitant drugs. When a clinically significant drug interaction has been reported and the mechanism of the pharmacokinetic or pharmacodynamics drug interactions has been identified for the same class of drugs of the investigational drug, with clinical drug interactions having been reported, clinical drug interaction studies should be carried out to examine the possibility of the drug interactions in question. With regard to combination therapy indicated in the drug labeling, etc., biological products that are to be used concomitantly with other drugs (low-molecular-weight medical products or biological products) should be evaluated in clinical studies, if necessary, for the possibility of interactions between the concomitantly used drugs, and evaluation on not only pharmacokinetic drug interactions, but also pharmacodynamic drug interactions should be considered.

5. Drug interactions in excretion
5.1 Drug interactions in urinary excretion

Renal clearance is determined by glomerular filtration, renal secretion from the blood to the urine and reabsorption from the urine to the blood at the renal tubules. Active transport mediated by transporters is known to be involved in secretion and reabsorption in the renal tubules. Therefore, drug interactions may occur at each process if drugs are actively secreted into renal tubules or reabsorbed at the renal tubules via transporters. In addition, drugs with low polarity are likely to be reabsorbed passively based on the physicochemical properties of the drugs in general and tend not to be easily excreted into urine. Weakly acidic or weakly basic drugs that are highly reabsorbed may affect the permeability from the urine and exhibit drug interactions caused by an alteration in their renal clearance due to changes in the ratio of non-ionic and ionic forms of drugs in the urine, when administered with drugs that alter the urinary pH. In patients with decreased renal clearance due to renal disease or aging, higher blood concentrations are often observed for drugs mainly excreted into urine compared with those in healthy adults with normal renal clearance. Therefore, particular caution is necessary to watch for enhancement of the drug efficacy or the occurrence of adverse effects associated with further increase in the blood concentrations resulting from drug interactions in the urinary excretion process.

Organic anion transporter (OAT) 1, OAT3 and organic cation transporter (OCT) 2 are transporters expressed on the basolateral membrane of the proximal tubular epithelial cells and transport drugs from the blood into the proximal tubular epithelial cells. If these transporters are inhibited, the blood concentration of substrates for these transporters may be elevated. P-gp, BCRP, multidrug and toxin extrusion (MATE) 1 and MATE2-K are transporters expressed on the brush border membrane that mediate excretion of drugs from the proximal tubular epithelial cells into the urine. When these transporters are inhibited, the blood concentration of drugs may be elevated, or in some cases, the concentration of drugs in the proximal tubular epithelial cells may be increased although there is no change in the blood concentration of drugs. It should be judged whether the investigational drug is a substrate or an inhibitor of these transporters, and whether clinical drug interaction studies are necessary (see Figs. 2-2, 2-3, 2-6, 2-7 of Section 11.2). As for other transporters that may contribute to urinary excretion, multidrug resistance-associated protein (MRP)2 and MRP4 mediate excretion of drugs from the proximal tubular epithelial cells into the urine.

5.2 Drug interactions in hepatobiliary transport

While transporters expressed on the sinusoidal membrane of the hepatocytes are involved in the drug uptake from the blood to the hepatocytes, transporters expressed on the bile canalicular membrane are involved in the efflux of parent drugs and/or their metabolites such as conjugated metabolites. Thus, the coadministration of drugs which affect the functions of these transporters may result in the drug interactions.

If OATP1B1 and OATP1B3, which are transporters expressed on the sinusoidal membrane of the hepatocytes and take up drugs from the blood into the hepatocytes, are inhibited, blood concentrations of substrates of these transporters may be elevated. It should be judged whether the investigational drug is a substrate or an inhibitor of these transporters, and whether clinical drug interaction studies are needed (see Figs. 2-4, 2-5 of Section 11.2). Drug interactions may occur in the presence of concomitant drugs affecting the activity of biliary excretion transporters on the canalicular membrane. In addition, as for transporters that mediate hepatic uptake and biliary excretion, OCT1 is expressed on the sinusoidal membrane of the hepatocytes and transports drugs from the blood into the hepatocytes and MRP2 is expressed on the canalicular membrane of the hepatocytes and mediates excretion of drugs from the hepatocytes into bile. If efflux transporters such as MRP2 are inhibited, the concentrations of drugs in the hepatocytes may be increased, although there is no change in the blood concentration of drugs. Furthermore, in the case of OATPs, MRP2 and bile salt export pump (BSEP) involved in the biliary excretion of endogenous substances such as bile acids and bilirubin, it is possible that their inhibition by

drugs increases the blood and tissue concentration of endogenous substances. Conjugates such as glucuronic acid conjugate are often excreted into the bile, deconjugated by enteric bacteria, and then reabsorbed from the GI tract as the unchanged drug (enterohepatic circulation). Drug interactions occurring during biliary excretion of conjugates may affect the residence time and AUC of the unchanged drug in the plasma.

6. Drug interaction studies mediated by transporters

6.1 General considerations in *in vitro* studies

For evaluation of transport using *in vitro* experimental systems for transporters, assessments with the use of typical substrates and typical inhibitors (see Tables 2-1, 2-2 of Section 11.3) should be carried out and a study of an investigational drug should be conducted with an experimental system that was confirmed to have a sufficient transport activity of the transporter in question.

Whether to implement clinical drug interaction studies to evaluate the possibility of the investigational drug is a substrate or an inhibitor of transporters should be determined according to the cutoff values based on *in vitro* data, etc. (see Fig. 2-1～2-7 of Section 11.2). Due to limited information on transporters compared with P450, the evaluation of transporters should be performed in consideration that the cutoff values may be changed based on future accumulation of scientific knowledge.

For evaluation of drug interactions mediated by transporters, findings reported for drugs that are similar in chemical structure to the investigational drug are informative. Because metabolites may cause interactions with concomitant drugs, evaluation of transporter-mediated drug interactions by metabolites may also be considered as necessary.

Necessity of *in vitro* studies using Caco-2 (for evaluation of intestinal absorption), transporter-expressing cell lines, other appropriate cells and membrane vesicles to determine the contributing transporter or the degree of contribution should also be considered when it is suggested that a transporter inform other than the isoforms which are described below to be evaluated has the major contribution to the intestinal absorption or clearance of the investigational drug, or when it is suggested that a transporter isoform other than the isoforms which are described below to be evaluated is inhibited by the investigational drug and thus intestinal absorption or clearance of concomitant drugs is affected.

6.2 Studies to examine drug interactions mediated by transporters involved in absorption（see Fig. 2-1~2-3 of Section 11.2）

Both P-gp and BCRP are important transporters that are expressed in the GI tract and may affect variations in the oral bioavailability. Because of this, the possibility of working as a substrate of P-gp

and BCRP should be evaluated for all investigational drugs by *in vitro* studies in principle. Because these transporters are also expressed in the liver, kidney and brain, they can affect the elimination and brain distribution of drugs. Therefore, drugs whose routes of administration are other than oral route need to be examined in some cases.

When examining the possibility that the investigational drug is a substrate or an inhibitor of P-gp and BCRP, *in vitro* studies should be performed using the experimental system with the transporter activity checked by using typical substrates and typical inhibitors (see Tables 2-1 and 2-2 of Section 11.3). For *in vitro* experimental systems, it is desirable to conduct bidirectional transcellular transport studies using Caco-2 cells or other cell lines over-expressing particular transporters. When conducting bidirectional transcellular transport studies, determination of the recoveries of the added drug on the acceptor side and on the donor side is also recommended.

Several types of transporters such as P-gp, BCRP and MRP2 are expressed in Caco-2 cells, involvement of each transporter can be qualitatively evaluated if typical inhibitors for the respective transporters can be used. If the use of typical inhibitors is not feasible, the cell lines overexpressing particular transporters can be used.

6.3 Studies to examine drug interactions mediated by transporters in the liver（see Fig. 2-1, 2-4 and 2-5 of Section 11.2）

Investigational drugs which are mainly eliminated via hepatic metabolism or biliary excretion (i.e., clearance via either route accounting for 25% or more of the total clearance) should be assessed to examine whether they are substrates for the hepatic uptake transporters OATP1B1 and 1B3.

When examining the possibility that the investigational drug is a substrate or an inhibitor of OATP1B1 and OATP1B3, *in vitro* studies should be performed using the experimental system whose transport activity is confirmed using typical substrates and typical inhibitors (see Tables 2-1 and 2-2 of Section 11.3). As i*n vitro* experimental systems, cell lines expressing OATP1B1 and OATP1B3 or hepatocytes can be used.

6.4 Studies to examine drug interactions mediated by transporters in the kidney（see Fig. 2-1, 2-6 and 2-7 of Section 11.2）

Investigational drugs whose major route of elimination is active renal secretion (renal secretion clearance accounting for 25% or more of the total clearance), should be assessed to examine whether they are substrates of OAT1, OAT3, OCT2, MATE1 or MATE2-K.

When examining the possibility that the investigational drug is a substrate or an inhibitor of OAT1, OAT3, OCT2, MATE1, and MATE2-K, *in vitro* studies should be performed using an experimental system whose transport activity is confirmed using typical substrates and typical inhibitors (see Tables 2-1 and 2-2 of Section 11.3). As i*n vitro* experimental systems, cell lines expressing OAT1, OAT3, OCT2, MATE1, and MATE2-K can be used.

7. Evaluation by clinical drug interaction studies

Clinical drug interaction studies should be carried out ethically and scientifically. It is important to obtain sufficient information from *in vitro* studies using human tissue-derived samples and expression systems of drug-metabolizing enzymes and transporters, and to implement clinical drug interaction studies efficiently while securing the safety of the subjects. For prediction of the drug interactions in humans based on the results of *in vitro* studies, etc., the modeling and simulation techniques are useful. And it is also useful to refer the data of the same class of drugs or the data of drugs with drug interactions of the same mechanism. For clinical drug interaction studies, it is necessary to prepare the study plan considering the safety of the subjects primarily, bearing in mind the adverse effects which are caused by the drug interactions.

7.1 Need for and timing of clinical drug interaction studies

When the possibility of drug interactions has been suggested for the investigational drug, it is recommended that a clinical drug interaction study(s) is conducted mainly in healthy volunteers, prior to phase III studies, in principle. Drug interaction studies should be conducted using clinical doses of the investigational drug, clinical index drug, inhibitor, and inducer. If significant drug interaction is observed, the drug interaction(s) with other drugs which would be frequently co-administered with the investigational drug should be investigated as needed considering their characteristics and the possibility of the drug interaction. If the investigational drug is developed for the objective of being used concomitantly with other drugs, as in the cases of development of combination products, and development related to indications for combination therapy, etc., clinical drug interaction studies should be carried out in combination with investigational drugs and corresponding concomitant drugs, in principle.

The results of clinical drug interaction studies are utilized for determination of the protocols for later phase clinical studies to consider the appropriate conditions of concomitant drugs. If *in vitro* drug interaction studies suggest the possibility of interaction with a concomitant drug, use of the concomitant drug in question should be contraindicated in principle, until its safety is demonstrated by a clinical drug

interaction study, etc. It may be useful to obtain information on interactions with concomitantly used drugs by population pharmacokinetic analysis to examine the influences of drug interactions in phase II or III studies for prediction of the pharmacokinetics taking into account inter-individual variations and evaluating the pharmacokinetics, efficacy, and safety of the investigational drug. If new scientific evidences on drug interaction are obtained after the drug approval, clinical drug interaction studies should be considered as needed.

7.2 Relevant indices of drug interactions and pharmacokinetic parameters to be evaluated

To evaluate the drug interactions quantitatively, the AUC of the investigational drug or the concomitant drug should be evaluated. Evaluation of the drug efficacy and adverse effects would provide useful indices for drug interactions for combinations with same concomitant drugs.

The presence/absence of drug interaction should be judged based on the results of clinical drug interaction studies, in terms of the 90% confidence interval of the geometric mean ratio of pharmacokinetic parameters obtained with and without co-administration of the interacting drug. When the 90% confidence interval of the geometric mean ratio falls in the range of 0.80-1.25, it is generally judged that there are no pharmacokinetic interactions between the drugs in question. Whether or not the above value does falls in the range, it should also be considered whether the drug interaction is a clinical concern based on the safety profile observed in clinical studies of the investigational drug. Also, the effects of interactions on pharmacokinetic parameters of the investigational drug and the concomitant drug, such as the C_{max}, trough concentration, time to reach C_{max} (t_{max}), clearance, distribution volume and half-life should be evaluated as needed.

If there is the possibility of clinically important drug interactions, information and alerts on drug interactions should be judged by consulting Chapter 8.

7.3 Study design

Clinical drug interaction studies are carried out using randomized cross-over design, add-on design (Evaluate the pharmacokinetics when the test drug is administered as a single agent first, and then evaluate the pharmacokinetics when the test drug and concomitant drug are administered), and etc. The parallel-group comparison design is not recommended usually because it is necessary to consider the influence of inter-individual difference. Comparison with a control group in the separate study (historical controls) is unjustifiable in principle.

Drug interaction studies can be conducted in an open-label manner, except in cases where it is important to evaluate pharmacodynamic endpoints, including adverse events that are susceptible to biases, such as in evaluation by blood pressure or observation of symptoms.

Subjects who have ingested ethical drugs, over-the-counter drugs, supplements, health food products, tobacco, or alcohol prior to the registration should be considered to be excluded from the clinical drug interaction studies because these substances may alter the activities of drug metabolizing enzymes and transporters.

If the clearance of the investigational drug is considered to be strongly influenced by drug metabolizing enzymes or transporters whose activities are altered by genetic polymorphisms (CYP2C9, CYP2C19, CYP2D6, UGT1A1, OATP1B1, BCRP etc.), the degree of drug interactions may vary according to the gene polymorphism. In this case, a study design using stratification by genotype may be useful (seeSection 7.9.5.1.).

7.4 Dose and route of administration

The dose and regimen of inhibitors or inducers used in the study should be those that maximize the possibility of drug interactions, and the expected or approved maximum doses and minimum intervals of administration should be used. For substrates, any dose in the linear range can be used. If the substrate has non-linear pharmacokinetics, the dose used should be determined with consideration of the therapeutic dose. A reduced dose of the substrate(s) may need to be considered due to safety concerns. In that case, the influences of altered dose or regimen in the drug interaction study including necessary detection sensitivity of the drug concentration measurement, should be discussed and described in the clinical study protocol and the clinical study report.

The route of administration chosen for clinical drug interaction studies is important. For an investigational drug, the route of administration generally should be the one expected in clinical practice. When multiple routes are being considered for the investigational drug, the need for metabolic drug-drug interaction studies by each route should be determined, depending on anticipated mechanism(s) of interaction and the degree of changes in the AUC of the investigational drug and metabolites. If only oral dosage forms will be marketed, studies with an intravenous formulation are not usually necessary.

7.5 Duration and timing of administration

In clinical interaction studies, it is desirable to examine drug interactions in the steady state at multiple doses for the investigational drug, if the investigational drug is the interacting drug of the drug metabolizing enzyme. In particular, a loading dose could be used at least several days for investigational

drugs that may cause enzyme induction or have shown time-dependent inhibition (TDI) in *in vitro* studies. In this case, it may be considered to achieve the target steady state concentrations earlier by adjusting dose and dosing interval taking into account the safety. However, when the interacting drug neither shows the possibility of TDI nor enzyme induction, etc., or when the investigational drug is expected to be used in a single-dose therapy in clinical practice, implementation of single-dose study is a possible option. In general, clinical drug interaction studies using a single dose design can be applied to investigational drugs that work only as substrates. If the expected drug interactions may cause prolonged fluctuation in the enzyme activities due to TDI or induction, etc., and become a clinical concern, it is recommended to evaluate recovery after the withdrawal of the interacting drug using a cross-over design including the period administering the affected drug alone, following the coadministration period. If the GI absorption of the interacting drug is influenced by gastric pH, it is useful to determine the extent of influence beforehand, for instance, from information on interactions between the interacting drug and gastric secretion inhibitor to accurately evaluate the influence on the metabolism process by separating the interactions in the absorption process.

Attention should also be paid to the influences of the timing of administration of the substrate and the interacting drug on interactions between these drugs. In clinical drug interaction studies, drugs should be administered at timing maximizing the possibility of drug interactions. However, the safety of the subject should be considered as much as possible. If the drug interactions occur for the most part during the first pass, the degree of drug interactions may be decreased when a longer interval is allowed between the administrations of the two drugs. However, it is also possible that more noticeable drug interactions occur when the two drugs are administered at different time points.

7.6 Selection of inhibitors for drug metabolizing enzymes and transporters

7.6.1 Clinical drug interaction studies using inhibitors of P450

For evaluation of the possibility that pharmacokinetics of an investigational drug is affected by P450 inhibition, a clinical drug interaction study is conducted with inhibitors of drug metabolizing enzymes involved in the metabolic pathway of the investigational drug, selected on the basis of the results of *in vitro* studies and clinical pharmacology(pharmacokinetic) studies. At that time, degree of inhibition should be considered. The degree of inhibition is set based on the degree of influence on the AUC of sensitive substrate in case of oral coadministration with interacting drug in clinical drug interaction studies. When an inhibitor is considered to increase the AUC by ≥5-fold (or a decrease in the CL/F to less than 1/5), the drug is termed as a "strong inhibitor". Likewise, an inhibitor that is considered to cause an increase in the AUC by ≥2-fold but <5-fold (or a decrease in the CL/F to <1/2 but ≥1/5) is termed as

a "moderate inhibitor", and an inhibitor that is considered to cause an increase in the AUC by ≥1.25-fold but <2-fold (or a decrease in the CL/F to <1/1.25 but ≥1/2) is termed as a "weak inhibitor". In selecting inhibitors to be used in clinical drug interaction studies, strong inhibitors of drug metabolizing enzymes involved in the elimination of the investigational drug should be used, and the implementation of clinical drug interaction studies using *in vivo* inhibitor of P450 (clinical index drug, see Table 1-5 of Section 11.3) is recommended; however safety of the subjects should be considered as much as possible. In cases that it is difficult to conduct clinical drug interaction studies using strong inhibitors from the point of view of safety concerns, the effects of moderate or weak inhibitors may be evaluated through clinical drug interaction studies paying attention to the safety of the subjects. When the necessity for consideration of dose adjustment is suggested from results of clinical drug interaction studies using strong inhibitors, the effects of other inhibitors on the same drug metabolizing enzyme should also be evaluated by the clinical drug interaction study considering the frequency of coadministration in clinical practice. Inhibitors other than those which have been evaluated in clinical drug interaction studies may be evaluated, as required, via phase II or phase III clinical trials or via modeling and simulation.

If major drug metabolizing enzymes of the investigational drug are not listed in Table 1-5 of Section 11.3, the inhibitory effects on the specific enzyme are examined using drugs that are used concomitantly in the clinical practice considering the safety of the investigational drug at blood concentrations over the therapeutic range and also considering the contribution of the specific metabolic pathway to the overall elimination of the investigational drug.

7.6.2 Clinical drug interaction studies using inhibitors of drug metabolizing enzymes other than P450 and transporters

If there is a risk that the investigational drug is metabolized by drug metabolizing enzymes other than P450 or is transported by transporters and causes drug interactions by inhibition in clinical practice, it is recommended to consider the feasibility of clinical drug interaction studies, considering the presence of known inhibitors towards the specific enzymes or specific transporters. When clinical drug interaction studies are conducted, the interactions should be evaluated according to the same procedures as those for the drugs metabolized by P450.

7.7 Selection of inducers for drug metabolizing enzymes

For evaluation of the possibility that pharmacokinetics of an investigational drug is affected by P450 induction, a clinical drug interaction study is conducted with P450 involved in the metabolic pathway of the investigational drug, selected on the basis of the results of *in vitro* studies and clinical pharmacology

(pharmacokinetic) studies. At that time, the degree of induction should be considered. The degree of induction is set based on the degree of influence on the AUC of sensitive substrate in case of oral coadministration with an interacting drug in the clinical drug interaction study. When an inducer is considered to reduce the AUC to 1/5 or less (or increase the CL/F ratio by >5-fold), the drug is termed as a "strong inducer". Likewise, an inducer that is considered to reduce the AUC to ≤1/2 but >1/5 (or increase the CL/F by ≥2-fold but <5-fold) is termed as a "moderate inducer", and an inducer that is considered to reduce the AUC by <1/1.25 but >1/2 (or increase the CL/F by ≥1.25-fold but <2-fold) is termed as a "weak inducer". In selecting inducers to be used in clinical drug interaction studies, strong inducers should be used to evaluate the maximum effect of interaction, and the implementation of clinical drug interaction studies using an *in vivo* inducer of P450 (clinical index drug, see Table 1-6 of Section 11.3) is recommended; however, safety of the subject should be considered as much as possible. Inducers other than those which evaluated in clinical drug interaction studies may be evaluated in phase II or phase III clinical trials or by using modeling and simulation if necessary. In the case of an investigational drug which has to be used concomitantly with a specific enzyme inducer from the viewpoints of indications and dosage, it is recommended that a clinical drug interaction study with the specific inducer be conducted with consideration for safety of subjects as much as possible, from the viewpoints of indications and dosage, in order to determine an appropriate treatment method (see Section 4.3).

7.8 Selection of substrates for drug metabolizing enzymes and transporters

For evaluation of the possibility that an investigational drug exhibits P450 inhibition or induction, a clinical drug interaction study is conducted with a substrate to be affected in its pharmacokinetics by the investigational drug considering results of the *in vitro* studies and clinical pharmacology (pharmacokinetic) studies. For assessing by clinical studies whether an investigational drug inhibits or induces a drug metabolizing enzyme (or a transporter) or not, a clinical drug interaction study should be performed using an index drug or a typical substrate for the transporter (Table 1-4 or 2-3 of Section 11.3) which has a high selectivity for specific drug metabolizing enzyme (or transporter) and its contribution to total elimination is high (sensitive substrate). In case of that it has been demonstrated in clinical drug interaction studies that an investigational drug inhibits or induces metabolism (or transport) of a index drug or sensitive substrate, it should be considered whether additional clinical drug interaction studies are added using a substrate for the specific enzyme (or transporter) which is very likely to be coadministered post-marketing (see Figs. 1-2 and 1-3 of Section 11.2, and Section 4.1).

7.9 Other considerations for evaluation by clinical drug interaction studies

7.9.1 Drugs metabolized by a single enzyme and multiple enzymes

The concentrations of drugs in the body that are metabolized by a single drug metabolizing enzyme become markedly high if the enzyme involved is inhibited. On the other hand, for drugs that are metabolized by multiple drug metabolizing enzymes, the elevation of concentrations in the body would be less, because the investigational drug is metabolized by other enzymes (alternate enzymes) even if the principal enzyme is inhibited. To predict drug interactions in the clinical practice, it is important to make an appropriate prediction of the relative CR of the enzyme to the overall elimination. For predicting the degree of these interactions, the analyzed results of appropriately designed drug interaction studies and the investigations with modeling and simulation are considered to be useful.

7.9.2 Drug interactions involving both drug metabolizing enzymes and transporters

Multiple mechanisms may be involved in drug interactions due to overlapping of the substrate specificities for drug metabolizing enzyme and transporter (complex drug-drug interactions). Overlapping of the substrate specificities for CYP3A and P-gp is a representative example. These drug interactions can be evaluated with the use of inhibitors that exert an inhibitory action against both P-gp and CYP3A such as itraconazole. However, caution is necessary in the interpretation of the study results, because it is impossible to identify the mechanism responsible for the change in AUC even if the interaction is evident.

It is also possible that the investigational drug causes interactions by inhibiting (or inducing) multiple enzymes and transporters, or by inhibiting a certain enzyme (or transporter) and inducing another enzyme or transporter simultaneously. In addition, if both drug metabolizing enzymes and transporters are inhibited by concomitant use of multiple drugs, the resultant influences may be even more complex and serious.

7.9.3 Cocktail substrate studies

Cocktail substrate studies can be used for evaluating the actions of the investigational drug on several different drug metabolizing enzymes and transporters in a single clinical drug interaction study. If designed appropriately, cocktail substrate studies allow examination of both inhibition (competitive inhibition and TDI) and induction. The substrates used in cocktail substrate studies should consist of index drug or sensitive substrates susceptible to interactions for each target enzyme (and transporter). For each index drug or substrate used, the effect of the investigational drug on AUC should be calculated. When the results of appropriately implemented cocktail substrate studies are negative (see Section 7.2), there is no need to carry out further evaluation of the enzymes or transporters in question. However, if

the results are positive and the drug interaction may become a clinical concern, further clinical drug interaction studies with a sensitive substrate (see Table 1-4 of Section 11.3) or a typical substrate(see Table 2-3 of Section 11.3) alone should be considered.

7.9.4 Evaluation by population pharmacokinetic analysis

Drug interactions that have not been evaluated in independent drug interaction studies may be investigated if the study plan is such as to allow evaluation of drug interactions in population pharmacokinetic analyses in phase II and/or III studies by collecting the information of concomitant drugs. It is important to appropriately determine the handling of assay samples, timing of collection, etc. in the clinical studies for that purpose.

7.9.5 Considerations for subjects with special background

7.9.5.1 Evaluation of drug interactions in consideration of genetic polymorphism

The degree of drug interactions (inhibition or induction) of the investigational drug on a certain target drug metabolizing enzyme or transporter may vary according to the genotype of the subject. In subjects in whom the major elimination pathway (drug metabolizing enzymes or transporters) is lacking or functioning poorly, the drug concentrations are generally high. If it is co-administered with drugs inhibiting the alternative pathway of metabolism or excretion of the investigational drug, the drug concentrations rise further, possibly causing safety issues.

Molecular species of drug metabolizing enzymes and transporters with pharmacokinetics that are greatly affected by the genetic polymorphism include CYP2C9, CYP2C19, CYP2D6, UGT1A1, OATP1B1 and BCRP. For drugs whose major elimination pathway is mediated by these drug metabolizing enzymes or transporters, it is useful to perform genetic polymorphism analysis prior to clinical drug interaction studies.

The type and frequency of genetic polymorphism need to be considered as well. In particular, the characteristics of these P450 isoenzymes should be borne in mind when implementing clinical drug interaction studies of investigational drugs whose major elimination pathway is mediated by CYP2C19 and CYP2D6; genetic polymorphisms that cause the defective activity of the former enzyme and marked reduction of the activity of the latter enzyme are frequent among East Asians.

7.9.5.2 Investigational drugs mainly applied to special population or patient populations with specific diseases

If the investigational drug is expected to be mainly administered to pediatrics or geriatrics patients or patients with renal dysfunction or hepatic impairment, drug interactions of the investigational drug can be evaluated also by population pharmacokinetic analyses appropriately designed, or by using PBPK models. Also, attention should be paid not to miss clinically significant drug interactions in these populations, e.g., by conducting examinations under the assumption of maximal effect in prediction by modeling and simulation.

7.9.5.3 Studies in population other than healthy volunteers

Clinical drug interaction studies are usually carried out in healthy volunteers, and drug interactions are often extrapolated to indicated patients in which the drug is indicated based on the results of studies in healthy volunteers. When it is difficult to perform studies in healthy volunteers, drug interaction studies may be performed in patients in whom the drug is indicated. In that case, there will be many restrictions in the study design such as study period, dose, and blood sampling schedule. Therefore, in the evaluation of drug interaction, it may be useful to supply information by using modeling and simulations if appropriate, as well as giving adequate consideration to inter-individual variability in patient populations.

8. Basic principles for provision of information and alerts on drug interaction

Pharmacokinetic information and information obtained from drug interaction studies during the drug development process appears to be valuable for proper use of drugs, when provided appropriately to professionals involved in patient care in actual medical practice through descriptions in package inserts or other means. When judging the contents of the information and alerts, attention should be focused on whether or not pharmacokinetic changes might affect the therapeutic effect or occurrence of adverse drug reactions. The general principles for reflection of information on pharmacokinetic drug interactions in package inserts are as follows.

8.1 Description in precautions for use in drug package inserts

Precautions for use should be included when concomitant use of other drugs may cause enhancement or attenuation of the pharmacological actions, enhancement of known adverse drug reactions, occurrence of new adverse drug reactions of the investigational drug or of the concomitant drugs, or aggravation of the primary disease, and if clinical precautions are necessary for these cases. When a drug interaction is associated with a pharmacokinetic change, the necessity of alerts should be considered based on the degree of change in the pharmacokinetic parameters (AUC and C_{max}, etc.)and dose response and

exposure-response relationship, etc. in drug activity. The alerting actions are classified into either "Contraindications For Co-Administration (Do not co-administer)" or "Precautions For Co-Administration (Be careful about concomitant use)." When the serious adverse drug reactions resulting from significant pharmacokinetic changes are anticipated realistically, and the seriousness of the consequence would outweigh expected therapeutic effects, it should be considered to make the combination contraindicated. When the therapeutic effect is clinically useful, however, the predicted exposure of the drug may exceed the exposure expected for approval dosage and administrations due to pharmacokinetic changes, and in situations when a management would be required to avoid serious clinical risk and concerns of patients, the combination should be "Contraindication For Co-administration" or "Precaution For Co-Administration" in accord with the seriousness of the risk.

In the section of "INTERACTIONS", at the beginning of the section, pharmacokinetic properties of the investigational drug related to drug interactions requiring attention in clinical practice should be described briefly, such as isoenzymes involved in the metabolism, an approximate extent of contributions of the enzyme, inhibition and induction potentials, and mechanisms of drug transport in absorption, distribution and excretion. These descriptions should be based on clinical pharmacokinetic information in principle. However, characteristics requiring attention in clinical practice, even if they were identified in the results of *in vitro* studies, should be described as needed. If the investigational drug affects the pharmacokinetics of other drugs via P450 (inhibitors or inducers), the potency of inhibition and induction should also be stated as needed (see Sections 7.6 and 7.7). The precautions for concomitant use with other drugs should be in user-friendly manner such as table form and described separately by type of interaction (mechanism, etc.). In the case of pharmacokinetic drug interactions, the non-proprietary name and, if necessary, information on metabolic enzymes, etc. should be described in "Drugs". In the case of pharmacodynamic drug interactions, therapeutic class, and if necessary, the non-proprietary name should be described in "Drugs".

Precautions to avoid the influences of drug interactions should be described in "Signs, Symptoms and Treatment". Further, the mechanisms underlying drug interactions and risk factors that may potentially raise a safety concern due to concomitantly administered medication should be described in the "Mechanisms and Risk Factors". If the mechanisms underlying interactions are unclear, the mechanisms should be stated as unknown.

If adjustment of the dosage regimen of the investigational drug is necessary to compensate the drug interactions, the adjustment should be described specifically in the "PRECAUTIONS CONCERNING DOSAGE AND ADMINISTRATION" based on the quantitative information collected from conducted clinical interaction studies, etc.

Important interactions with biotechnical and biological products or foods and beverages should also be described in the same manner.

When a clinically significant adverse drug reaction had been caused unambiguously by pharmacokinetics drug interactions in the same class of drugs, and clinical interaction studies are not conducted as to the investigational drug, however, pharmacokinetic interactions of the same mechanism is suggested for the investigational drug by appropriate modeling and simulations etc., it should be considered to describe alerts in the package inserts considering the possibility of the concomitant use in clinical practice. Although in this case pharmacokinetic changes are used as an index, the level and contents of the alerts should be described in consideration of the clinical significance including changes in the efficacy and safety, and how the drug interaction should be managed. In the description of alerts of this category, the use of modeling and simulations should be clearly specified.

8.2 Description in "Drugs" in the section of "INTERACTIONS"

In "Contraindication For Co-Administration", all drugs should be described by both non-proprietary names and representative brand names. In "Precautions For Co-Administration", drugs in this category should be described by the non-proprietary names. For drug interactions mediated by CYP3A in "Precautions For Co-Administration", many drugs require alerts and the grade of each alerts varies depending on the therapeutic effect and pharmacokinetic properties of concomitant drugs. In the package inserts of drugs inhibiting or inducing CYP3A, the strength classification of inhibition or induction should be described at the beginning in the section "INTERACTIONS" and each of non-proprietary names of about three representative concomitant drugs (drug metabolized by CYP3A) requiring special attention should be listed in "Drugs" in "Precautions For Co-Administration", in consideration of possibility of concomitant use in clinical practice. Then the package inserts of the drugs affected by CYP3A inhibition or induction should have a description that the drug is primarily metabolized by CYP3A at the beginning of the section of "INTERACTIONS", and the strength classification of CYP3A inhibition or induction and each of non-proprietary names of about three representative concomitant drugs (CYP3A inhibitors or inducers) requiring precautions should be listed in "Drugs" in "Precautions For Co-Administration", in consideration of possibility of concomitant use in clinical practice. In alerts of drug interaction via P450 isoenzymes other than CYP3A, P450 isoenzymes involved may be described, however, the strength classification of inhibition or induction should be described only when necessary. In alerts of drug interaction with drug metabolizing enzymes other than P450 and transporters, only the non-proprietary names of concomitant drugs should be described. However, the names of metabolizing

enzymes and transporters should be described on top of the non-proprietary names as needed when there are other drugs requiring special attention in clinical practice.

8.3 Description in the section of "PHARMACOKINETICS"

In the section of "PHARMACOKINETICS", the basic pharmacokinetic parameters, the mechanism of interactions and *in vivo* or *in vitro* study results to be evidence should be described in order to grasp the pharmacokinetic characteristics of the investigational drug in humans. Pharmacokinetic parameters including total clearance, distribution volume, absolute bioavailability, and urinary excretion ratio are important to grasp the pharmacokinetic characteristics. In the development of drugs for oral administration, data obtained by intravenous administration, if available, should be described in the pertinent sections on absorption, excretion, etc., as needed. As matters related to the mechanisms of interactions, quantitative information on the major elimination pathway, enzymes involved in that pathway, and the degree of their contribution, inhibition and induction of drug metabolizing enzymes, and mechanisms of drug transport in absorption, distribution and excretion, should be described in the pertinent sections on metabolism, excretion, etc. When providing information concerning data, it should be specified whether the data were derived from *in vitro* studies or clinical drug interaction studies, whether they were obtained by actual measurement or represent estimates obtained from simulation, etc., in a clear distinctive manner. Information on drug interaction alerted in the section of "INTERACTIONS" from the clinical drug interaction studies should be provided in the section of "PHARMACOKINETICS" section appropriately. Data of *in vitro* studies, etc., should be supplemented for the mechanism of interactions and risk factor as needed. When describing the results of clinical drug interaction studies, information of the dosage and administration used in studies as well as changes in pharmacokinetic parameters should be provided so that the degree of interactions can be quantitatively determined. Study results should be illustrated as a quantitative, simple outline of the changes in the AUC, C_{max}, etc., using narrative text, tables and/or figures. Further information on the detailed study design and data should be provided using materials other than the package inserts. In any such instance of provision of information, the evidence should be clearly stated, e.g., by citing the literature in the package insert. Drug interactions not alerted in the section of "INTERACTIONS" should be described only when they are particularly important, in such cases as a high possibility of concomitant use in clinical practice.

9. Relevant guidelines etc.

This guideline shows general principles of investigational methods of study and alerting related to drug interactions. Although previously issued guidelines, guiding principles, etc., include descriptions of studies of drug interactions, the present guideline organizes the contents of such guidelines and incorporates current new findings and concepts. In the evaluation of the individual drugs, refer to descriptions of previously issued guidelines and regulatory documents (as shown below references) as needed.

Reference

ICH Guideline

1) Clinical Safety Data Management: Definitions and Standards for Expedited Reporting (ICH E2A Guideline), Notification No. 227 of the Evaluation and Licensing Division, PMSB, dated March 20, 1995.

2) Post-approval Safety Data Management: Definitions and Standards for Expedited Reporting (ICH E2D Guideline), Notification No. 0328007 of the Safety Division, PFSB, dated March 28, 2005.

3) Pharmacovigilance Planning (ICH E2E Guideline), Notification No. 0916001 of the Evaluation and Licensing Division, PMSB, dated September 16, 2005.

4) Structure and Content of Clinical Study Reports (ICH E3 Guideline), Notification No. 335 of the Evaluation and Licensing Division, PMSB, dated May 1, 1996. Structure and Content of Clinical Study Reports Questions & Answers (R1), Office Memo, dated October 18, 2012.

5) Dose-Response Information to Support Drug Registration (ICH E4 Guideline), Notification No. 494 of the Evaluation and Licensing Division, PMSB, dated July 25, 1994.

6) Handling of Data on Drug Products from Clinical Studies Conducted in Overseas Countries, Notification No. 739 of the Evaluation and Licensing Division, PMSB, dated August 11, 1998. Ethnic Factors in the Acceptability of Foreign Clinical Data (ICH E5 Guideline), Notification No. 672 of the Evaluation and Licensing Division, PMSB, dated August 11, 1998. Ethnic Factors in the Acceptability of Foreign Clinical Data Questions and Answers, Office Memo, dated February 25, 2004. Ethnic Factors in the Acceptability of Foreign Clinical Data Questions and Answers (Part 2), Office Memo, dated.

7) Guideline for Good Clinical Practice (GCP) (ICH E6 Guideline), Ordinance of the Ministry of Health and Welfare No.28, dated March 27, 1997. Enforcement of Good Clinical Practice, PAB Notification No. 430, dated March 27, 1997

8) Studies in Support of Special Populations: Geriatrics (ICH E7 Guideline), Notification No. 104 of the New Drugs Division, PAB dated December 2, 1993. E7 Studies in Support of Special Populations: Geriatrics Questions & Answers, Office Memo, dated September 17, 2010.

9) General Considerations for Clinical Trials (ICH E8 Guideline), Notification No. 380 of the Evaluation and Licensing Division, PMSB, dated April 21, 1998.

10) Clinical Investigation of Medicinal Products in the Pediatric Population (ICH E11 Guideline), Notification No. 1334 of the Evaluation and Licensing Division, PMSB, dated December 15, 2000. Clinical Investigation of Medicinal Products in the Pediatric Population Questions & Answers, Office Memo, dated June 22, 2001.

11) Addendum: Clinical Investigation of Medicinal Products in the Pediatric Population (ICH E11 (R1) Guideline), Notification No. 1227-(5) of the Pharmaceutical Evaluation Division, PSEHB, dated December 27, 2017

12) Definitions for Genomic Biomarkers, Pharmacogenomics, Pharmacogenetics, Genomic Data and Sample Coding Categories (ICH E15 Guideline), Notification No. 0109013 of the Evaluation and Licensing Division, PMSB, Notification No. 0109002 of the Safety Division, PFSB, dated January 9, 2008.

13) Biomarkers Related to Drug or Biotechnology Product Development: Context, Structure and Format of Qualification Submissions (ICH E16 Guideline), Notification No. 0120-(1) of the Evaluation and Licensing Division, PMSB, Notification No. 0120-(1) of the Safety Division, PFSB, dated January 20, 2011.

14) Guideline on Genomic Sampling and Management of Genomic Data (ICH E18 Guideline), Notification No. 0118-(1) of the Pharmaceutical Evaluation Division, PSEHB, dated January 18, 2018.

15) Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals (ICH M3 (R2) Guideline), Notification No. 0219-(4) of the Evaluation and Licensing Division, PMSB, dated February 19, 2010. Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals Questions & Answers (R2), Office Memo, dated August 16, 2012.

Other notifications, etc.

Guidelines and documents in Japan (Pharmacokinetics-related)

1) Guidelines for the Design and Evaluation of Oral Prolonged Release Dosage Forms, Notification No. 5 of the First Evaluation and Registration Division, PAB, dated March 11, 1988.

2) Guidelines on Nonclinical Pharmacokinetic Studies, Notification No. 496 of the Evaluation and Licensing Division, PMSB, dated June 26, 1998.

3) Clinical Pharmacokinetic Studies on Pharmaceuticals, Notification No. 796 of the Evaluation and Licensing Division, PMSB, dated June 1, 2001.

4) Guidance for Conducting Microdose Clinical Studies, Notification No. 0603001 of the Evaluation and Licensing Division, PMSB, dated June 3, 2008.

5) Studies utilizing Pharmacogenomics, Notification No. 0930007 of the Evaluation and Licensing Division, PMSB, dated September 30, 2008.

6) Partial revision of Guidelines for Bioequivalence Studies of Generic Products, Notification No. 0229-(10) of the Evaluation and Licensing Division, PMSB, dated February 29, 2012. Revision of Questions & Answers, on Guidelines for Bioequivalence Studies of Generic Products Office Memo, dated February 29, 2012.

7) The Guidelines for the Validation of Analytical Methods of Drug Concentration in Biological Samples in Drug Development, Notification No. 0711-1 of the Evaluation and Licensing Division, PMSB, dated July 11, 2013

8) Joint MHLW/EMA Reflection Paper on the Development of Block Copolymer Micelle Medicinal Products, Notification No. 0110-1 of the Evaluation and Licensing Division, PMSB, dated January 10, 2014. Joint MHLW/EMA Reflection Paper on the Development of Block Copolymer Micelle Medicinal Products Questions & Answers, Office Memo, dated January 10, 2014.

9) The Guidelines for the Validation of Analytical Methods of Drug Concentration (ligand-binding assay) in Biological Samples in Drug Development, Notification No. 0401-1 of the Evaluation and Licensing Division, PMSB, dated April 1, 2014

10) Clinical Pharmacokinetic Studies on Pharmaceuticals –Evaluation of Clinical Pharmacokinetic Studies and Drug Interaction: Explanation of Notification-, Jiho Inc., issued January 15, 2003.

Package insert-related

1) Instructions for Package Inserts of Prescription Drugs, etc., Notification No. 0608-1 of the PSEHB, dated June 8, 2017.

2) Points to Consider regarding the Instructions for Package Inserts of Prescription Drugs, etc., Notification No. 0608-1 of the Safety Division, PSEHB, dated June 8, 2017.

Overseas guidance, etc.

1) FDA:Guidance for Industry Food-Effect Bioavailability and Fed Bioequivalence Studies (2012,2)

2) EMA: Guideline on the use of pharmacogenetic methodologies in the pharmacokinetic evaluation of medicinal products (2012,8)

3) EMA: Guideline on the investigation of drug interactions (2013,1)

4) FDA:Guidance for Industry Clinical Pharmacogenomics:Premarket Evaluation in Early-Phase Clinical Studies and Recommendations for Labeling (2013,1)

5) FDA: Guidance for Industry In Vitro Metabolism and Transporter Mediated Drug-Drug Interaction Studies DRAFT GUIDANCE (2017,10)

6) FDA: Guidance for Industry Clinical Drug Interaction Studies-Study Design, Data Analysis, and Clinical Implications DRAFT GUIDANCE (2017,10)

10. Glossary

1) Substrate: A drug that is subject to metabolism or transport by transporters

2) Distribution volume: The distribution volume is small when it is about the same as extracellular fluid volume or less (approx. 0.25 L/kg or less in humans), and the volume is large when it is 0.8 L/kg or more in humans.

3) Concomitant drug: When two or more drugs are used, each drug is called a concomitant drug, in the broad sense. In the narrow sense, a concomitant drug is a drug that is added to the basic drug treatment.

4) Interacting drug: In pharmacokinetic drug interactions, a drug that affects the pharmacokinetics of other drugs when administered concomitantly. For instance, in the case of metabolism, the affecting drug may inhibit or induce drug metabolizing enzymes.

5) Affected drug: In pharmacokinetic drug interactions, a drug whose pharmacokinetics is affected by a concomitant drug. For instance, in the case of metabolism, the metabolism of an affected drug may be decreased by inhibition of the drug metabolizing enzymes or increased by induction of the drug metabolizing enzymes by the interacting drug.

6) Investigational drug: A medicinal product or a drug under development that is investigated as to its potential to act as an affecting drug or an affected drug

7) Index drug: A drug that has been demonstrated in multiple clinical studies to have a high specificity for the enzymes etc., and represents the changes in the pharmacokinetics. Index drugs need to be possible to quantify, and to be shown to have high safety if they are to be used in clinical studies.

8) Drug metabolized by a single enzyme: A drug metabolized mainly by a single drug metabolizing enzyme. The total metabolic clearance of this drug is markedly influenced by the activity change of a drug enzyme by drug interaction, and thus the risk in that case is high.

9) Drug metabolized by multiple enzymes: A drug metabolized by multiple drug metabolizing enzymes. The total metabolic clearance of this drug is less susceptible to the activity changes of enzymes caused by drug interactions, and therefore the risk in that case is small.

10) Transporter: A carrier that is inserted in the biological membranes and transports drugs into and out of cells.

11) Selective inhibitor, selective substrate: A drug that rather strongly inhibits a specific drug metabolizing enzyme etc. A drug that is metabolized or transported selectively by a specific drug metabolizing enzyme etc.

12) Typical inhibitor, typical substrate (Tables 2-1 to4 of Section 11.3): A typical inhibitor may inhibit multiple drug metabolizing enzymes or transporters and a typical substrate may be a substrate for

multiple drug metabolizing enzymes or transporters so that it is not necessarily a selective inhibitor or a selective substrate.

13) Strong inhibitor, moderate inhibitor, weak inhibitor: When a drug which is considered to increase the AUC of sensitive substrates by ≥5-fold (or a decrease in the CL/F to less than 1/5), the drug is termed as a "strong inhibitor"., a drug that is considered to cause an increase in the AUC by ≥2-fold but <5-fold (or a decrease in the CL/F to <1/2 but ≥1/5) is termed as a "moderate inhibitor", and a drug that is considered to cause an increase in the AUC by ≥1.25-fold but <2-fold (or a decrease in the CL/F to <1/1.25 but ≥1/2) is termed as a "weak inhibitor" (see description in Section 7.6).

14) Strong inducer, moderate inducer, weak inducer: A drug that is considered to reduce the AUC of sensitive substrates to ≤1/5 (or increases the CL/F ratio by >5-fold) is termed as a "strong inducer", a drug that is considered to cause a decrease in the AUC to ≤1/2 but >1/5 (or an increase of the CL/F by ≥2-fold but <5-fold) is termed as a "moderate inducer", and a drug that is considered to reduce the AUC to ≤1/1.25 but >1/2 (or increases the CL/F by ≥1.25-fold but <2-fold) is termed as a "weak inducer" (see description in Section 7.7).

15) Sensitive substrate, moderate sensitive substrate: A substrate susceptible to pharmacokinetic drug interactions whose AUC increases by ≥5-fold (or a decrease in the CL/F to <1/5) when co-administered with a "strong inhibitor", and a substrate moderately susceptible to pharmacokinetic drug interactions whose AUC increases by ≥2-fold but <5-fold (or a decrease in the CL/F to <1/2 but ≥1/5) when co-administered with a "strong inhibitor" (see description in Section 7.8).

11. Appendices

11.1 List of figures and tables

[Figures]

Drug metabolizing enzymes

Transporters

[Tables]

Drug metabolizing enzymes

Transporters

11.2 Decision trees

Figure 1-1: Evaluation of the possibility of the investigational drug as an affected drug (Identification of the enzymes involved in the metabolism of the investigational drug)

Is it presumed from the results of _in vitro_ metabolism studies[a] and clinical pharmacokinetic studies that a particular enzyme contributes by at least 25% in the total elimination of the investigational drug[b]?

Yes or inconclusive

No → No clinical drug interaction study mediated by drug metabolizing enzymes is required.

In clinical drug interaction studies, are there any drug interactions for which dose adjustment should be considered by concomitant use of strong inhibitors of the enzyme in question?

No → No further clinical drug interaction study is required.

Yes

Consider the necessity of further clinical drug interaction studies with other inhibitors/inducers of the enzyme in question.[c]

a) The targeted drug metabolizing enzymes are CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A (CYP3A4 and CYP3A5). However, when the contribution of primary isoenzymes of P450 is small, other isoenzymes of P450 (e.g., CYP2A6, CYP2E1, CYP2J2, CYP4F2), phase I enzymes other than P450 (e.g., MAO, FMO, XO, alcohol dehydrogenase, aldehyde dehydrogenase), and phase II enzymes (if the investigational drug is mainly metabolized by UGT) should also be examined.

b) In the following cases, major metabolites of the investigational drug should also be evaluated in the same manner. When the investigational drug is a pro-drug, and the main action is exerted by the active metabolite, when a pharmacologically active metabolite is produced and the _in vivo_ pharmacologic effect of the metabolite estimated from the _in vitro_ activity and the AUC of the unbound form of the metabolite accounts for at least 50% of the entire pharmacologic effect, or when it is suspected that adverse effects may be induced by the metabolite, the enzyme involved in the major production pathway and elimination pathway of the metabolite in question should be identified, and studied in the same manner.

c) Inhibitor(s)/inducer(s) should be selected taking into consideration possible concomitant use with the investigational drug. Clinical drug interaction studies with inducers are required when the risk of clinically significant drug interactions is inferred by modeling and simulations (e.g., if the validity

of the PBPK model is confirmed and the results of the clinical studies can be described consistently) or other procedures based on the results of clinical drug interaction studies with inhibitors.

Figure 1-2: Evaluation of the possibility of the investigational drug inhibiting drug metabolizing enzymes

Does the investigational drug or metabolites[b] inhibit drug metabolizing enzymes in *in vitro* studies[a]?

No → No clinical drug interaction study mediated by enzyme inhibition is required.

Yes

Is the R value[d] calculated from the inhibition parameters (reversible inhibition: K_i, TDI: K_I, k_{inact})[c] ≥ 1.02 (reversible inhibition), ≥ 11 (reversible inhibition in the small intestine), or ≥ 1.25 (TDI[e])?

No → No clinical drug interaction study mediated by enzyme inhibition is required.

Yes[f]

Is the AUCR≥ 1.25 in the mechanistic static pharmacokinetic (MSPK) model[g] or physiologically based pharmacokinetic (PBPK) model?

No / inconclusive → No clinical drug interaction study is required when there is a clear reason for the discrepancy from the judgment based on the R value.

Yes

In clinical drug interaction studies, are there obvious effects on the pharmacokinetics of the index drug for the enzyme in question?

No → No further clinical drug interaction study is required.

Yes

Consider the necessity of further clinical drug interaction study(ies) with other substrate(s) (selected taking into consideration the therapeutic range and possible concomitant use with the investigational drug) of the enzyme in question.

a) The targeted drug metabolizing enzymes are CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A (CYP3A4 and CYP3A5), UGT1A1, and 2B7. Drug metabolizing enzymes other than P450 are examined if they are involved in the major elimination pathway of the investigational drug and primary concomitant drugs.

Concentration setting should include concentrations of $50 \times C_{max}$ (unbound concentration) or $0.1 \times$ dose/250 mL.

In the case of P450, the presence or absence of time-dependent inhibition should also be examined. *In vitro* enzyme inhibition studies use human liver microsomes, human hepatocytes, microsomal fraction of the expression system (recombinant cells) of the target enzymes, etc.

b) Enzyme inhibition effects of major metabolites: It is desirable to investigate the enzyme inhibition effects of metabolites which is less polar than the parent(unchanged) drug whose AUC accounts for at least 25% of the parent drug; metabolites which is more polar than the parent drug whose AUC accounts for at least 100% of the parent drug; and metabolites with a chemical structural alert(s) for time-dependent inhibition (TDI) whose AUC accounts for at least 25% of the parent drug and at least 10% of the total AUC of drug-related substances.

When investigating the inhibitory effect of a metabolite, the concentration should be set in a range including $50 \times C_{max}$ (unbound concentration) of the metabolite, similarly to the case for the unchanged drug.

c) If the investigational drug is metabolized rapidly in the reaction mixture, a marker substrate with a sufficiently high metabolic rate as compared with the investigational drug should be used to minimize reduction in the concentration of the investigational drug, and the Ki (inhibition constant: dissociation constant of the inhibitor from the enzyme-inhibitor complex) should be evaluated. Table 1-1 shows the representative marker reactions of P450 isozymes used in the *in vitro* studies. Literature should be referred to for the concentrations of the marker substrates to be used in *in vitro* studies. Usually, the concentration should be around K_m value or below.

The range of investigational drug concentrations may be set according to the expected site of enzyme inhibition (liver, small intestine), method of administration, dosage form, and pharmacokinetic parameters (C_{max} or AUC). However, the concentration range is usually set to include $50 \times C_{max}$ (unbound form) or $0.1 \times$ dose/250 mL, and the K_i value is calculated for cases of concentration-dependent inhibition. When the inhibitory effect is enhanced by pre-incubation in *in vitro* metabolism studies, it should be judged that there is time-dependent inhibition (TDI), and the k_{inact} value (maximum inactivation rate constant) and the K_I value (the concentration of the inhibitor that yields a 50% rate of the maximum inactivation) should be estimated. The estimated or measured value of the concentration of the unbound drug in the reaction mixture is used if the concentration of the unbound form of the investigational drug in the reaction mixture is expected to be markedly lower than the total concentration of the investigational drug. This applies also to cases where the investigational drug is likely to be prominently adsorbed to the test tube wall.

Positive control experiments should be conducted using *in vitro* marker drugs (inhibitors, Table 1-2) and compared to the values of inhibition parameters (reversible inhibition: K_i, TDI: K_I, k_{inact}) in literature evaluated in the same manner to confirm the validity of the study system.

d) In case of reversible inhibition, the R value is determined from the *in vitro* inhibition constant (K_i) and the maximum concentration [I] of the inhibitor (investigational drug or metabolite) achieved *in vivo*, according to Formula 1.

Formula 1

$R = 1 + [I] / K_i$

[I]: C_{max} (unbound concentration) or $[I]_g$: dose/250 mL

The maximum unbound concentration of the inhibitor in the systemic circulation should be used as [I], and a cutoff value of 1.02 should be used for the R value. When calculation of the Ki value is necessary, the ratio of binding to microsomes, etc., should be estimated or measured, as needed, to achieve correction for the Ki value based on the unbound concentration. When the protein binding ratio in blood is 99% or higher, in principle, unbound fraction should be set as 1%. In the case of orally administered drugs, the possibility of inhibition of P450 (example: CYP3A) that is highly expressed in the GI tract should be borne in mind. The use of dose (molar dose)/250 mL as the maximum concentration in the GI tract, $[I]_g$, may reflect the maximum concentration of the inhibitor more appropriately than the systemic blood concentration. If $[I]_g$ is used, a cutoff value of 11 should be used for the alternate R value ($R = 1 + [I]_g / K_i$).

e) Time-dependent inhibition (TDI)

The standard *in vitro* method of TDI evaluation involves pre-incubation of the investigational drug in the study system before adding the substrate. If the formation rate of the metabolite of the substrate decreases in a time-dependent manner, the parameters (k_{inact} and K_I) of TDI in *in vitro* studies should be calculated. The R value of TDI is dependent on the degradation rate constant (k_{deg}) of the inhibited enzyme, as well as on the concentration of the inhibitor and the parameters of TDI (k_{inact} and K_I) (Formula 2). (Cutoff value of 1.25 should be used for the R value.)

Formula 2

$R = (k_{obs} + k_{deg}) / k_{deg}$ where $k_{obs} = k_{inact} \times 50 \times [I] / (K_I + 50 \times [I])$,

In case of CYP3A in the GI tract, $k_{obs} = k_{inact} \times 0.1 \times [I]_g / (K_I + 0.1 \times [I]_g)$

[I]: C_{max} (unbound concentration) or $[I]_g$: dose/250 mL

K_I: concentration of the inhibitor that yields 50% of the maximum inactivation rate

k_{deg}: Degradation rate constant of the enzyme,

k_{inact}: maximum inactivation rate constant, k_{obs}: apparent inactivation rate constant

f) It is acceptable to directly proceed to the clinical drug interaction studies if the accuracy of the model-based predictions is considered insufficient.

g) MSPK model

Formula 3

$$AUCR = \left[\frac{1}{(A_h \times B_h \times C_h) \times f_m + (1 - f_m)}\right] \times \left[\frac{1}{(A_g \times B_g \times C_g) \times (1 - F_g) + F_g}\right]$$

A, B, and C in the formula 3 denote TDI, induction, and reversible inhibition, respectively, as described in the following supplemental table. F_g is the fraction of the investigational drug reaching the portal blood after absorption into the intestinal enterocytes, which is decreased when the investigational drug is metabolized there. Also, fm denotes the fraction of intrinsic metabolic clearance of the substrate mediated by P450 affected by inhibitor (or inducer) relative to the whole metabolic clearance of the liver.

Formula 3 (Supplemental table)

Time-dependent inhibition (TDI)	$A_h = \dfrac{k_{deg.h}}{k_{deg.h} + \dfrac{[I]_h \times k_{inact}}{[I]_h + K_I}}$	$A_g = \dfrac{k_{deg.g}}{k_{deg.g} + \dfrac{[I]_g \times k_{inact}}{[I]_g + K_I}}$
Induction	$B_h = 1 + \dfrac{d \cdot E_{max} \cdot [I]_h}{[I]_h + EC_{50}}$	$B_g = 1 + \dfrac{d \cdot E_{max} \cdot [I]_g}{[I]_g + EC_{50}}$
Reversible inhibition	$C_h = \dfrac{1}{1 + \dfrac{[I]_h}{K_i}}$	$C_g = \dfrac{1}{1 + \dfrac{[I]_g}{K_i}}$

The subscripts "h" and "g" denote the liver and gastrointestinal tract, respectively, while $[I]_h$ and $[I]_g$ denote the concentration of the investigational drug in the liver hepatocytes and intestinal enterocytes, respectively. In addition, "d" is the conversion factor obtained from the linear regression to the control data set.

Figure 1-3: Evaluation of the possibility of the investigational drug inducing drug metabolizing enzymes

```
┌─────────────────────────────────────────────────────────────────────────────┐
│ Does the investigational drug or metabolites induce drug metabolizing enzymes │
│ in in vitro studies[a)]?                                                       │
│ Evaluate with the methods of 1) to 3): 1) mRNA level[b)], 2) Correlation       │
│ method[c)], 3) R value[d)]                                                      │
└─────────────────────────────────────────────────────────────────────────────┘
```

Yes[e)]   No → ┌─────────────────────────────────────┐
                        │ No clinical drug interaction study   │
                        │ mediated by enzyme induction is   │
                        │ required.   │
                        └─────────────────────────────────────┘

```
┌─────────────────────────────────────────────────────────────────────────────┐
│ Is the AUCR≤0.8 in the mechanistic static pharmacokinetic (MSPK) model[f)] or  │
│ physiologically based pharmacokinetic (PBPK) model?                            │
└─────────────────────────────────────────────────────────────────────────────┘
```

Yes   No → ┌─────────────────────────────────────┐
       inconclusive   │ No clinical drug interaction study   │
                       │ is required when there is a clear   │
                       │ reason for the discrepancy from the │
                       │ judgment based on the R value, etc. │
                       └─────────────────────────────────────┘

```
┌─────────────────────────────────────────────────────────────────────────────┐
│ In clinical drug interaction studies, are there obvious effects on the         │
│ pharmacokinetics of the marker drug for the enzyme in question?                │
└─────────────────────────────────────────────────────────────────────────────┘
```

Yes   No → ┌─────────────────────────────────────┐
                       │ No further clinical drug interaction │
                       │ study is required.   │
                       └─────────────────────────────────────┘

```
┌─────────────────────────────────────────────────────────────────────────────┐
│ Consider the necessity of further clinical drug interaction study(ies) with    │
│ other substrate(s) (selected taking into consideration the therapeutic range   │
│ and possible concomitant use with the investigational drug) of the enzyme in   │
│ question.                                                                      │
└─────────────────────────────────────────────────────────────────────────────┘
```

a) Target drug metabolizing enzyme: CYP1A2, CYP2B6, CYP3A（CYP3A4 and CYP3A5）

   Add CYP2C9 etc., if necessary.

   Concentration setting should include concentrations of $50 \times C_{max}$ (unbound form) or $0.1 \times$ dose/250 mL.

In *in vitro* enzyme induction and down-regulation studies, use of changes in the mRNA expression level of the target gene as an evaluation item is recommended in order to avoid overlooking the enzyme induction effect because of enzyme inhibition by the investigational drug, using primary cultured hepatocytes (fresh or cryopreserved). Positive control experiments should be conducted using *in vitro* marker drugs (inducers, Table 1-3) to confirm the validity of the study system.

The concentration range of the investigational drug in the *in vitro* induction study varies according to their *in vivo* pharmacokinetics and should be set to include more than three levels of concentrations, including the maximum concentration predicted for hepatocytes *in vivo*, to determine induction parameters (EC_{50} and E_{max}). Usually, in the case of drugs that exert influences

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on hepatic enzymes, the concentration range should be set to include $50 \times C_{max}$ (unbound form) obtained in the steady state after administration of the maximum therapeutic dose or $0.1 \times$ dose/250 mL.

b) Evaluation based on mRNA level: The mRNA level is compared with that of the control (vehicle), and enzyme induction in *in vitro* studies is regarded as positive when increases in the mRNA level are concentration-dependent, showing an over 100% increase. When the observed concentration-dependent increases in mRNA expression are less than 100%, the results can be regarded as negative as long as the increase corresponds to less than 20% of that in the reaction with a positive control.

c) Evaluation using correlation method: Evaluation based on the results using positive or negative control by calculating relative induction score (RIS), $E_{max} \times [I]/(EC_{50} + [I])$, or $[I]/EC_{50}$ value using EC_{50} and E_{max} obtained in *in vitro* studies.

   [I] : C_{max} (unbound concentration)

   EC_{50}: Concentration that yields 50% of the maximum effect, E_{max}: Maximum induction effect

   Although it is possible to set arbitrary cutoff values using known positive control or negative control drugs in the evaluation based on the mRNA level and using the correlation method, the criteria should be set on the basis of experience with inducing drugs (positive control) and non-inducing drugs (negative control) supported by sufficient clinical evidence.

d) Evaluation based on the calculation of R value (Formula 4):

   Formula 4

   $R = 1/(1 + d \times E_{max} \times 10 \times [I]/(EC_{50} + 10 \times [I]))$

   [I] : C_{max} (unbound concentration)

   EC_{50}: Concentration that yields 50% of the maximum effect, E_{max}: Maximum induction effect,

   d: Conversion factor

   For evaluation based on the cutoff values, d = 1 is used. If R is 0.8 or less, the investigational drug in question is regarded as the enzyme-inducing drug.

e) May directly proceed to the clinical drug interaction studies if the accuracy of the model-based predictions is considered insufficient.

f) See Formula 3 (Figure 1-2, note g).

Figure 2-1: Evaluation of the possibility of the investigational drug being a substrate of transporter
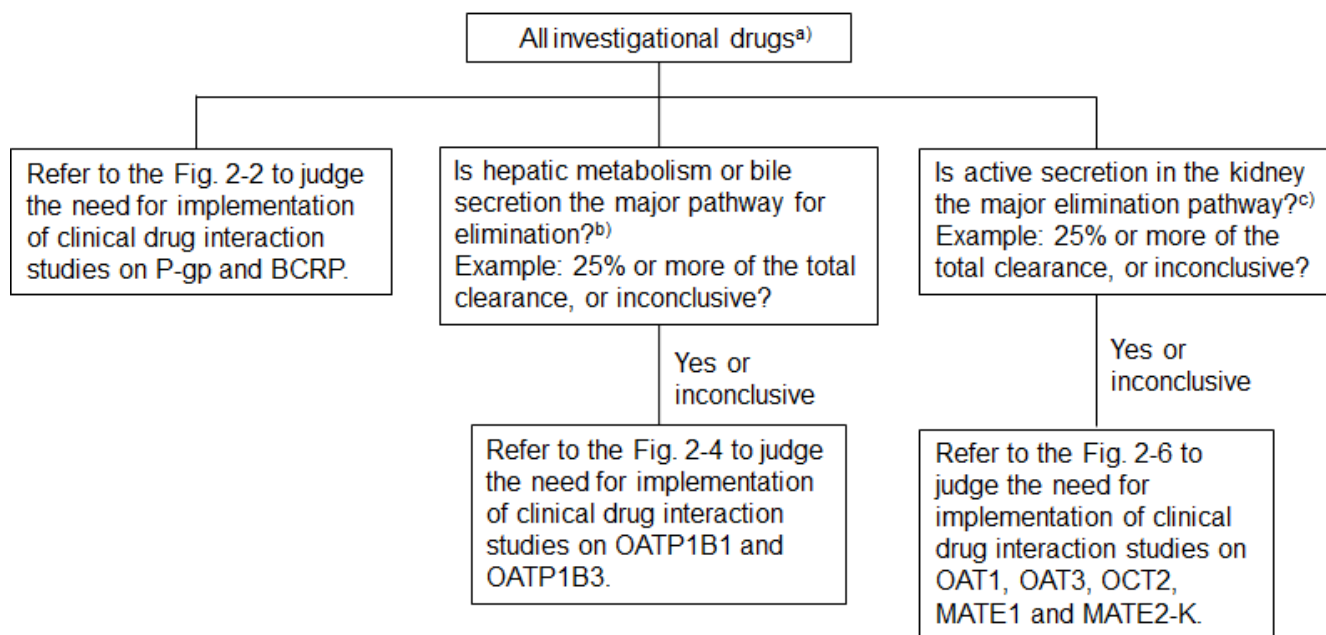
a) If necessary, it is considered to evaluate the transporter-mediated drug interaction for metabolites.

b) When an investigational drug for which hepatic elimination is important (hepatic metabolism clearance or biliary secretion in an unchanged drug accounts for 25% or more of the total clearance), it should be examined whether the drug is a substrate of OATP1B1 and OATP1B3. Biliary secretion clearance can be estimated from preclinical data (*in vitro* experiment with hepatocytes or *in vivo* ADME study using radiolabeled drugs) and data of non-renal clearance.

c) When there is an investigational drug for which renal tubular secretion is important (renal secretion clearance accounts for 25% or more of the total clearance), *in vitro* experiments should be performed to determine whether the drug is a substrate of OAT1, OAT3, OCT2, MATE1, and MATE2-K. The percentage of renal secretion clearance (%) in total clearance can be estimated using the formula $(CL_r\text{-}f_u*GFR)/CL_{total},$ assuming that there is no renal reabsorption (CL_r: Renal clearance, f_u: Fraction of unbound drug in blood, GFR: Glomerular filtration rate, CL_{total}: Total clearance)

Figure 2-2: Evaluation of the possibility of the investigational drug being a substrate of P-gp or BCRP
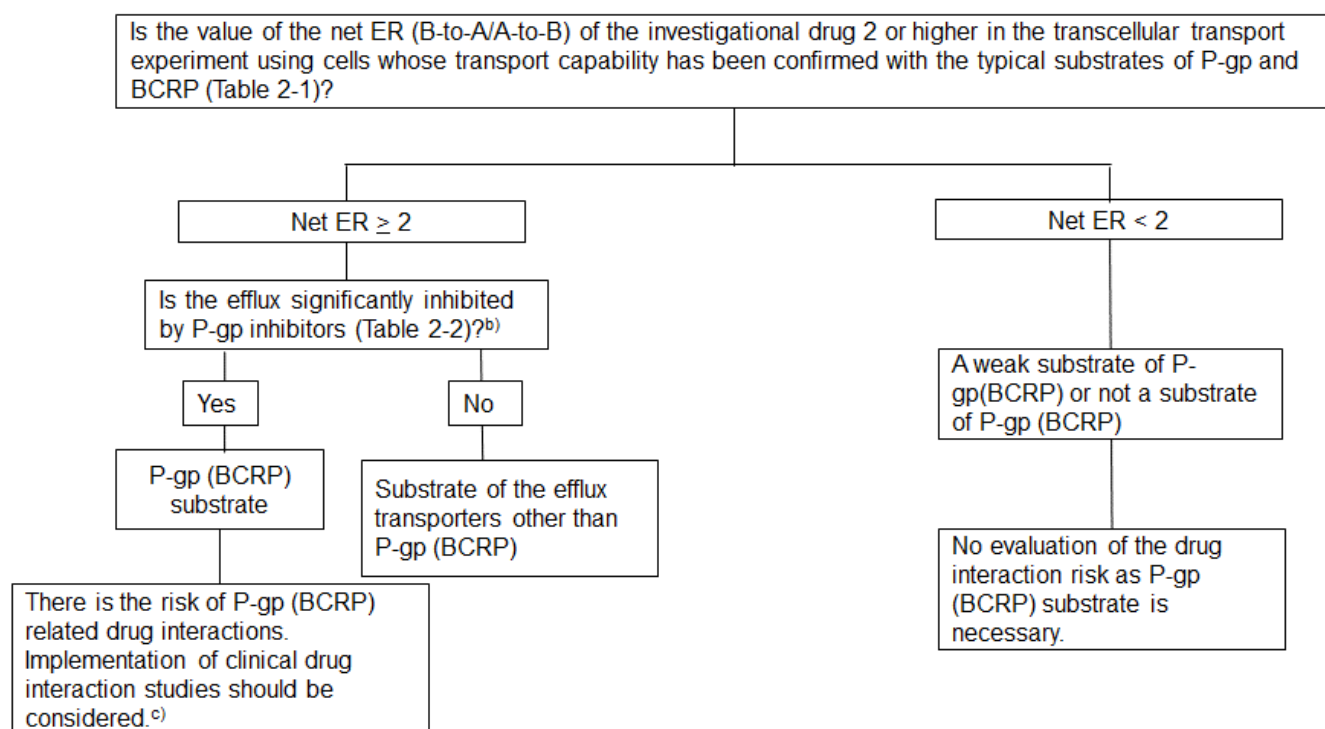
a) When evaluating the transport via efflux transporters such as P-gp and BCRP, permeability of the investigational drug from the apical side (A) to the basal side (B) should be compared with the permeability of the drug in the opposite direction, i.e., from B to A, and the efflux ratio (=B to A/A to B ratio, ER) should be calculated from the ratio of the permeability from B to A to that from A to B. If transporter-expressing cell lines are used, correction using the ER of non-expressing cells should be applied to calculate the net ER [= (ER of expressing cells)/(ER of non-expressing cells)]. If efflux transporter (P-gp etc.)-expressing cell lines are used, transport activity should be confirmed with the net ER of typical substrates as the index (Table 2-1), while if Caco-2 cells are used, transport activity should be confirmed with the ER of typical substrates (Table 2-1) as the index. If a net ER of 2 does not seem to provide clear results based on the previous experience with the cell lines used, a net ER other than 2 may be used as the cutoff value, or the relative ratio to the positive control may be used. In that case, an appropriate value should be set based on the investigational results of positive control (Table 2-1).

b) Net ER is around 1 or clearly reduced.

c) Because P-gp is involved in GI absorption, renal tubular secretion and brain distribution, the need for clinical drug interaction studies should be judged taking into consideration intestinal availability

(FaFg), presence/absence of renal tubular secretion, and the risk of CNS toxicity. If the FaFg is more than 80%, it is presumed that 1.25-fold or higher increase of AUC will not take place only by the P-gp inhibition in the GI tract. In the case of BCRP substrates, because a genetic polymorphism in BCRP (c.421C>A) with decreased transport function *in vivo*, whose allele frequency is relatively high in the Japanese, may cause inter-individual variability in their pharmacokinetics, examination of whether the drug plays as a substrate of BCRP *in vitro* using this decision tree is recommended. The experimental method should be in accordance with that of P-gp substrate studies. Typical substrates and inhibitors are shown in Table 2-1 and 2-2, respectively. If the investigational drug is a BCRP substrate, it is currently difficult to design clinical drug interaction studies using *in vivo*-usable typical inhibitors (Table 2-4). Therefore, in this case, only a description that the drug is a BCRP substrate should be provided at the moment.

Figure 2-3: Evaluation of the possibility of the investigational drug being an inhibitor of P-gp or BCRP
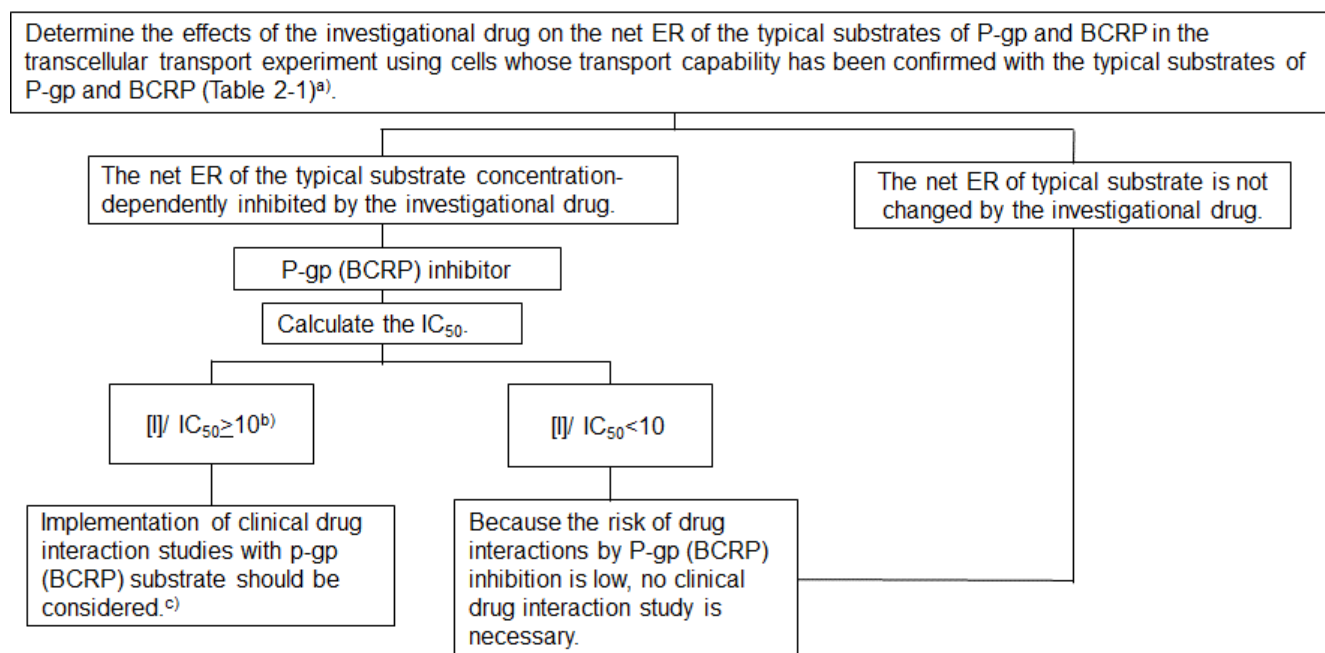

a) When evaluating the transport via efflux transporters such as P-gp and BCRP, permeability of the investigational drug from the apical side (A) to the basal side (B) should be compared with the permeability of the drug in the opposite direction, i.e., from B to A, and the flux ratio (=B to A/A to B ratio) should be calculated from the ratio of the permeability from B to A to that from A to B. If transporter-expressing cell lines are used, a correction using the ER of non-expressing cells should be applied to calculate the net ER [= (ER of expressing cells)/(ER of non-expressing cells)]. If efflux transporter (P-gp etc.)-expressing cell lines are used, transport activity should be confirmed with the net ER of typical substrates as the index (Table 2-1), while if Caco-2 cells are used, transport activity should be confirmed with the ER of typical substrates (Table 2-1) as the index. It should be verified that the net ER is reduced by the addition of known inhibitors to the extent that can be theoretically estimated by the concentration of the added inhibitor and the Ki value.

b) [I] should be set based on the expected maximum concentration (maximum single dose of inhibitors/250 mL) of the investigational drug in the lumen side of the GI tract. ER should be used as the index in the calculation of IC_{50}. The concentration of the typical substrate should be set as a value sufficiently lower than the Km value (Table 2-1). ER should be used as the index in the calculation of IC_{50}. When a correction using the ER of non-expressing cells cannot be performed

due to the effect of endogenous transporters in the evaluation using expressing cells, calculation using the ER of expressing cells alone is acceptable in some cases.

c) The typical substrate to be used *in vivo* should be selected in Table 2-3.

Figure 2-4: Evaluation of the possibility of the investigational drug being a substrate of OATP1B1 or OATP1B3
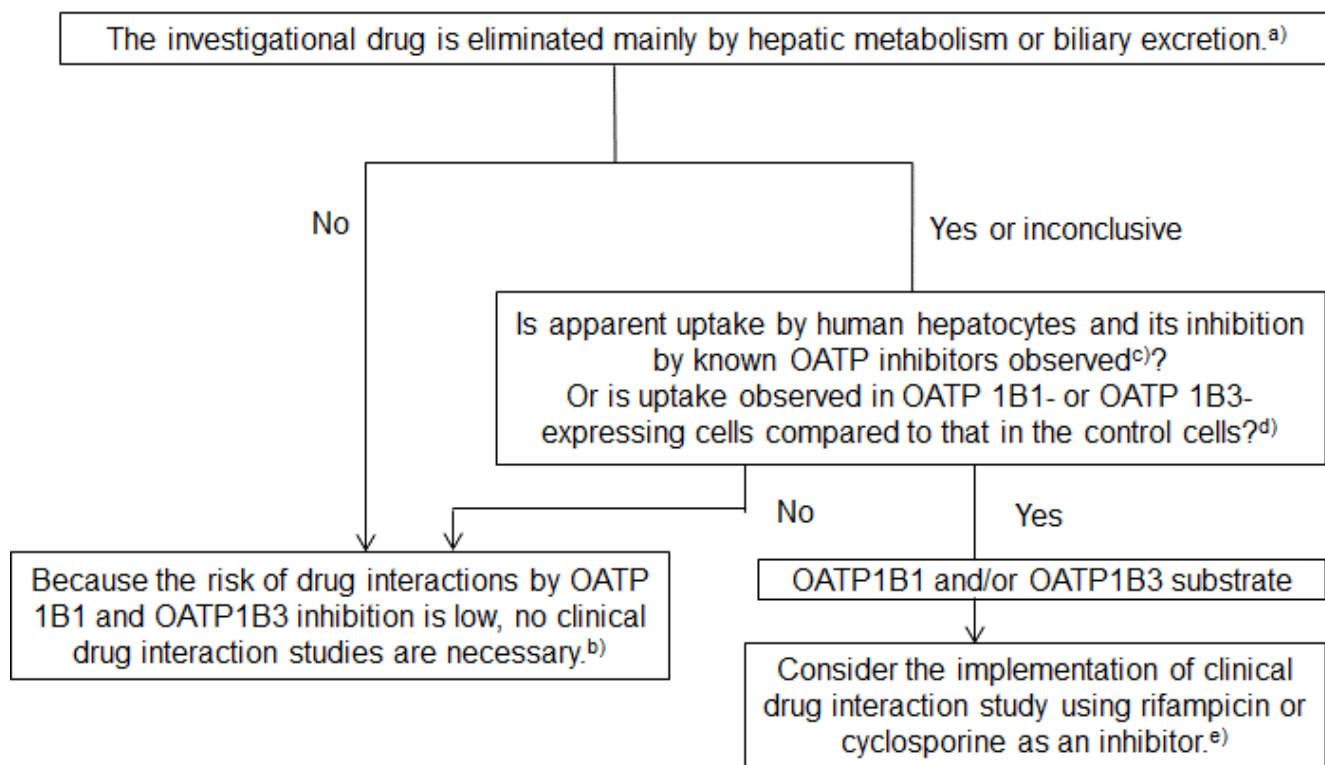
a) See Fig. 2-1.

b) Cases where the contribution of passive diffusion is high, leading to masked transport by OATP1B1 and/or OATP1B3, are included.

c) Human hepatocytes whose OATP1B1 and/or OATP1B3 transport activity has been confirmed should be used. The investigational drug is judged as a substrate of OATP1B1 and OATP1B3 when there is an uptake of the typical substrates (Table 2-1) and inhibition by typical inhibitors (Table 2-2) to the extent that can be theoretically estimated by the concentration of the added inhibitor and the K_i value.

d) When cell lines expressing OATP1B1 and OATP1B3 are used, the uptake of the investigational drug should be determined using cells in which the ratio of the uptake in the typical substrate (Table 2-1) in transporter-expressing cell line to that in a non-expressing cell line (uptake ratio) has been confirmed to be not less than 2-fold, and that uptake is inhibited by known inhibitors (Table 2-2) to the extent that can be theoretically estimated by the concentration of the added inhibitor and the K_i

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value. Under that condition, when the same condition as the above-described typical substrate is satisfied for the investigational drug, the investigational drug is judged as a substrate of OATB1B1 and OATP1B3. However, based on the previous experiences of the cell lines to be used, when it cannot be determined whether the investigational drug is a substrate or not based on the threshold value of the uptake ratio (the uptake ratio of the transporter-expressing cells to non-transporter-expressing cells is 2), another threshold value of the uptake ratio may be used. In the case where the investigational drug uptake into the transporter-expressing cells is less than 2-fold as compared to the uptake into the non-transporter-expressing cells due to nonspecific adsorption of investigational drug to culture equipment and cells etc, the investigational drug can be judged to play as a substrate if it is verified that the uptake is inhibited by a typical inhibitor to a degree that would allow theoretical estimation from the K_i value and the concentration of the added inhibitor. In the case of highly lipophilic compounds, it should be borne in mind that the uptake may be difficult to be detected in the cells expressing the transporter.

e)   Since rifampin exerts the inducible effect after its repetitive administration, single dose of rifampin should be performed for the inhibition of OATPs.

Figure 2-5: Evaluation of the possibility of the investigational drug being an inhibitor of OATP1B1 or OATP1B3

```
┌─────────────────────────────────────────────────────────────────────┐
│ Determine the effects of the investigational drug on the uptake of    │
│ the OATP1B1 and/or OATP1B3 typical substrates using cells expressing  │
│ the transporter or human liver cells whose transport capability has   │
│ been confirmed with the typical substrates of OATP1B1 and/or          │
│ OATP1B3 (Table 2-1).a)                                                 │
└─────────────────────────────────────────────────────────────────────┘
                                │
        ┌───────────────────────────────────────────────┐
        │ Is it $1+f_{u,b} \cdot I_{inlet,max}/K_i \geq 1.1$?b) │
        └───────────────────────────────────────────────┘
              │                                    │
             Yes                                   No
              │                                    │
┌──────────────────────────────┐   ┌──────────────────────────────┐
│ Implementation of clinical    │   │ Because the risk of drug      │
│ drug interaction studies      │   │ interactions by OATP1B1 and   │
│ with in vivo typical          │   │ OATP1B3 inhibition is low,    │
│ substrates of OATP1B1 and     │   │ no clinical drug interaction  │
│ OATP1B3 (Table 2-3) should    │   │ studies are necessary.        │
│ be considered.                │   │                               │
└──────────────────────────────┘   └──────────────────────────────┘
```

a) When hepatocytes are used in *in vitro* experimental systems, it should be confirmed that there is a clear uptake of the typical substrates (Table 2-1) and inhibition by typical inhibitors (Table 2-2) to the extent that can be theoretically estimated by the concentration of the added inhibitor and the K_i value. When cell lines expressing OATP1B1 and OATP1B3 are used, the uptake of the investigational drug should be determined using cells in which the ratio of the uptake in the typical substrate (Table 2-1) by transporter-expressing cell line to that by a non-expressing cell line (uptake ratio) has been confirmed to be not less than 2-fold, and that uptake is inhibited by known inhibitors (Table 2-2) to the extent that can be theoretically estimated by the concentration of the added inhibitor and the K_i value. It is recommended that substrates for OATP1B1 and OATP1B3 be selected taking into account the drugs that are concomitantly administered in the clinical situation. If the selection entails difficulty, a typical substrate for OATP1B1 and OATP1B3 (Table 2-1) may be utilized. For the determination of K_i value of the investigational drug, typical substrates can be selected from Table 2-1 and their recommended concentration should be sufficiently lower than their K_m value. Also, when calculating the K_i value, preincubation for 30 minutes or more is performed.

b) $I_{inlet,\ max}$ is the estimated maximum concentration of the inhibitor at the inlet to the liver, and it is equal to the $C_{max} + (k_a \times dose \times F_a\ F_g/Q_h)$. C_{max} is the maximum blood concentration of the inhibitor, dose is the dose of the inhibitor, F_aF_g is the intestinal availability of the inhibitor, k_a is the absorption rate constant of the inhibitor, and Q_h is the hepatic blood flow rate (example: 97 L/hr/70kg). If the

F_aF_g and k_a values are unknown, 1 and 0.1 min^{-1} can be used as the values for the F_aF_g and k_a, respectively, because false-negative prediction can be avoided by the use of theoretically maximum values. $f_{u,b}$ is blood unbound fraction of drugs. In the case of drugs whose $f_{u,b}$ values are less than 0.01 or protein binding are very high, resulting in an inaccurate determination of the $f_{u,b}$ value, it should be assumed that the $f_{u,b}$ equals 0.01 to avoid false-negative predictions.

Figure 2-6: Evaluation of the possibility of the investigational drug being a substrate of OAT1, OAT3, OCT2, MATE1 or MATE2-K
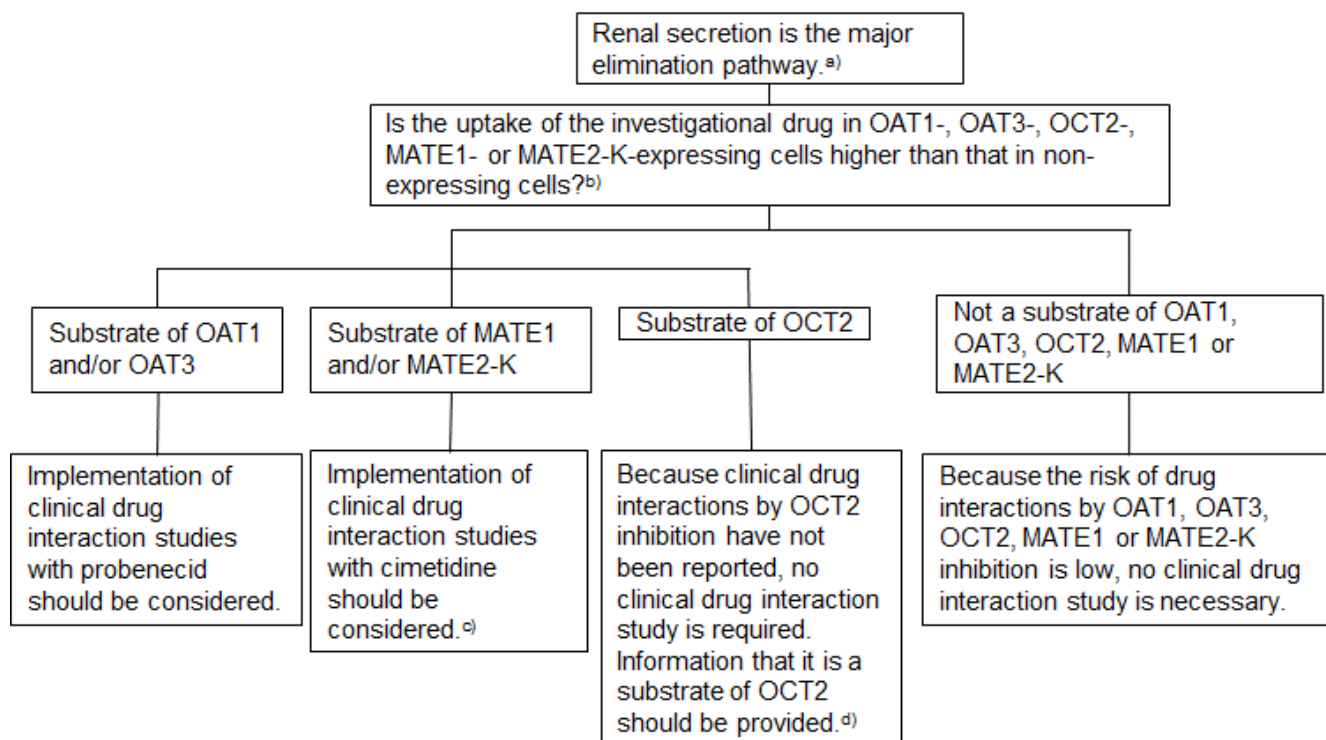
a) See Fig. 2-1.

b) The uptake of the investigational drug should be determined using cells in which the ratio of the uptake in the typical substrate (Table 2-1) by transporter-expressing cell line to that by a non-expressing cell line (uptake ratio) has been confirmed to be not less than 2-fold, and that uptake is inhibited by known inhibitors (Table 2-2) to the extent that can be theoretically estimated by the concentration of the added inhibitor and the K_i value. The investigational drug is judged as a substrate of the target transporter when the uptake of the investigational drug into transporter-expressing cell line has been confirmed to be not less than 2-fold as compared to the uptake into the non-transporter-expressing cells, and that uptake is inhibited by known inhibitors (Table 2-2) to the extent that can be theoretically estimated by the concentration of the added inhibitor and the K_i value. However, if previous experiences with the cell lines used suggest that an uptake ratio of 2 (ratio of uptake by the transporter-expressing cells to that by non-transporter-expressing cells) does not provide clear results, another threshold value of the uptake ratio may be used. In the case where the investigational drug uptake into the transporter-expressing cells is less than 2-fold as compared to that into the non-transporter-expressing cells due to adsorption of investigational drug or any other reasons, the investigational drug can be judged to play as a substrate if it is verified that the

uptake is significantly reduced by a typical inhibitor to a degree that would allow theoretical estimation from the K_i value and the concentration of the added inhibitor. In the case of highly lipophilic compounds, it should be borne in mind that the uptake may be difficult to be detected in the cells expressing the transporter.

Meanwhile, in the cases of MATE1 and MATE2-K, since the driving force is the proton gradient with a lower proton concentration inside the cells, it is possible to evaluate the efflux transport activity *in vivo* by the *in vitro* uptake study after acidifying the intracellular compartment compared with the extracellular compartment (e.g., preincubating MATE-expressing cells with ammonium chloride, or alkalifying the extracellular pH to approx. 8.4 in the uptake experiment). It is also possible to use membrane vesicles prepared from MATE1- or MATE2-K-expressing cells instead of MATE1- or MATE2-K-expressing cell lines. In such instances, acidification of the intra-vesicular compartment is necessary to provide driving force of the transport.

c) Since MATE1 and MATE2-K are involved in the renal excretion of drugs, it should be borne in mind that the kidney concentration may be increased even if the blood concentration remains unchanged.

d) If the investigational drug is an OCT2 substrate, it is currently difficult to design clinical drug interaction studies using *in vivo*-usable typical inhibitors (Table 2-4). Therefore, in this case, only a description that the drug is an OCT2 substrate should be provided at the moment.

Figure 2-7: Evaluation of the possibility of the investigational drug being an inhibitor of OAT1, OAT3, OCT2, MATE1 or MATE2-K
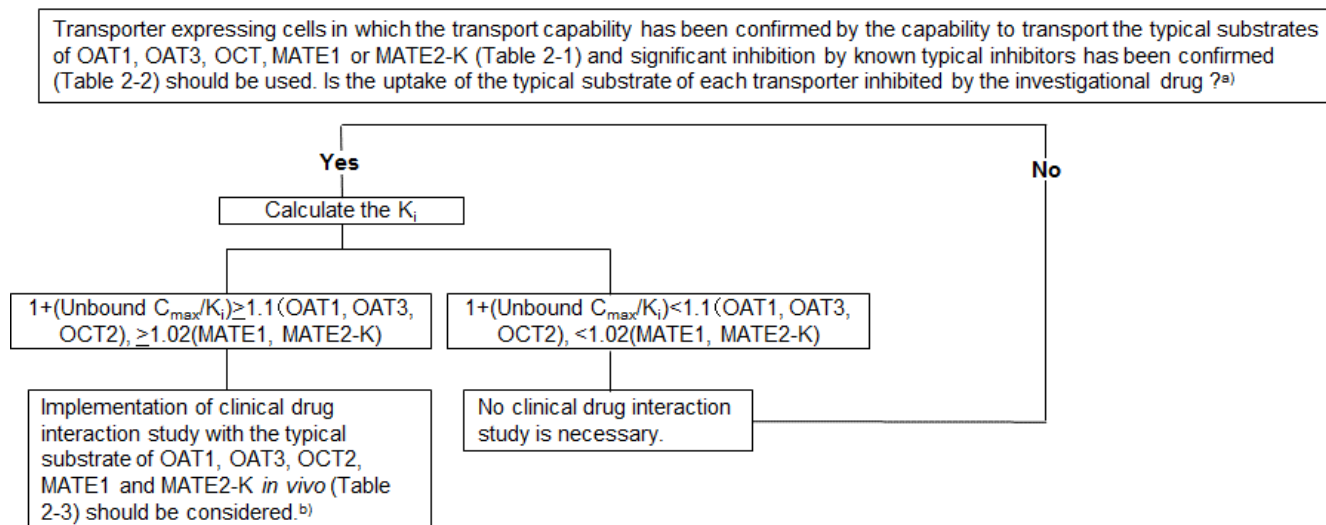
Transporter expressing cells in which the transport capability has been confirmed by the capability to transport the typical substrates of OAT1, OAT3, OCT, MATE1 or MATE2-K (Table 2-1) and significant inhibition by known typical inhibitors has been confirmed (Table 2-2) should be used. Is the uptake of the typical substrate of each transporter inhibited by the investigational drug ?[a]

Yes

No

Calculate the K_i

$1+$(Unbound C_{max}/K_i)$\geq$1.1 (OAT1, OAT3, OCT2), $\geq$1.02 (MATE1, MATE2-K)

$1+$(Unbound C_{max}/K_i)$<$1.1 (OAT1, OAT3, OCT2), $<$1.02 (MATE1, MATE2-K)

Implementation of clinical drug interaction study with the typical substrate of OAT1, OAT3, OCT2, MATE1 and MATE2-K *in vivo* (Table 2-3) should be considered.[b]

No clinical drug interaction study is necessary.

a) The uptake of the investigational drug should be determined using cells in which the ratio of the uptake in the typical substrate (Table 2-1) by transporter-expressing cell line to that by a non-expressing cell line (uptake ratio) has been confirmed to be not less than 2-fold, and that uptake is inhibited by known inhibitors (Table 2-2) to the extent that can be theoretically estimated by the concentration of the added inhibitor and the K_i value. Meanwhile, in the cases of MATE1 and MATE2-K, since the driving force is the proton gradient with a lower proton concentration inside the cells, it is possible to evaluate the efflux transport activity *in vivo* by the *in vitro* uptake study after acidifying the intracellular compartment compared with the extracellular compartment (e.g., preincubating MATE-expressing cells with ammonium chloride, or alkalifying the extracellular pH to approx. 8.4 in the uptake experiment). It is recommended that a substrate for the transporter be selected taking into account the drugs that are concomitantly administered in the clinical situation. If the selection entails difficulty, a typical substrate for the transporter (Table 2-1) may be utilized. For the determination of K_i (IC_{50}) value of the investigational drug, typical substrates can be selected from Table 2-1 and their recommended concentration should be sufficiently lower than their K_m value (see Table 2-1).

b) Inhibition of MATE1 and/or MATE2-K may cause an increase in the kidney concentration while causing no changes in the blood concentration.

11.3 Examples of substrates, inhibitors, and inducers

Table 1-1 Examples of *in vitro* P450 enzyme marker reactions

Enzyme	Marker reaction
CYP1A2	phenacetin *O*-deethylation, 7-ethoxyresorufin-*O*-deethylation
CYP2B6	efavirenz hydroxylation, uupropion hydroxylation
CYP2C8	paclitaxel 6α-hydroxylation, amodiaquine *N*-deethylation
CYP2C9	*S*-warfarin 7-hydroxylation, diclofenac 4'-hydroxylation
CYP2C19	*S*-mephenytoin 4'-hydroxylation
CYP2D6	bufuralol 1'-hydroxylation, dextromethorphan *O*-demethylation
CYP3A*	midazolam 1'-hydroxylation, testosterone 6β-hydroxylation

* CYP3A inhibition should be evaluated using multiple marker substrates which differ in the substrate binding site on the enzyme.

Table 1-2 Examples of *in vitro* P450 inhibitors

Enzyme	Inhibitor
CYP1A2	α-naphthoflavone, furafylline*
CYP2B6**	sertraline, phencyclidine*, thiotepa*, ticlopidine*
CYP2C8	montelukast, quercetin, phenelzine*
CYP2C9	sulfaphenazole, tienilic acid*
CYP2C19**	*S*-(+)-N-3-benzyl-nirvanol, nootkatone, ticlopidine*
CYP2D6	quinidine, paroxetine*
CYP3A	itraconazole, ketoconazole, azamulin*, troleandomycin*, verapamil*

* Time-dependent inhibitors.

**At present, there is no known selective inhibitor that can be used *in vitro*. Although the inhibitors cited here are not selective, they can be used with other information such as metabolic activity of the substrate in a single enzyme system.

Table 1-3 Examples of *in vitro* P450 inducers

Enzyme	Inducer*
CYP1A2	omeprazole, lansoprazole
CYP2B6	phenobarbital
CYP2C8	rifampicin

CYP2C9	rifampicin
CYP2C19	rifampicin
CYP3A	rifampicin

* This table only provides examples, and does not provide an exhaustive list.

Table 1-4. Examples of *in vivo* P450 substrates (clinical index substrates)

Enzyme	**S**ubstrates
CYP1A2	caffeine, tizanidine
CYP2B6 *	-
CYP2C8	repaglinide
CYP2C9	（tolbutamide）, s-warfarin
CYP2C19	lansoprazole **), omeprazole
CYP2D6	（desipramine）, dextromethorphan, （nebivolol）
CYP3A	midazolam, triazolam

Drugs unapproved or discontinued in Japan are shown in parentheses.–

* Examples of clinical index substrates of CYP2B6 cannot be presented at present.

** lansoprazole is a substrate moderately susceptible to pharmacokinetic drug

interactions whose AUC increases by >2-fold but <5-fold (or a decrease in the

CL/F to <1/2 but >1/5) when co-administered with a strong inhibitor.

S-lansoprazole is a sensitive substrate, whose AUC in CYP2C19 extensive

metabolizer (EM) subjects increases by ≥5-fold (or a decrease in the CL/F to <1/5)

when co-administered with a strong inhibitor.

Substrates other than lansoprazole are substrate susceptible to pharmacokinetic

drug interactions whose AUC increases by ≥5-fold (or a decrease in the CL/F to

<1/5) when co-administered with a strong inhibitor.

Table 1-5. Examples *of in vivo* P450 inhibitors (clinical index inhibitors)

Enzyme	**Strong index inhibitors, *Moderate index inhibitors**
CYP1A2	Fluvoxamine
CYP2B6**	-
CYP2C8	clopidogrel, （gemfibrozil）
CYP2C9	fluconazole*****
CYP2C19	Fluvoxamine

| CYP2D6 | （fluoxetine）, mirabegron**, paroxetine |
| CYP3A | clarithromycin, erythromycin**, fluconazole**, itraconazole, verapamil** |

Drugs unapproved or discontinued in Japan are shown in parentheses.

**Examples of clinical index inhibitors of CYP2B6 cannot be presented at present.

Table 1-6. Examples of *in vivo* P450 inducers (clinical index inducers)

Enzyme	Strong index inducers, *Moderate index inducers
CYP1A2**	
CYP2B6	rifampicin *
CYP2C8	rifampicin *
CYP2C9	rifampicin *
CYP2C19	rifampicin
CYP3A	phenytoin , rifampicin

** Examples of clinical index inducer of CYP1A2 cannot be presented at

present.

Table 2-1 Examples of *in vitro* typical substrates of transporters

Transporters	Gene	Typical substrate	K_m value*
P-gp	*ABCB1*	digoxin[a)]	73-177 μM (Caco-2 cell)
		fexofenadine[b,c,d)]	150 μM
		loperamide	(1.8-5.5 μM)
		quinidine	1.69 μM
		talinolol[c)]	(72 μM)
		vinblastine[c)]	19-253 μM
BCRP	*ABCG2*	2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)[c,e)]	
		coumestrol	
		daidzein	
		dantrolene	
		estrone-3-sulfate[b,f)]	2.3-13 μM
		genistein	
		prazosin[e)]	
		sulfasalazine	0.7 μM
OATP1B1, OATP1B3	*SLCO1B1, SLCO1B3*	cholecystokinin octapeptide(CCK-8)[g)]	3.8-16.5 μM (1B3)
		estradiol-17β-glucuronide [h)]	2.5-8.3 μM (1B1), 15.8-24.6 μM (1B3)
		estrone-3-sulfate [i)]	0.23-12.5 μM (1B1)
		pitavastatin[c,e,f,j)]	1.3-6.7 μM (1B1), 3.25μM (1B3)
		pravastatin[c,f,k)]	11.5-85.7μM (1B1)
		telmisartan[l)]	0.81 μM (1B3)
		rosuvastatin[c,f,j,k)]	0.802-15.3 μM (1B1), 9.8-14.2 μM (1B3)
OAT1	*SLC22A6*	adefovir	23.8-30 μM
		p-aminohippurate	4-20 μM
		cidofovir	30-58 μM
		tenofovir	14.6 – 33.8 μM
OAT3	*SLC22A8*	benzylpenicillin[b,c)]	52 μM
		estrone-3-sulfate [j,m)]	2.2-75 μM

		pravastatin[b,c]	27.2 μM
MATE1, MATE2-K	*SLC47A1, SLC47A2*	metformin[n]	202-780 μM (MATE1), 1050-1980 μM (MATE2-K)
		1-methyl-4-phenylpyridinium (MPP+)[n]	100 μM (MATE1), 110 μM (MATE2-K)
		tetraethylammonium (TEA)[n]	220-380 μM (MATE1), 760-830 μM (MATE2-K)
OCT2	*SLC22A2*	metformin[n]	680-3356 μM
		1-methyl-4-phenylpyridinium (MPP+)[n]	1.2-22.2 μM
		tetraethylammonium (TEA)[n]	33.8-76 μM

* Figures in parentheses represent the K_i or IC_{50} values.

a) Substrates of OATP1B3

b) Substrates of OATPs

c) Substrates of MRP2

d) Substrates of MRP3

e) P-gp substrate

f) NTCP substrate

g) Selective substrate of OATP1B13 (vs. OATP1B1)

h) It has been confirmed that the K_i value calculated when performing the inhibition study using this substrate is similar to the K_i value calculated from the combination of the substrate and inhibitor in which clinical drug interactions were confirmed.

i) Selective substrate of OATP1B1 (vs. OATP1B3). However, it is reported that the K_i value from the inhibition study using this substrate tends to be estimated to be larger than K_i value calculated from the combination of the substrate and inhibitor in which clinical drug interactions were confirmed. Therefore, there is a need to pay attention to an interpretation of the results of inhibition study.

j) Substrate of BCRP

k) Substrate of OAT3

l) Selective substrate of OATP1B1 (vs. OATP1B1)   Consider addition of albumin into experimental system to decrease the nonspecific adsorption.

m) Substrates of OATP1B3

n) Substrates of OCTs and MATEs.

Table 2-2 Examples of *in vitro* typical inhibitors

Transporters	Gene	Inhibitor	K_i or IC₅₀ value*
P-gp	*ABCB1*	ciclosporine[a]	0.5-2.2 μM
		elacridar (GF120918)[b]	0.027-0.44 μM
		ketoconazole[c]	1.2-6.3 μM
		quinidine[d]	3.2-51.7 μM
		reserpine[e]	1.4-11.5 μM
		ritonavir [f]	3.8-28 μM
		tacrolimus [f]	0.74 μM
		valspodar (PSC833)[e,k]	0.11 μM
		verapamil[d]	2.1-33.5 μM
		zosuquidar (LY335979)	0.024-0.07 μM
BCRP	*ABCG2*	elacridar (GF120918)[g]	0.31 μM
		fumitremorgin C [k]	0.25-0.55 μM
		Ko134[,k]	0.07 μM
		Ko143[,k]	0.01 μM
		novobiocin [k]	0.063 - 0.095 μM
		sulfasalazine [k]	0.73 μM
OATP1B1, OATP1B3	*SLCO1B1, SLCO1B3*	ciclosporine[c,e,g,i]	0.24-3.5 μM (1B1)[i], 0.06-0.8 μM (1B3)
		estradiol-17 β -glucuronide[b,e]	2.5-8.3 μM (1B1), 15.8-24.6 μM (1B3)
		estrone-3-sulfate [b,c]	0.2-0.79 μM (1B1), 97.1 μM (1B3)
		rifampici[C]	0.48-17 μM (1B1), 0.8-5 μM (1B3)
		rifamycin SV	0.17-2 μM (1B1), 3 μM (1B3)
OAT1, OAT3	*SLC22A6, SLC22A8*	benzylpenicillin	1700 μM (OAT1), 52 μM (OAT3)
		probenecid[f]	3.9-26 μM (OAT1), 1.3-9 μM (OAT3)
MATE1, MATE2-K	*SLC47A1, SLC47A2*	cimetidine [d,j]	1.1-3.8 μM (MATE1), 2.1-7.3 μM (MATE2-K)
		pyrimethamine	77 nM (MATE1), 46 nM (MATE2-K)
OCT2	*SLC22A2*	cimetidine[h,j]	95-1650μM
		1-methyl-4-phenylpyridinium (MPP+)[h]	(1.2-22.2 μM)
		tetraethylammonium (TEA)[h]	144 μM

* Figures in parentheses represent the K_m values.

a) Substrates of MRP2, BCRP, NTCP, and OATPs.

b) BCRP inhibitor

c) NTCP inhibitor

d) OCTs inhibitor

e) MRP2 inhibitor

f) OATPs inhibitor

g) P-gp inhibitor

h) Substrate and inhibitor of MATEs

i) Preincubation of the inhibitor with transporter-expressing cells (human hepatocytes) before inhibition study has been reported to decrease the K_i value

j) OAT3 inhibitor

k) Selective inhibitor

Table 2-3 Examples of *in vivo* typical substrates of transporters

Transporter	Gene	Typical Substrate*
P-gp	*ABCB1*	aliskiren, ambrisentan, colchicine, dabigatran etexilate,digoxin, evelolimus, fexofenadine[a], lapatinib, maraviroc, nilotinib, saxagliptin, sirolimus, tolvaptan, topotecan
BCRP	*ABCG2*	imatinib, rosuvastatin[b], sulfasalazine
OATP1B1 OATP1B3	*SLCO1B1* *SLCO1B3*	asunaprevir, atorvastatin, bosentan, docetaxel, fexofenadine, glibenclamide, nateglinide, paclitaxel, pitavastatin[c], pravastatin[d], repaglinide, rosuvastatin[b], simvastatin acid
OAT1 OAT3	*SLC22A6* *SLC22A8*	adefovir, bumetanide, cefaclor, ceftizoxime, ciprofloxacine, famotidine, fexofenadine, furosemide, ganciclovir, methotrexate, oseltamivir carboxylate, zidovudine
MATE1, MATE2-K, OCT2	*SLC47A1, SLC47A2, SLC22A2*	metformin

a) It has been reported that OATP1B1, OATP1B3, MRP2 and MRP3 contribute to the hepatic clearance, and OAT3, MATE1 and MATE2-K contribute to the renal clearance of fexofenadine.

b) It has been reported that BCRP contributes to the intestinal absorption, OATP1B1, OATP1B3 and NTCP contribute to the hepatic uptake, and OAT3 contributes to the renal clearance of these drugs. These drugs have also been shown to be substrates of P-gp and MRP2 *in vitro*.

c) Pitavastatin is also a substrate of P-gp, MRP2 and BCRP *in vitro*.

d) MRP2 and OAT3 contribute to biliary and renal excretion, respectively.

Table 2-4 Examples of *in vivo* typical inhibitors of transporters

Transporter	Gene	Typical Inhibitor*
P-gp	*ABCB1*	amiodarone, azithromycin, carvedilol, clarithromycin[a], ciclosporine A[a], itraconazole, lapatinib, lopinavir/ritonavir, quinidine, ritonavir, （saquinavir/ritonavir）, （telaprevir）, （tipranavir/ritonavir）, verapamil
BCRP	*ABCG2*	curcumin[b], eltrombopag
OATP1B1, OATP1B3	*SLCO1B1, SLCO1B3*	（atazanavir/ritonavir）, clarithromycin, ciclosporine A[c], erythromycin, lopinavir/ritonavir, rifampicin[d], simeprevir
OAT1, OAT3	*SLC22A6, SLC22A8*	probenecid
MATE1, MATE2-K, OCT2	*SLC47A1, SLC47A2 SLC22A2*	cimetidine, dolutegravir, (pyrimethamine), trimethoprim, vandetanib

± Drugs such as unapproved or discontinued in Japan are shown in parentheses.

a)　These drugs inhibit OATP1B1 and OATP1B3 at clinical blood concentrations.

b)　A supplement

c)　This drug has been reported to also inhibit intestinal P-gp at clinical blood concentrations.

d)　Single-dose administration is necessary to eliminate the influence of induction of the transporter by repeated administration.

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 representing 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.

27) van Giersbergen PL, Treiber A, Schneiter R, Dietrich H, Dingemanse J.: Inhibitory and inductive effects of rifampin on the pharmacokinetics of bosentan in healthy subjects. Clin Pharmacol Ther. 2007;81:414-

9.

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).

<Case Example 1 (A drug inhibiting CYP3A)>

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.

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

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

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		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.