by two years later, on in vivo evaluation.

And the science has progressed, and we have a look at the literature; we have various workshops to continue to look at emerging issues, and we also have meetings with this Committee. For example, in 2003, we have discussed standardization procedures, including classification of CYP3A inhibitors, and, at that time, we also discussed the need to evaluate a new molecular entity of inhibition of potential on P-gp.

That same year, we also discussed additional CYPS that may be needed to be evaluated in addition to the major CYPs that at that time we have recommended to evaluate, including CYPs 1A2, 2C9, 2C19, 2D6, and 3A.

And in October 2004, we published a concept paper incorporating all the discussions and the literature up to that point, and, again, we discussed at this Committee in that the relevant principal drug interactions we touched upon P-gp and transporter-based interactions.

But based on the recommendation of the Committee, and also we have received about a dozen comments from the public because our comment paper was posted on the net, so based on those comments, we have published this draft guidance last

month, and this will be for public comment for about two months, and we do plan to finalize it early next year.

So the key message is that this new draft guidance, as we discussed, for the first time that we think transport and transporter-based interaction is key in addition to metabolism and metabolism-based interactions to risk-benefit assessment.

We discussed earlier in '99 guidance that we should use an integrated approach, starting with in vitro, followed by in vivo for CYP-based interactions, and we're now trying to use this approach for transporter-based interactions as well.

We have a lot more detailed discussion on study design data analysis, because they're key to proper labeling, and this is one of the reasons that our guidance has grown from 20 pages to about 51 pages is because we give detailed recommendations.

Since our guidance, and we discussed the exposure changes due to interaction with multi pharmacokinetic based, so we need to emphasize again the clinical significance of these PK changes, but they 50 percent increase in AUV, a hundred percent increase, what are their clinical

significance will need to be based on exposure-response relationship.

So for two different drugs, the same extent of interaction may be different.

But we also have a classification of CYP inhibitors of the major CYPs, and pharma's white paper in 2003 proposed to classify CYP3A, and we have expanded to classify all CYP -- all major CYP enzymes that was recommended to the evaluate the gene, and in addition, we also extended the classification of the substrate. So we have designated

sensitive substrates or substrates and their therapeutic range for each of the CYPs.

Again, we mentioned that labeling language needs to be useful and consistent and needs to be conforming with the new labeling rule that was published January of this year, and which went into effect in June. So we discuss when a drug interaction will need to be put on the highlights section, which is the new section for our professional labeling.

So what's new in this guidance? We discussed detailed study designs, including specific inhibitors, substrates, inducers for each CYP, and we have tables in this draft

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guidance. And these tables are also listed on the Web site, which is online in May. And we will be updating regularly, and we already updated once after this was online.

For transporters, we only did this for PTP phase and what we have recommended substrates, inhibitors, and inducers for both in vitro and in vivo evaluation.

However, for other transporters, such as organic anionic transporter peptides in breast cancer with the protein, associated protein, organic -- transporters, we only have very general recommendations on substrate inhibitors, inducers, and we did them separate like the others and specific recommendations for in vitro versus in vivo.

Based on this Committee's recommendation that we should have a model or decision tree to see when we need to conduct an in vivo study, based on in vitro data, so we have detailed appendices to each delineating when we need to do an in vivo study of the substrate of a CYP enzyme or an inhibitor or an inducer.

And we also proposed criteria, too, for further in vivo study; for example, phospho concentration with the inhibition constant, and we set up a threshold; we proposed

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a threshold for public comment. So if the threshold is more than .1, then you can do an in vivo study.

Similarly, for inducer we said if the intrigue and in-lab activities is more that 40 percent of your positive results, then you can do a study.

So we try to do it similar decision treatment. This is again also based on the Committee's recommendation for evaluation of Pg-P based interactions.

So we have two decision trees, one each to determine if an in vivo study is necessary, if the in vitro data show that it was substrate.

But how do we determine that it is a substrate? So we're coming up with some recommendations, and this will be discussed in more detail for substrate or an inhibitor.

We only mentioned very briefly on inducer for Pg-P. I mentioned earlier we have proposed classifications for CYP inhibitors and substrates. This will be helpful for study design and cross labeling at level and inducing, which you'll see in later slides. However, we have not had a similar classification system for either Pg-P or other transporters.

There are other new issues that discuss this draft guidance. We have discussed the importance of protocol restrictions, such as the subject intake of juice or dietary supplements when evaluating clinical interaction. This will be applicable to both a CYP enzyme and transporter interaction.

At the last Committee meeting, we have discussed whether there may be a need to do a multiple inhibitor study, so you can block the major metabolic pathways. And in this guidance, we have to delineate more on what -- under what conditions a multi-inhibitor study to block metabolic pathways may be needed.

It's -- some of example that we'll illustrate here and by the other speakers. We also may need to consider blocking off metabolic pathways and transporter pathways.

We had a lot of questions from sponsors or reviewers on when would it be appropriate to use a cocktail approach. So you can have a mixture of substrates that you take -- the subject will take with the new drug to evaluate the new drug's induction or inhibition potential.

So we have discussed when would this study be appropriate, and also the results for labeling.

And we have recently more requests on whether a cocktail study, including transporter substrates, along with CYP substrates, is appropriate, and we'll hear -- discuss more within the separate settings, not today.

So why do we want to study transporters? Why transporter-based interaction is important for risk-benefit assessment?

Later on, you will hear more clinical examples or expert opinion from other speakers, but we know from this rapidly growing literature -- we know transporters being involved in distribution transport of drugs, and they're important for intake, efflux uptake cells, which use energy and here's the pusher that represents your energy. But we also need to consider the concurrent event that you may have metabolic pathways and transporter pathways; either they are concurrent in the same direction or they're in opposite direction, and what is the net effect.

As you can see from this diagram, there are many transporters that we identified as present in major organs and tissues, such as the small intestine, liver, kidneys, brain. We can see a Pc-P is in all major organs.

So what is the role of Pc-P transporters-based interaction? And I think based on what we see in the publication now, it's only the tip of the iceberg. We have a lot more to learn.

If you look at this -- a recent survey. This we look at the bio system, we're in track for citation of these -- either the proteins or the genes that you can code in these transporters cited in papers or patents. You can see that the MDR1, the publication -- the citation that you see has almost doubled in the last 10 years. The other transporters, BCRP, OCT, MRP2, or OAT, OATP1B1, those

citations are smaller, but they're growing much more rapidly than MDR1.

So based on the growing data, publications, discussions on P-gp or other transporter-based interactions, how do we evaluate are the important. As Dr. Lesko mentioned earlier this morning, do we just wait for it to happen, some interactions that we could not explain as CYP-based, and would we say perhaps are transporter-based. Do we wait for them to happen or do we predict and try to anticipate a possible interaction.

So we have proposed decision trees to evaluate transporter-based interactions. And since we know the most, the most data evaluation, so we proposed -- the decision

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tree is based on P-gp, and perhaps this can be a model for evaluation of all the transporters.

So I will show you two decision trees, and the first one is to determine when we need to evaluate in vivo, if the in vitro indicated that it was an inhibitor of P-gp.

So our recommendation is to use bi-directional transporter in vitro assays and look at the next flux of a probe substrate when it's given with this investigational drug. So if the concentration of drug that we're evaluating increased, but the net flux of probe substrate does not change, we think it's probably important for non-inhibitors.

However, if the net flux decreased with increased concentration of this drug, then we think it's possible of an inhibitor, but we'd like to determine the IC50 or KI.

Our initial decision tree was based on absolute IC values to see the next step, and we have feedback, because we posted on the Net that we should compare this IC50 or KI to plasma concentration for these extended exposures.

So here we put out something for comment, and this is following the proposal that was used for a CYP inhibitor. So if the concentration compared to the IC50 is less than

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.1, then we don't think that it is an inhibitor, and an in vivo study would not be needed.

However, if it's more than .1, then we think an in vivo interaction with the P-gp substrate, such as Digoxin, is recommended.

And I mentioned earlier, after we put out this draft guidance, we already had received early comments from individual sponsors who thought that .1 perhaps was too arbitrary. Is it better than our initial recommendations that just look at IC50 or KI up to 10 micromoles, and the way we get input.

This is very similar to a CYP enzyme to us. We have discussed many times I over IC50, whether it should be one or two. That was our original recommendation, or should it be .1. And in addition, there is some comments whether we needed to do bi-directional transporter perhaps on efflux change.

In our guidance, we said if the in vitro data are showing that the entity is an inhibitor of P-gp, then we recommend an in vivo study with Digoxin and may be an

appropriate substrate. And this has been discussed with the Committee three years ago, and we have Committee endorsement

to use Digoxin, part of the reason because the Digoxin plasma concentration has a very nice spectrum of change that increases with an inhibitor of P-gp, and decreases with inducers of P-gp.

And in addition, the result of this interaction study we have is relevant from both meaning.

So what I just discussed, the evaluation of a new drug and an inhibitor. So here's the decision tree to determine whether an investigative drug is a substrate and whether an in vivo study will be needed based on the results.

So again, we recommend to use a bi-directional transport, and we look at net flux ratio of this drug itself. If it's less than two, we think it's a poor or non-substrate. If it's more than two, then we look at it again, because there's a possibility of other transporters that's affecting this transporter.

So we say if efflux is significantly inhibited by one or more P-gp inhibitors -- it's not, then we think the other efflux transporters -- it's not P-gp that are responsible. And further in vivo study to determine which efflux transporters are involved may be warranted.

And this is one of the questions I would ask the

Committee: what other transporters that we need to look at to realize that there's a difference in transport is not P-gp constrained.

So the -- if the answer is the efflux is significantly inhibited by a P-gp inhibitor so we say it's likely a P-gp substrate, then we think an interaction study with a P-gp inhibitor may be warranted.

And again, we have receive early comments that why we use the ratio of two. We already have feedback. Why don't you use 1.5 because that's what one laboratory is using. And we got another feedback that said why don't you use three, because we use that.

So again, this is a number -- something we need to discuss as perhaps there is some suggestion that maybe we should use the criteria that we use for CYP induction; we say you have a positive control, and use the percentage of that number as a threshold to determine whether you need to follow up for study, although the group that suggested a percent value did not actually tell us what percentage it should be -- 40 percent, 50 percent, 60 percent.

And in addition, there was also a discussion on there is an exception. There may be a drug that follows all this

-- it's a substrate, but it's highly metabolized and highly Verapamil, so P-gp would be transported. It's not a rate limiting factor, so we don't need to do the study. We're noting that exception in our decision tree.

So now comes the most difficult part: if we decided that a new drug is a substrate, what should we do next? In our current draft guidance, we have put in -- we said if it's a substrate perhaps was evaluation with a P-gp

inhibitor, ritonavir, cyclosporine, verapamil, may be appropriate.

And we know that cyclosporine affects multiple transporters, not just P-gp. It could be OATP1B1, and here's just -- I just listed some of the substrates that are not 3A but not P-gp substrates, but they're OATP1B1 substrates. But cyclosporine has a large increase when it's given together.

So cyclosporine, although it's not specific, but it's a general inhibitor of many transporters and in recent submissions we have seen cyclosporine being used in the evaluation of the threshold potential that some of the statins. Some other studies were conducted in patients, and we've recently seen studies conducted in subjects, and the

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results are included in the labeling.

So this I'm sure will -- the previous question we'll have more discussion later.

So what if a new molecular entity is a substrate for both P-gp and CYP3A, and our recommendation and guidance is perhaps we should use a strong inhibitor for both, such as ritonavir. Ritonavir is like cyclosporine. It inhibits multiple metabolic pathways, multiple transporters. Here just to give an example that was for substrate 3A, you can see the strong 3A inhibitors -- Indinavir, ketoconazole -- and the tremendous increases -- even Erythromycin -- is four- to six-fold increases. However, ritonavir shows a 49-fold increase with possibly an additional increase for other transporters.

So noted that our recommendations these are not specific inhibitors for P-gp; however, it inhibits multiple transporters so a negative result perhaps to tell us not to worry about the unexpected interaction of the substrates.

So how do we label transporter-based interactions? Before I talk about -- give you some examples, I want to mention the proposal that we have is guidance on CYP-based interactions.

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We have proposed that we classify a substrate and inhibitors, and this will have implications on how we label. And I'll give you an example with Eletriptan, which is listed in our table as a sensitive substrate because Ketoconazole increases the AUC more. Anything more than five-fold is classified as a sensitive substrate.

So in the labeling we say it should not be used within -- at least 72 hours because it's a strong inhibitors. And here only Ketoconazole studies were conducted, and the others were not conducted but were not studied but because we classified Eletriptan as a sensitive substrate, so we can label with the other strong inhibitors.

What about how we -- the implication of classifying inhibitors and how that impacts the labeling?

So another example is Telithromycin. It increased the AUC more than six-fold, so anything more than five-fold is classified as a strong 3A inhibitor.

So in the labeling we said it's a strong inhibitor and the use of Atrovastatin or these sensitive substrates or

substrate within the therapeutic range should not be used together. And notice that the ones I circled they are not studied, but because we have classified Telithromycin as a

strong inhibitor, so we can classify -- so we can label with the drugs that we have not studied.

So do we have sufficient data and understanding in order to class label drugs that are inhibitors for substrates of transporters? And I'll use some recent examples to -- whether we have enough information.

Here's one drug where the in vitro has shown that it's not a substrate or an inhibitor for P-gp in normal dose, so we put that in the labeling. However, we did not extrapolate to other substrates. And here we have also this under clinical pharmacology. There's no clinically significant drug interactions were observed when this drug was given with Digoxin. If you -- based on the in vitro action, this study is not necessary. However, as we have seen in many of our recent submissions, even the in vitro study is showing no potential for inhibition, we still see those studies conducted, partly because they been the focus, and Digoxin is the important drug; although we wouldn't have recommended a study.

But you can see that we have put in transporter information here, but we have not discussed other substrates.

Pramipexole here is organic-based, and here we have under the precaution labeling, we've talked about Cimetidine. It's a known inhibitor of renal transport tubular secretions. The drug is mostly screened at the change in the GFR and the renal clearance function -- of the GFR. And here it says, when it's given with organic bases, if you're cationic transport system it constantly increases in the AUC.

So this is the other drug's effect on Pramipexole. Again, Probenecid also other drug's effect on Pramipexole through a different transporter. So here notice that we talk about transporters, but we did not have a specific transporter identified. And in the same labeling we actually extrapolate to other drugs, but that's because in the population, the PK analysis, the other drug that we classified was that being clear was the cationic transport system or anionic transporter and it was stated in the labeling.

So, in other words, there was no extrapolation except the patients actually were taking these drugs in the clinical trials.

So the next one is the most recently approved drug.

This was approved for smoking cessation and it was approved in May of this year. There was a lot of information under clinical pharmacology, and many of them showing the in vitro data shows that it does not inhibit other drugs, but it was followed with a clinical study anyway.

And in vitro studies show that it is a substrate and that it was followed in the clinical study.

What I want to show here is it actually identified what specific transporter instead of the other labeling that it only says cationic transport system, but here it actually identified OCT2. So we talk about this drug's effect on OCT2 substrate, such as Metformin, or other drugs, such as the inhibitors on this drug, such as Cimetidine. And in the implications there are many other transporters being evaluated. Other OATs -- they're all in the review, which is on the Internet.

But we are seeing more and more studies on transporters included in these submissions, and our question is whether we need to have more general discussion so that we have a standardized approach to help us in the labeling.

Earlier I had mentioned that multiple inhibitor interactions may be relevant in recommending us to study.

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But here, I just want to cite a literature study, and this I think will be also illustrated by other speakers, where Rapaglinide increased by Gemfibrizol and Itraconazole to a differing standard. Rapaglinide is a substrate for 2CA and 3A. Gemfibrizol is a 2CA inhibitor and 3A inhibitor, although the other 2CA inhibitor, Trimethazine [ph.], is only to about 1.8. So there's a possibility of an additional effect and additional studies showing that it metabolizes gluconyride [ph.] and also inhibits 2CA.

What's not understandable is the synergistic effects. This is April, and this is 1.4 to 1.6. However, you're seeing an 18- to 19-fold increase when the three drugs are given together.

So our current labeling did warn about the use in patients, and here specifically we said Gemfibrizol and Intraconazole had a synergistic metabolic inhibitor effect; and, therefore, if you are already on Rapaglinide or Gemfibrozol, you should not take Intraconazole, and as well -- also we discussed later Gemfibrizol as a metabolite of transporters of OATP1B1. So even our labeling has not been updated on specific information, but yet the recommendation

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will be very similar.

So in summary, on the transporter part of our draft guidance, we felt that P-gp is the most well developed system that we could evaluate in vitro and in vivo, and we've seen that information to be increasingly included in the labeling, and many studies with Digoxin have been conducted as an inhibitor and also there's studies where it's listed as a substrate.

At the last advisory committee meeting with you, we have recommended that we need to have agreed upon criteria to evaluate in vitro and have proposed a system, and I'll describe it again later. We see Digoxin as a clinically relevant substrate, but right now we only have general, non-specific transport inhibitors recommended for evaluation. There are other issues that we considered, including whether the change in systemic exposure, if those transporters are relevant to the change of the tissue, which is brain, and I think other speakers may address that

question.

As far as other transporter-based interactions which are not P-gp based, we see the in vitro methodologies being developed, and I think we'll hear more from some of the other presentations today. And I have shown you the information has been included in the labeling, for example, the LCT and OAT information is already included, and we have additional information that for -- extrapolated to similar inhibitors or substrate for those transporters. However, we think we -- more standardized procedures we have proposed a system to evaluate P-gp based interaction, and we'd like to ask the subcommittee whether we use a similar system for other transporters. The short-term recommendation may be a drug or therapeutic class specific or some other drug we know the class of drugs that -- for example, statins. You know, maybe of them are OAT and 1B1 substrates. We may warn about interactions with OATP1A1 inhibitors, and I haven't mentioned about BCRP [ph.] but I know we will discuss it later. Many of the drugs that we know through substrates or BCRP, and I know the sponsor had already been studied and whether we will also recommend it because of similar drugs in the same class.

So the question for the Committee number one is, are the criteria that for determining whether an investigational drug is an inhibitor for P-gp and whether an in vivo interaction study is needed as described in the following figure are appropriate. This is the figure we discussed earlier.

Our second question is are the criteria for determining whether an investigational drug is a substrate for P-gp and whether an in vivo drug interaction study is needed. Again, in this decision tree whether this is appropriate.

And third, this is regarding the recommendation of substrate inhibitor to use in vivo or whether our recommendation is appropriate. It's based on whether it's a P-gp substrate or it's a substrate for both P-gp and CYP3A.

And finally, we know many studies on other transporters are ongoing, so does the current knowledge base support recommendation of drug interaction studies for other transporters such as I only listed a few. I'm sure there will be additional transporters that may be applicable.

And I'd like to mention this is the initial application where we have the first generation of decision trees on in vitro evaluation and based on the feedback, we have a revised decision tree, and this is the one that was published last month, and this is an important Web site where we are able to update our recommendation of substrate inhibitor, inducers, and also decision trees on a more regular basis.

Finally, I'd like to acknowledge the drug interaction working group. It's a very large group, and we have subs to work on different issues. For example, right now, we have a

group to look at multiple drug interactions, and these are underlined are those who worked on the final draft on the P-gp based interactions.

CHAIRMAN VENITZ: Thank you, Shiew-Mei. Before I open the floor for questions, I was reminded to make sure that everybody introduce themselves. Just introduce yourselves for the record.

MS. ZHANG: I'm Lei Zhang. I'm on the FDA.

MR. STRONG: John Strong from the FDA.

CHAIRMAN VENITZ: Okay. Thank you. Now, I open the floor up for questions. Any clarification questions for Shiew-Mei.

Shiew-Mei, I have a question on your slide number 15. This is where you talk about the renal interaction, and you mentioned it was only an extrapolation, but were other drugs studied in the population?

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DR. HUANG: Which one?

CHAIRMAN VENITZ: Slide number -- I'm sorry it's on page number 15, slide 30.

DR. HUANG: Okay. Thirty.

CHAIRMAN VENITZ: And I wanted to make sure that I understood what you were referring to. Could you explain?

DR. HUANG: I'm sorry. You said the extrapolation?

CHAIRMAN VENITZ: Yeah, tell me about the extrapolation; what you did there?

DR. HUANG: Okay. There are additional information in the labeling where it says a population pharmacokinetic analysis indicated other cationic transport inhibitors, such as -- and there's a list of drugs that may cause about 20 percent increase. That's based on population analysis and can include a drug based on their cationic.

CHAIRMAN VENITZ: So that was in addition to the base that you had?

DR. HUANG: Right. But those are extrapolated because they're a population PK analysis.

CHAIRMAN VENITZ: Which you wouldn't have done if you had known this prospective information?

DR. HUANG: I mean we would --

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CHAIRMAN VENITZ: Could you have generalized the information on PK?

DR. HUANG: I think we need to consider that now that we know there are different isoforms, and that's why we're showing here other examples. The most recent example where we actually identified specific transporter, OCT2. So there are various transporters, and I think later on will be discussed more in detail.

Right now, we only said all the transporter systems, but just like a CYP enzyme not all CYPs acted alike. Not all organic cationic transporter systems or inhibitors will work alike. And I think once we have the specific information, I think we should include it as we did.

CHAIRMAN VENITZ: Then my second question is on slide 34. This is when you are talking about the Rapaglinide inhibitor interactions.

DR. HUANG: Which one -- please go to the slide.

CHAIRMAN VENITZ: Slide number 34, and I'm wondering how did you use this information to search the inhibitors?
DR. HUANG: Well, they're affecting different pathways -- one is on 2CA and one is 3A.
CHAIRMAN VENITZ: Okay. So you have a 43?

DR. HUANG: A 1.4 and 1.6.
CHAIRMAN VENITZ: Okay. So what would you have expected if there was no such thing?
DR. HUANG: I think it will be very difficult because they're a different pathway, and that's why we have a separate working group working on multiple inhibitors. When you're working on different pathways, you don't just multiply them together, and that's -- we're actually discussing at the Science Day on how do we project what will be the increase in concentration.
CHAIRMAN VENITZ: Who are independent processes, and we have no synergy, what?
DR. HUANG: It would be --
CHAIRMAN VENITZ: Okay. So whatever the product --
DR. HUANG: Yeah. It's more than --
CHAIRMAN VENITZ: -of the two numbers is that would be what you would expect if both of them, in fact, are independent processes, which is similar to what --
DR. HUANG: Okay. But when we multiply them together, they're about 10-fold.
CHAIRMAN VENITZ: Okay. By that --
DR. HUANG: So you think 10 and 20 are about similar?

CHAIRMAN VENITZ: Well, I mean you said one and a half, I mean we can quibble with the numbers, but the range --
DR. HUANG: Okay.
CHAIRMAN VENITZ: -- but my point is this to me is not necessarily synergistic, because I would have expected the product of the two which is just what happens when you get the effect, and maybe they were special here. I can get you it.
DR. HUANG: Okay.
CHAIRMAN VENITZ: Any other questions. Again, I thank you again, Shiew-Mei.

BOEHRINGER INGELHEIM EXPERIENCE/OPINION: TRANSPORTER-BASED DRUG INTERACTIONS

CHAIRMAN VENITZ: Our next speaker is Dr. Taub, and he is with the Boehringer Ingelheim Pharmaceuticals in the Drug Metabolism AK.
DR. TAUB: Thank you for inviting me to give this talk today. I will talk about Boehringer Ingelheim's experience with transporter-based drug interactions, and at the end, I'll list some of my own opinions that address that shall be proposed during the talk.

So the outline is fairly simple. I'm going to talk a little about some background information concerning drug transporters, drug transporter interactions, the similarities between P-glycoprotein and Cytochrome P3A4, some in vitro techniques that we use at BI and some data

using those techniques, and I'm going to intersperse the talk with some examples of clinical transporter-based drug interactions.

So this is a fairly comprehensive slide. I think you've seen the similar one in Shiew-Mei's presentation. Transporters are expressed in a variety of different areas. As Shiew-Mei pointed out P-gp is expressed in all sites on this slide, the intestine, brain, liver, kidney. Transporters can work to efflux certain compounds, as you see, for example, the intestine, P-gp, BCRP, and other transporters' uptake of compounds, such as the intestine, P-gp, OATPA, and the hepatocytes, the OATP family, NTCP and so on.

It's safe to say that transporters contribute to the absorption, distribution, and elimination of drugs, metabolites, various endogenous molecules by nutrients, and the tissue entry of drugs can be either facilitated or

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inhibited by the activity of the transporters.

So why are transporters important? Well, in addition to absorption, distribution, and elimination, they can also facilitate the access of certain drugs to metabolites, again, for example, in the liver, it's a very important process.

Understanding the pharmacokinetics, the pharmacodynamics of certain drugs certainly requires the knowledge of drug transporter interactions. This is something that's becoming more and more apparent. Every month that goes by, every review, there are papers that are published.

As with CYP450, these interactions of transporters differ between species and the consideration here is how are we going to be able to predict clinical outcome from in vitro studies and through clinical studies using animals.

From a drug-drug interaction perspective, we know that that DDIs can cause variability to exposure, essentially can cause toxicity, and in certain cases therapeutic failures. And certainly they can also originate from drug transporter interactions as well as classic drug interactions.

So the question-the principal question that we ask

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ourselves is what do we need in order to be able to predict whether and to what extent the biological fate of drugs is influenced by drug transporters and the challenge that, on face, is well, it's likely a compound is going to interact with multiple transporters, and its likelihood is going to increase for newer drugs that are structurally related to those that are already known to interact with transporters.

So you saw on the first slide, there are quite a few transporters. Well, how many actually exist? The last time I looked into it, there were 48 BTB finding set genes identified and approximately 300 solute carriers, so this is quite a few transporters. Obviously, we don't have the resources to evaluate all of those and probably not the need either. So out of these which ones would we consider the most important to evaluate.

Well, the general consensus, as I understand, is

similar to the new 450s not all these transporters are relevant. So on hand -- this side of the slide, you can see the transporter and at the top of P-gp we know most about the protein. There are some alternative names listed there -- that's listed there. We know the major transport is a possibility. Hopefully, the comment there remains with the

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transporters.

And the analogies I'll present in the next slide to P-gp would be CYP3A and 455 and CYP3 and 4 enzymes has been responsible for most of -- approximately 50 percent of the drugs that link available oxidated metabolism.

So coming up close behind P-gp would be OATPC, PCRP, MRP2, OATPB, and also we could argue with OATPA and OATPA. Again, this is necessarily I mean more or less everybody's understanding of the transporters field, but it's something that's been discussed internally with VI taking into consideration before with various resources involved.

So the similarity would be to the second tier of transporters the other CYP enzymes that are considered most important -- 2C9, 2D6, 1A2 to 2C19 and also more and more important 2C8 genes.

So it's been published and it's generally understood that P-gp and 3A4 have quite a few similarities. Just briefly they've been expressed as epithelium, both frontline and a defense against antibiotics. They both show a broad substrate's selectivity, and it has been promiscuous, and a cooperative action for drug degradation [ph.] for overlapping substrates. Certifications have been detected

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in the P-gp 3A4, but the impact on pharmacokinetics is doubtful. Generally, what we consider to be minor. Both are reducible via PXR, and they're both occasionally demonstrated in typical or sigmoidal kinetics, for example, cooperativity and activation.

So quite, clearly, the effects of consequences on pharmacogenetics by 3A4 P-gp can only be applied to understanding of the activities of both the enzyme and the transporter.

So what Shiew-Mei proposed in her talk was evaluating whether or not a compound was a P-gp substrate by doing bi-directional transport experiments across a model, so this is the -- you may agree with the type of these experiments similar to the one in which most people will use such a thing so the cells are seated on a semi-transparent, permeable filter membrane. They form tight junctions and then you monitor the transport of the drug in the apical to basal direction and the basal to apical direction, and you calculate the permeability and then compare the permeability to the efflux direction for the secretory transport and the permeability in the apical direction and the secretory transport.

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And, you know, I understand this is somewhat debatable what number you use to evaluate this ratio. It signifies that you have an efflux transporter substrate. Some people use 1.5, two, or three, but any rate what we generally use

is the cutoff near two.

So what other considerations are there concerning P-glycoprotein? It's definitely a very complex protein. There are multiple binding sites on P-gp. It was four that have been quoted, although selective probes for each of these binding sites has not yet been identified. In a paper that we published in my lab last year, we looked at the effects of Ketoconazole on the last in transport that caused that BT failure. One set was using kidney cells, which expressed the P-gp in the protein. And we showed that at low concentrations the Ketoconazole actually activated the P-glycoprotein efflux transporter, and at higher concentrations it inhibited the transporter. This is somewhat -- I give the analogy to the CYP450 field is shown for 2C1.

So with a compound that we're working on with similar properties as Ketoconazole also has a differential effect on P-gp. It's been tested. We have it for a high

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concentration compared to the effects of P-gp at the blood brain barrier, we have a relatively lower concentration.

But what's clear is that substrate cooperativity and allosteric binding can complicate the determination of secretory transport of P-gp substrates and express in cell lines such as MDCK-MDR1 and Caco-2.

Inhibition to P-gp can also potentially alter the pharmacokinetics and possibly the pharmacokinetic profile of the drug. It has yet to be shown. I've been asked about this in the past, but it hasn't been shown in the literature with respect to a clinical correlate for these data observed the possibility of the activation.

Nevertheless, possibly due to expression of other transporters, we certainly need to be cautious comparing data between cell lines and expressed P-gp.

So what I'm going to present now -- just trying to go through this briefly is a clinical study for the office have shown the effects of the P-gp inhibitor on P-gp activity clinically.

So Loperamide, we know that Loperamide is a potent opiate used as a anti-diarrheal. It's available over the counter, and we also know that there are no central nervous

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system effects at low doses.

But Loperamide is -- in this study was given concomitantly with Quinidine at 600 milligrams, the AUC of Loperamide increased about two and a half fold. As you can see in the draft that's presented on the left side of the slide, when the authors of this paper -- the study directors of this paper administered Quinidine and then an hour later administered Loperamide, they then measured the change in baseline carbon dioxide response as a surrogate marker for CNS depression, it shows significant CNS depression. Again, Loperamide was co-administered. So they proved that Quinidine inhibited P-gp mediated effects of Loperamide.

So this is a good example of transporter-mediated drug interaction with potential toxic effects.

So switching now to the investigation of the activity

of other types of transporters, this is a system that's used by my lab. This is something about Richard Kim at Stanford University, and so this is expression of OATP, as I used as an example. You can also express other transporters in HeLa cells, using Vaccinia-based transfection system.

So this utilizes the efficient bacteriophage T7 RNA polymerase. I like to refer to it as a modular system

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whereby these are modular transporters because they don't have to have a lot of different cell lines. But they're able to transfect and they're constantly kept in a state of heating and seating, and I just used HeLa cells, and then you can evaluate transporters. So there's only one cell line to passage, and the way it works is we have a cDNA that expresses the transporter, which won't, by itself, enter the cell. This can be used with a lipofectin technique, or inhibit the cell. And at the same time, or just shortly thereafter, we administer this Vaccinia virus which recognizes the T7 promoter and causes cytoplasmic expression of the transporter which is then shuttled to the surface and you can conduct studies by new cells. So it's a transient transfection system, and it works actually quite nicely.

This is an example in this slide here of OATP-A expression in HeLa cells and it's known the effects of Fenadine is a substrate for OATP-A, with the first slide the effects of the Fenadine that's expressed -- I'm sorry. OATP-A is expressed in the gut, and you can see here that compared to the effect of control, the OATP-A expressed in the cells demonstrates actual uptake affects Fenadine.

So you can see here a clinical correlate to this data

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in OATP-A is expressed via the intestinal epithelium, and the authors of this paper wanted to investigate if the administration of grapefruit juice had any effects on Fexofenadine uptake. And you can see here compared to control, which is the co-administration of water, the co-administration of grapefruit juice has significantly increased the AUC about three-fold change in the AUC for effects of Fenadine in the presence of 300 mil or actually in that case 1,200 ml of grapefruit juice.

So those are some of the uptake transporters and the ways in which we study that in the lab. In addition to MRP1, which is the same as P-gp, as you see here, expressed in canalicula upgrade of hepatocytes, there are other transporters that we know of and there are three -- BCRP, MRP2. And some of the ways in which we can study this involve having or creating cells that express these transporters and created inside-out vesicles and looking for the uptake of a probe substrate into the vesicle. And I got this slide from a company called SOLVO Biotechnologies, who makes this particular product.

So you can see here they took Sf9 cells, which is an insect cell line, expressed human BCRP, which stands for

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breast cancer resistance protein, and then created an inside-out vesicle. We purchased them and then used them in the lab to evaluate the influence of Sulfasalazine, which is

a BCRP inhibitor, on the uptake of -- on the probe BCRP substrate, which is Methotrexate.

And you can see here that Sulfasalazine inhibits the uptake of Methotrexate to BCRP-expressing Sf9 cells, with an IC fifty of about .4 micro moles.

So going into the literature, what's the clinical results of BCRP inhibition? I did find this one paper from 2002, where the author is looking at the effect of GF120918, which is a known BCRP inhibitor on the Topetecan, which is the BCRP substrate.

And you can see here where they administered one gram of GF120918, the AUC of oral Topetecan increased at least two fold. So this is showing that BCRP inhibition can actually result in a change in pharmacokinetics of BCRP substrates. You can see on the right-hand side that this wasn't just passing noise. This has affected actually of the -- all patients.

So one thing that we wanted to do internally was to look into the literature and try to find out well, how many

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papers have -- how many clinical reports have been published looking at the different transporters, and there are quite a few. And this actually was done last year, so we know these numbers have changed. But what we found was that overwhelmingly the number of reports looking at potential drug interactions involving transporters cover P-gp. So you can see about 180 reports, and then everything falls off quite dramatically after that.

So just taking P-gp as an example, we categorized them into the number of studies showing no effect and the number of studies actually showing -- this is using the University of Washington database where the outlook again is greater than 20 percent change in pharmacokinetics, and pharmacokinetic practice.

So quite a few of these studies looking at the input of P-gp on the transporter interactions, demonstrating greater than 20 percent change in pharmacokinetics. That's summarized at the bottom. About 120 of those studies fall into P-gp inhibition and a maximum effect is about an 18-fold increase in AUC. And about 40 of these studies show a maximum effect for, with respect to induction, 80 percent reduction in AUC.

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Now, admittedly, some of these results could be due to the decline effects on P-gp and CYP3A4 induction or inhibition. But what we saw is that P-gp effects often exceeded a two-fold increase or decrease in exposure. That's usually considered acceptable variability in a pharmacokinetic study.

So at the bottom the key question is well, what's the current regulatory perspective on the design and implementation of clinical studies to investigate potential drug transporter interactions, and that's what we're here to discuss.

So, in conclusion, 3A4 and P-gp demonstrate many similarities. They're both equally important to consider in most R&D programs. There are some indications of which they

are very important to consider would be CNS diseases, cancer, liver-targeted indications.

So to what extent are frequency to clinical drug interactions or toxic effects involving transporters occur? This is something that we definitely need to consider carefully in R&D programs.

Clearly, the selection of appropriate transporter probe substrates and inhibitors is a critical issue.

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Unfortunately, this area is still really not that well defined for many transporters.

And I presented the in vitro methods that are used in my lab. And I know from talking with scientists from other companies that people have different ways of studying the same problem, so the question would be how are we going to get different data for the same type of study for the lab, and we need to try to standardize this.

And the last bullet that I put in red is something that perhaps you're not so familiar with, but we in industry have to deal with this that legal barriers in the form of patents exist that restrict our freedom to operate to mechanistically evaluate certain transporters.

So I put together a couple of slides that are my response to the questions that should be proposed. So the first question is the criteria for determining whether an investigational drug is an inhibitor of P-gp or whether an in vivo drug interaction study is needed, as you demonstrated in one flow chart, is that appropriate?

So the question that comes to mind when I look at that is well, how relevant is the I to KI relationship. This was originally established and included the cell and this was

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important for CYP450 inhibition. How relevant would this be for transporter interactions? Certainly this area is not as well defined for transporters, even for P-gp as it for the CYP450s. You also have to consider well, what are we going to use for the I values for Plasma C Max as we use for the 450 analysis or an estimated GI concentration of drug and show the P-gp that's expressed in tests.

And also there's a difference when we're evaluating for microsomes. This I value typically comes from -- I'm sorry. The KI value comes from microsome studies and P-gp comes from cells, so is this something that we actually can compare.

The second bullet point there is something that you may already went over during her presentation, so I don't need to go into this, but just, as I said, is this an arbitrary value, so it's defined to KI greater than .1, admittedly. It was previously 10 micro molar. Is .1 any less arbitrary, and this is considered and introduced.

The other thing I think is kind of important to this is some of the most potent inhibitors of P-gp are compounds that are not commercially available. They may not be suitable for evaluation in clinical studies.

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And concerning Ritonavir and Cyclosporine and other proposed inhibitors, these compounds have been know to

inhibit many transporters, so, at this point, it's not very exactly clear how the lack of specificity would affect results of the clinical drug interactions.

This is my last slide here. With respect to the question for determining whether an investigational drug is a substrate of P-gp, and whether an in vivo drug interaction study is needed. It's certainly a reasonable concern that flux ratios greater than two represent a value that is too liberal here, but that's not really the word I'm looking for. Maybe conservative is a better word, but too strict perhaps and could lead to too many positive results.

This is something that pharma members are in the process of getting together and should have a consensus opinion fairly soon.

Again, the question would be would it be expected that any developing compound with a flux ratio of greater than two be evaluated clinically, using P-gp inhibitors to determine potential drug interaction and also it should be mentioned -- it's also important to consider not only the efflux ratio but the transcellular passive permeability of

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the compound. It may be that if that's high, it would be reasonable or logical to conduct a clinical DDI study.

So a sort of closing statement and the general concern I think that many open questions still exist regarding the complexity of the transporter field and how we can appropriately link in vitro data to the potential clinical outcome, even for the CYP450 area for which the in vitro correlation with drug interactions is better characterized but not always able to correctly predict clinical drug interactions.

And the current knowledge base doesn't really yet support the recommendation of drug interaction studies involving other transporters, such as OATPC, MRB2, BCRP, OCTs.

That's it.

CHAIRMAN VENITZ: Thank you, Dr. Taub. Any questions by the Committee?

DR. WATKINS: Just a clarification. On the slide that showed the drug-drug interactions from the University of Washington's database, those were all human studies? Were there actually PK endpoints?

DR. TAUB: Yes.

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DR. WATKINS: That's some big numbers.

DR. LESKO: Mitch, I actually had a question on that same slide. It's in that last column. I was wondering what the number of drugs were with the effect of eliciting a greater change than 20 percent. In other words, what would happen if I put not a hundred percent there? How many actual numbers would pop up, would you guess?

DR. TAUB: I mean that's really a hypothetical question because if you can't put a hundred percent, and it's all to keep the data, but they're only categorized according to change of 20 percent.

DR. LESKO: Oh, I was kind of looking for an area under curve change. That's a PK. Does that -- does that

mean PK is measured by blood levels?
DR. TAUB: Yes.
DR. LESKO: Okay. Well, they don't give that information. I was trying to get sense of how significant these interactions are by putting an arbitrary hundred percent increase on the pharmacokinetics as based on an area of --
DR. TAUB: I think it would be an interesting feature of the database.

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DR. LESKO: That's selected.
CHAIRMAN VENITZ: Any other questions?
DR. TAUB: Because that's actually the --
DR. LESKO: Yeah. Okay. The other thing about your pharma, I think on the last slide you said something about pharma is developing a consensus. Is there any active partnership or consortium that is sharing data on transporter methodologies, such as cell line systems, such that collectively pharma, partners, whoever they are, could make some recommendations on some standardized approaches to these things?
DR. TAUB: Not to all pharma as such. It's important that it is an open pharma problem.
DR. LESKO: Yeah.
DR. TAUB: I mean I think there's certainly is some companies that an FDA consortium and act as sort of a --
DR. LESKO: Okay. Nothing more. Thanks.
DR. BARRETT: I know we're going to get into this when we go to the questions, but just in the previous slide, when you were making comments, and you mentioned some of these other potent inhibitors or compounds not being commercially available, but I know it's just a statement, but is it your

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opinion that or is it practice at your company that you would use the most potent agent? I know the kind of de facto guidance has been to approximate the worst case scenario by using a potent inhibitor.
But I don't know that that's necessarily relevant for all therapeutic areas. I don't, you know -- and I know we're going to discuss it as a group, but just your opinion as far as the choice of an agent there. Do you de facto choose the most potent?
DR. TAUB: I think you're going to have to balance it between potency and selectivity, which is really the problem with a lot of transporter field in the selectivity of the substrate, and the transporter. It's almost impossible to find one and the selectivity of an inhibitor that's just going to inhibit transporters and it's almost impossible to find that sort of example. I think I was able to directly -- quinidine is used, but it's also a fairly potent 2D6 inhibitor.
So I mean arguably you could design your studies that will be able to influence the probe substrate that you're using. I mean the answer to your question directly is you'd want to use the most potent compound that also is the most

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definite --

DR. BARRETT: I guess that's --
DR. TAUB: I guess that's where the problem lies.
DR. BARRETT: These are more kind of methodologic, but I see this tied into the class effect or the desire to have a class effect labels so that you can kind of add the other agents that might be relevant therapeutically with a drug of choice, but I don't see kind of an a priori alignment where you would choose that to be your probe agent, an agent which you would have some certainty it would be potentially co-administered with your developmental program.
DR. TAUB: Yeah, I don't think. I think we could still design the study appropriately, but probably using the two in answer to that.
DR. BARRETT: Okay.
DR. GIACOMINI: I'm going to be covering organic cations and organic anion kind of interactions clinical interactions and there are many more than are in that database, so I'll mention the data. I just thought I should tell you this.
DR. TAUB: Okay.
DR. GIACOMINI: Okay?

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DR. TAUB: Thank you.
CHAIRMAN VENITZ: No further questions? Okay. Thank you again, Dr. Taub.
And according to our schedule, you're ready for a break. A short in the first part of the afternoon, so let's take a 15 minute break, and let's reconvene at 3:15 p.m.
[Break]

CLINICAL SIGNIFICANT TRANSPORTER-BASED INTERACTIONS

CHAIRMAN VENITZ: Can we reconvene, please? Let's get started, please?
Okay. Let's go ahead and start with our next presentation. Our next presenter is Dr. David Greenblatt. David is Chair and Professor at the Department of Pharmacology and Experimental Therapeutics at Tufts University, and he is going to talk about the clinical significance of drug transporter-based drug interactions. David.
DR. GREENBLATT: Thank you very much. I appreciate the chance to meet with you, and somebody is going to hook up the slides. Thanks.
I wish I could help, but I'm on this thing, and I have no idea how to work this thing. Good. Thank you.

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Okay. What I wanted to do is my contention here is that with regard to assessing the importance of transporter-based interactions and the development of guidelines and approaches to interpreting in vitro data, we are about 10 years behind where were are with CYPs.
So what I wanted to do is hypothetically roll the clock back 10 years and look at -- pretend that we're giving this talk on the clinical significance of CYP-based interactions and think about where we were then compared to where we are now with transporters and then look at the evolution of what we learned over the next decade to try to forecast where we're going with transporters and maybe avoid

some of the pitfalls and errors and mistakes that we've made along the way.

So roll the clock back to 1996 and try to remember what the state of the art was back then, and I think that we were coming to the conclusion that we were getting many new clinical entities in the '80s and '90s that were improved and were major therapeutic advances -- effective treatments for diseases that were previously poorly treated or inadequately treated. So we have really a many therapeutic breakthroughs in the '80s and '90s and along the bottom are

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examples of drug classes and there are many others.

But along with that, we bought the obligatory secondary pharmacologic properties of these new clinical entities. They have the capacity to induce or inhibit the metabolism of other drugs.

So we discovered to our dismay many new kinds and new categories of drug interactions, and there were a lot of them and some of them were very large and clinically important and, for example, all the drug interactions involving the SSRIs, the Azole antifungal agents, the intra Antiretroviral drugs, et cetera.

So that was an emerging revelation of the 1990s.

The second thing is that polypharmacy in general was good. We were able to keep alive and also maintain a good, a positive quality of life for many patients with serious medical disease, because we were able to combine these highly potent drugs for their combined therapeutic benefit, but also the combined potential effects with regard to drug interactions.

So the number of drug interactions that we recognized increased and increased, and finally we came to the realization that we just had too many drug interactions to

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memorize. Clinicians were complaining I simply can't learn all this. Where can I look it up? Where is there a Web site? What do I do? And so we had to kind of come up with a structure to understand the drug interactions. So we developed the framework of the understanding of the CYPs and what the substrates were and what the inhibitors are, and used that as a framework upon which we could hang the results of individual drug interaction studies and help clinicians come to grips with it.

Okay. So that was very valuable.

And finally, we were well on the way to developing a rather sophisticated in vitro -- set of in vitro models involving obviously microsomes, recombinant enzymes, liver slices, hepatocytes. And we were very encouraged and excited by the outcome of these in vitro studies and what we could learn from various in vitro models, and we had the hope and maybe the fantasy that these models might actually provide predictive estimates as to what kinds of drug interactions could happen, not happen, uncertain as to whether they could happen, and we might be able to use these as a guideline to planning clinical studies or maybe even predicting what would happen without even doing a clinical

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study.

Okay. So that's about where we were in 1996 with respect to CYPs, and I would suggest that that's approximately where we are now with regard to transporter-based drug interactions. Okay.

Now, let's come back to the present and look at what the last decade has taught us about CYPs so we can see where we might be going with transporters.

First of all, it's pretty clear that when there's a bad drug interaction that leads to a serious adverse event or in particular a death, that obviously is very bad for the patients affected, but it's bad for everybody. Okay. It gets in the newspapers, and it leads to a cycle of blame. Regulators get blamed for approving a dangerous combination. The sponsor, the industrial sponsor gets blamed for propagating a dangerous lethal, drug on the public, and practicing physicians get blamed for stupid prescribing and giving drugs to patients without adequate understanding of the science behind it. So this is very bad for everybody, and makes us all look bad. Okay.

On the other hand, when you look at drug interactions in general, in particular bad drug interactions, they're

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actually quite unusual and bad drug interactions that are lethal or potentially very harmful, they are very rare given the denominator of potential drug interactions and you've probably seen tables like this, and this made me go back and exhume my college math and come back to binomial coefficients. But if we take in the left column the number of drugs co-prescribed to a given patient, the corresponding entry in the right column is the number of possible drug-drug interaction pairs that you get by taking each of the drugs that they're on two at a time.

So by the time you get to let's say an elderly patient with multiple medical disorders, taking seven, eight, nine, 10 drugs at a time, the number of possible drug interactions that you get by taking a possible two by two pair is huge.

Yet, the number of drug interactions, let alone important drug interactions, is small. So drug interactions are unusual, and, in fact, most of the time when drugs are co-prescribed, there are no drug interactions. That's the most common outcome. Two drugs are prescribed; there's no interaction.

Occasionally, you can get an interaction which can be demonstrated if you do a careful pharmacokinetic study, to

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study the clearance of the substrate with and without the inhibitor, and you can show a change in clearance.

These kinds of studies most of the time demonstrate interactions that are clinically unimportant and we talked about the 20 percent threshold here and I think most of the time 20 percent will not be clinically important.

So we have occasional drug interactions that can be demonstrated but are of no practical importance. Either the change in exposure to the substrate is not big enough to make a difference or that change is buried in much larger intrinsic individual variability and genetics in response.

Sometimes, unusual, we come up with interactions that are clinically important in that either you have to monitor more closely or you have to make an adjustment in the dosage of either the substrate or the inhibitor or the substrate or the inducer. It's pretty unusual.

And really rare is the Ketoconazole Terfanidine type of interaction, where it's a hazardous interaction, potentially life threatening and the combination is absolutely contraindicated. That is rare.

Now this is a hypothetical construct, but there's actually some data to this effect, and it came out of

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Germany from this study in which those questions were actually asked in a population of patients whose drug therapy was carefully monitored, and they started out with some 9,400 patients receiving 223,446 drugs and they started going down the line as to what the possible pairs of -- possible drug interactions were. Then B are the actual drug combinations that might have caused an interaction. C was the subset of those where there was actually data available on what the drug combination did. D was the -- were the number of pairs where a drug interaction was actually possible, and then E came down to a real interaction that was manageable, and F, finally, 74 cases, less than one percent, a half a percent of those exposures, actually had a drug combination where there was potentially hazardous interaction so this I think is consistent with this table that most of the time there's no interaction and the rare ones are very rare. The hazardous ones are very rare. Okay.

Thirdly, to our dismay, we have learned that the in vitro systems have major drawbacks, limitations, and biases. And I think we're all pretty much aware of what those problems are. You hope that your in vitro system will

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provide data in the form of the left half of the equation, which will allow you to predict what will happen in vivo; that is, the right half of the equation. And the right half of the equation represents a clinical study in which the area under the curve with a substrate with co-administration of the inhibitor is expressed as a ratio to the area in the control state. It's the change in AUC, the full increase in AUC.

So what you hope will be true is that you don't have to do that study, because you can use the in vitro data in which you get a KI and compare that to I in brackets which is the extent of exposure of the enzyme to the inhibitor and you hope that one plus I over KI will be predictive of that AUC ratio in vivo.

Well, it didn't work very well, okay, mainly because we continue to be left with the core questions of what does either KI or IC50 when measured in vitro actually mean in vivo, and secondly what does I in brackets -- what is that entity? What is the extent of exposure of the enzyme to the inhibitor? And those questions still are not completely answered so that we are left with an in vitro model that works pretty well at the extremes.

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So, for example, if you take -- let's Ritonavir, which we talked about as the -- probably the worst case scenario and an inhibitor of CYP3A. If you do a typical in vitro study on the left, and get an IC50 above Ritonavir versus CYP3A, and, you know, it's probably in part a mechanism-based inhibitor, but either way you look at it, the usual systemic exposure to Ritonavir on the lower right of that left-hand panel greatly exceeds the IC50 or KI.

So, therefore, this is an extreme case where you predict the drug interaction, and sure enough if you do the study, on the right-hand side of the graph, with the same substrate given in the controlled state and with Ritonavir, you get a gigantic interaction. Okay. That's easy.

We're also reasonably confident at the other end when I over KI is small. Now, I'm using here the ratio 0.5. Maybe that's a little somewhat more liberal than it should be. I know the current guidance is 0.1. My own feeling is that 0.1 is maybe a little aggressive, but whatever you choose, we feel pretty good about I over KI values bigger than five predicting the high probability of an interaction and then low values making it unlikely. But we still don't know what to do in the middle. And, also, by the way, I

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encountered or was hit with the first really first negative that I could remember seeing and that has to do with Bupropion and CYP2D6, and this data isn't published. It's on the GSK Web site.

If you look at the in vitro data for Bupropion and Hydroxy-Bupropion versus CYP2D6, the I over KI values are very small and you would not predict an interaction. Yet, Bupropion significantly inhibits CYP2D6 in vivo. So that's a very troublesome false negative and apparently an exception to this scheme whether you use .1 or .5 and that -- you know that worries me a bit.

But, in any case, that aside, we still don't know what to do in the middle, even 10 years later in 2006. Okay.

And finally, we again we have to -- in interpreting actual clinical interactions, we need to get our focus on the things that matter, and that means either or both of these highly potent inducers or inhibitors. We're really worried about the Ketoconazoles and Ritonavirs. We're really worried about the Rifampin that produce two-, five-, 10-, 20-, 50-fold changes in AUC.

We don't want to pollute the clinician's attention by focusing on the plus or minus 10 percent, 20 percent, 30

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percent change, and, yes, there may be interactions, but they're just -- it is not likely that they'll be important.

And, of course, we need to focus on the substrate victims, the things that are being interacted with, with the narrow therapeutic ranges -- Warfarin, phenetoin, whatever. Narrow therapeutic ranges. Okay.

Now, let's try to apply what we've learned about CYPs to the transporting system.

Okay. First of all, the same thing: the in vitro models have significant drawbacks and limitations and

biases. And I think you can categorize those as specificity problems and confounding due to passive diffusion. So we're really worried about the specificity of cell-based models with respect to expression of individual transporters. Okay. Probably most of the older data using KCO2 [ph.] cells basically were illustrating what happens with multiple co-expressed transporters or multiple co-existing transporters.

And the more sophisticated we get in specificity of cellular models, the more we're able to be reasonably certain that we're studying individual transporters as opposed to a mix.

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Secondly, of course, we're very worried about the specificity of the index substrates as well as for one for a specific transporter and many of the substrates are not at all specific, and likewise the inducers and the inhibitors are not necessarily specific for a given transporter.

And finally, we've heard talk about passive diffusion. I mean there are many cases in which a drug may be transported, the ratio may be two or three, but the passive diffusion in either direction completely overwhelms the importance of the transporter. A lot of energy has been expended on looking at, for example, CNS efflux transport of anti-depressants, and you can demonstrate that some of these drugs are substrates for efflux transport, but it doesn't make any difference because even in the absence of -- even with normal transport, the brain-plasma ratios are far in excess of one. Okay?

So transport has to be considered in the context of passive diffusion and lipid solubility, et cetera. Now, here's a study on Ketoconazole just to illustrate this.

It's in a KECO2 transwell [ph.] model and the transported substrate is Rodamine123 [ph.] and we're looking at Ketoconazole as a inhibitor of transport of that

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particular substrate.

So the question is we thought at this point or it was thought at this point that this probably, the KECO2, probably expresses mainly P-glycoprotein and that Rodamine probably is mainly a substrate for P-glycoprotein and that Rodamine probably is mainly a substrate for P-glycoprotein transport. So we get an IC50 of 2.7.

Okay. What on earth does that mean? Well, first of all, as has been mentioned, when trying to interpret the IC50 of transport modulators, we have to continuously recognize that there is a huge difference between the exposure of enteric mucosal cells as opposed to blood-brain barrier mucosal cells and probably also the same for hepatobiliary cells.

So the interpretation of IC50 with regard to inhibition and ultimately the predictability of induction will depend on the numbers that you get in the context of the level of exposure.

So if you take Ketoconazole; dissolve 200 milligrams of Ketoconazole and a hundred mL of water and swallow it, the enteric exposure to the Ketoconazole is huge. It far

exceeds any IC50.

But on the other hand, if you let Ketoconazole get to the systemic circulation, where the total plasma concentration is one, two, or three micrograms per mL or three, four, five micro molar and the unbound concentration is probably one fiftieth to one one hundredth of that, then that complicates what this IC50 means.

Now, here -- and I'm not suggesting here -- and this is just an illustration of this, and I'm not for the moment or any -- in any way suggesting that a rat model is of value in understanding of what happens to humans, but nonetheless this illustrates in the context of P-gp conduction the difference between blood-brain barrier exposure and enteric exposure to Ritonavir and Dexamethasone given enterically to rats. And in this study, we looked at the relative increase in P-gp expression relative to vehicle control for enteric P-gp where the expression increase was a factor of nearly three as opposed to the blood-brain barrier increase where the whole expression increase was a factor of about 1.2 to 1.3 and that probably is due to the difference in exposure to the inducer.

Now, getting back to the issue of passive diffusion as opposed to efflux transport, this is of particular

importance for the blood-brain barrier. Now this is a study of -- really a very good model of efflux transporter P-gp, efflux transport activity and that is the brain plasma ratio in ABCB1 minus, minus, and that is normally called P-gp knockout mice relative to wild type animals; okay?

And in this animal model, you can demonstrate -- the authors could demonstrate a significant increase in the brain-plasma ratio for morphine in the knockout animals compared to controls.

But still in the controls the brain-plasma ratio was greater than one first of all.

Secondly, we know that morphine is an effective CNS drug in humans, who express P-gp in the blood-brain barrier, and in wild type animals, who express P-gp in the blood-brain barrier. So this is a case in which yes, there is transport and that can be demonstrated in isolated cell models, but passive diffusion probably overwhelms transport.

The two human studies that I could find in which morphine pharmacodynamics were studied in the presence and absence of P-gp inhibition in humans one showed no difference in pharmacodynamics and another showed a small

difference of the type that I would suggest is not clinically important.

Furthermore, we don't have any epidemiologic data to suggest that morphine toxicity is enhanced with co-administration of P-gp inhibitors.

So, again, you can get an IC50 and you can -- in a carefully controlled study maybe demonstrate enhanced pharmacodynamic effects, maybe not. But there's really no evidence that that is of clinical importance.

Here's another interesting study on the brain -- the CSF to free plasma, unbound plasma ratio of Ritonavir on the left and Sequinovir [ph.] on the right. A very difficult study to perform in humans, with and without Ketoconazole.

And you can see for both of these anti-retrovirals, it looks as if co-administration of Ketoconazole increases the CSF to free-plasma ratio, consistent with inhibition of efflux transport.

First of all, this is not consistent with the in vitro data for Ketoconazole showing the IC50 of about, you know, two to three micro molar; and, second, I know of no data that the -- either the CNS efficacy or CNS toxicity of either Ritonavir or Sequinovir are modified in one way or

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another by co-administration of Ketoconazole or any P-gp modulator.

The next problem is and Mitchell talked about this and Dr. Huang as well: the need to or the isolation of the relative importance of CYP3A modulation versus transport modulation for the large number of compounds that are dual substrates or inhibitors or inducers, and we know that there is a large degree of crossover there. It's not obligatory, but there is a large degree of crossover, so when we look at what's happening with let's say Lopenavir, which is probably a dual substrate, and Ritonavir, which is probably a dual inhibitor, the clinical study shows that even small -- a low level of exposure to Ritonavir increases the systemic exposure of Lopenavir by factor of about hundred-fold. And, in fact, that's so desirable that we don't call it an interaction. You know, we call it augmentation, you know, whether it's good or bad, we change the nomenclature. Okay.

But is this due to P-gp inhibition or CYP inhibition or some combination of the two? How do we isolate the relative contribution and does it really matter?

And finally, the most important thing: getting back

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to the issue of clinical importance is whether there's an established exposure response relationship with the victim. This is absolutely critical in assessing clinical importance, so if we go to let's say the Fexofenadine citrus interactions -- this is similar to what Mitchell showed previously.

Obviously, co-administration of regular strength grapefruit juice, orange juice, or apple juice in the three lower curves presumably through inhibition of uptake transport of Fexofenadine depresses plasma levels and systemic exposure to the Fexofenadine. That obviously is statistically significant in a pharmacokinetic sense, but is it clinically important, and I would say that at this point, it may or not be depending on what we know about the exposure response relationship of Fexofenadine, and my understanding is we don't know that much about the exposure response relationship for that particular drug. So I would suggest that labeling -- I saw a proposed label of Fexofenadine and citrus -- that may be too aggressive at this point.

So my contention here in evaluating what has been proposed, and in particular the bottom half of the proposed guidance trees in which in vitro I over KI values for partition or relative partition ratios then trigger a clinical decision. I think that it's too soon for that, and in looking at the questions that the Committee will be considering, it reminded me of this little story which I put down here, and that was in the context of I think one of the Clinton campaigns, the first Clinton campaign. And, as you know, in the winter, early on in the campaign, the State of New Hampshire is inundated by reporters and the media and press -- this is taciturn conservative laid-back, sparse New Hampshire.

So all of the media inundates New Hampshire, and they're trying to get stories about prior to the first primary, you know, how do you feel about this and who is -- or so. The story I heard, and maybe you've heard it before is that some reporter, a female reporter from New York, was trying to get some background color on the locals, the New Hampshire locals, and she was trying to interview them just to get something that would be entertaining.

So she came upon this crusty old geezer of about 70 who was sitting in a barber shop, reading a newspaper, waiting to have his steel gray flat top cut. Okay? I can empathize with these guys because I'm a crusty old Massachusetts geezer myself.

So she goes up to this guy, and, "excuse me, sir." You know, trying to engage him in discussion, and, you know, "have you lived your whole life in New Hampshire?" And he looks up at her, very surprised, and says, "not yet." So that's my feeling about where we are with respect to the questions posed and the guidance.

I think we're not there yet, and I would urge you to be not too aggressive in your labeling, in your guidance until we have the information to support it. I think we're not there yet, and I will stop there. Thank you.

CHAIRMAN VENITZ: Thank you, Dr. Greenblatt. Any clarification questions by the Committee?

Dr. Greenblatt will be assisting us on our discussions. Any questions right now? Okay. Thank you, again.

CLINICAL SIGNIFICANT INTERACTIONS OF OATP1B1 AND THEIR TRANSPORTER-BASED INTERACTIONS.

CHAIRMAN VENITZ: And our last speaker for today is our very own Committee member, Dr. Kathleen Giacomini. She's going to tell us about Clinically Significant Interactions of OATP1B1 and Their Transporter-Based Interactions.

DR. GIACOMINI: Thank you. We've got a point to write. Thanks.

Okay. Good. So today I'm going to not be talking about P-gp, which is really the focus of a lot of the discussion today. I'm going to talk specifically about OATP1B1, and I'm going to talk about selected kidney

transporters, and I'll be addressing two questions. The first one is, are these transporters important for drug-drug interactions? And the second one is, what is the evidence for that?

And then I'm going to give some suggested, not really recommendations, but suggestions for further discussion maybe at a future time, and we might want to consider how important you think this evidence is that I'm presenting as discussion for not so far in the future.

So let's see. I think Mitch said earlier there are around 300 site carrier families, solute carrier super family members in the human genome. Not all of them interact with drugs. Many of them are very specific -- mitochondrial transporters which are involved in the uptake

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of amino acids, glucose, nutrients, et cetera.

So the ones that have been receiving a lot of attention for drug-drug interactions are the OATPs -- and sorry not only for drug-drug interactions but for drug disposition and response and OATPs, organic anion transporting polypeptides.

So I'll be focusing on one of these OATPs, OATP1B1.

The other group of transporters that receives a lot of attention in the literature and has for over the years are the OCTs, OATs, and OCTNs. These are organic cation transporters and organic anionic transporters in the kidneys. And I'll spend a little bit of time at the end talking about those and addressing the same group with the questions, whether I'll feel they're important for drug-drug interactions and what the evidence is.

So let me start with OATP1B1. And this is just a diagram of the hepatocyte, and you can see there are a number of transporters on the basal lateral membrane here, including some OATPs, and there's OATP1B1, OATP1B3 and OATP2B1 are ones that I think again Mitch discussed and those are the ones that have received a lot of attention in terms of how they interact with various drugs.

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I'll focus mostly on OATP1B1, but I'll say a few words about OATP1B3 and 2B1.

These transporters -- well, yeah, so let me get to the question. So are they -- is OATP1B1 important for drug-drug interactions? What's the evidence, and I'll go with in vitro evidence first and then move from in vitro to in vivo evidence.

So in in vitro, there are a whole lot of studies that have been performed showing a variety of different molecules, structurally diverse molecules interact with transporters. And here are some of the substrates for OATP1B1. You can see there's the penincillins, a whole bunch of statins, Rapaglinide, Rifampin -- a number of those compounds. OATP1B3 interacts with Digoxin. It also has a number of statins interacting with it, and OATP2B1 also expressed on the same membrane interacts with a number of these compounds.

So the take-home message here is that, yes, a lot of structurally diverse molecules interact with these

transporters so they are potentially important drug-drug interactions.

The second take-home message here is that because of

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their location on the sinusoidal membrane as the hepatocyte, they are particularly important, and that's because they are gatekeeping the enzymes that reside in the liver. So the interactions there may be particularly important. If a drug can't get into the liver, you're going to get a double kind of an interaction. A, it didn't get into the liver, so you have a disposition interaction, and, B, it's not going to be metabolized because it's not getting in. So they're taking drugs; they're still hepatocytes.

The other take-home message here is -- and I think this has been pointed out by the other speakers is that there is a lot of overlapping substrate specificity.

We don't have very distinct transporters for particular molecules, so that creates an in vitro situation.

So I think the evidence here in vitro is that potentially these transporters may be important for drug-drug interactions.

Now, let's look at the in vivo evidence here.

So when you think about in vivo evidence, and you want to know whether a transporter or a protein is particularly important for a drug-drug interaction or even important for

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the disposition of drugs, there are two levels of evidence; one at the genetic level -- what kind of information can you get, for example, from knockout mice or polymorphism in humans. And the second are chemical inhibition studies. Those will also tell you whether a particular transporter is at work, if you can get a drug-drug interaction in which in particular you have a specific inhibitor. That will give you in vivo evidence that a transporter is playing a role.

So what do we have for OATP1B1? Well, knockout mice for the OATPs are not applicable. So the wonderful data that we had on P-glycoprotein or MPR1, which I think paved the way to all of us being to understand that this transport protein was very important in drug to all of us beginning to understand that this transport protein was very important in drug to sufficient death and usually this is for the OATPs. And it can't exist because we -- there aren't species orthologues of each one of the OATPs. So there is mouse OATP1B1. And there's human OATP1B1, and then in the mouse or in the rodent there are different OATPs and even mouse and rats differ from one another.

So knockout mice information is not applicable for us to get some hint as to whether these transporters may play

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an in vivo role or drug-drug interaction study.

Polymorphism in humans, however, have -- there have been recent studies in the last four or five years, and I'm going to show you some of those in which there are increasing examples, increasing numbers of examples where polymorphisms in OATP1B1 appeared to play a role in drug disposition, which suggests that that particular transporter

may be important for drug-drug interactions.

Chemical inhibition studies suffer from, and I think David pointed it out nicely in his study -- we also saw it from Mitch -- and that is that their specific inhibitors are there, so you have to interpret those data.

But I'm going to show you what's there as well.

So here is -- let's go to polymorphism. So there have been a number of polymorphisms of OATP1B1 that have been identified and then studied in cellular-based assays. And this is a clinical study with Rapaglinide is shown.

So Rapaglinide, which is an anti-diabetic drug, and you can see these are individuals who are homozygous for 521C variant, and these are individuals who are homozygous at position 521, but they have the T allele. The T allele is the common allele.

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This variance, it changes the T to the C, changes in amino acid from Valine at a third position to Alanine. It's been shown in a number of cellular studies to not take up drugs very well when you have the "C" [ph.], and so you can see that the individuals who have 521CC have higher area under the curve, and I think here it's about 200 percent increase in their area under the curve when they have this particular variant.

That suggests that OATP1B1 is probably playing a role in Rapaglinide plasma disposition. They also looked at ABCB1 for Rapaglinide and there was no effect of certain common ABCB1 or P-glycoprotein variance there. They also looked at CYP3A and 5. There's really no effect there.

So the effect appears to be specifically for OATP1B1 and particularly for that 521C to T change.

There's another study, this is less compelling to me, where they looked at Fexofenadine. Again, the same variant at the 521C and again in the same direction. The people with the 521C who have the alanine at stat position are having -- are clearing the drug in a poorer fashion and having a higher systemic exposure than the individuals with 521T.

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I'm going to skip to this slide and then go back. For Pravastatin that same variance, 521C again, but this is another drug, 521C and the individual who are heterozygous for 521TC again have higher plasma levels than individuals who are homozygous for 521TT.

So those are three different drugs with the particularly -- the same data and there's in vitro data that support -- that correspond to this; that is, if you have the alanine at that position or if you have the 521C, you don't take up these drugs as well.

They're also promoter region variance in OTP1B1, and on this one particular promoter region variance, which shows again some type of a phenotypic difference. And this variance is in the untrans -- the five times UTR, and you can see that individuals who were heterozygous for the GA have higher plasma levels in this case of Pravastatin, the individuals who are homozygous for the G allele.

Skipping back. One more study with Pravastatin and

this is a haplotype. This haplotype contains the 521C. It also contains that promoter region variance, and here you see heterozygous again have higher plasma levels of Pravastatin than the people who are not carriers of this

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particular star seven.

So let me summarize. So there's compelling evidence, and there's some nice reviews in this area, but there's compelling evidence with a variety of different drugs that genetic variance in OATP1B1 is playing a role in drug disposition. So Pravastatin, Rapaglinide, Fexofenadine, Atrosensin [ph.], and Resuvestatin [ph.] have all gotten studies in the literature showing the same trend as with the star five or the 521C allele.

So what about chemical inhibition studies? Well, the chemical inhibition studies again are compelling in that the magnitude of the effects are large, but not specific for the transporter, and here's the example that Shiew-Mei presented at the very beginning. Again, Rapaglinide plasma levels, together with these dirty inhibitors; so Itraconazole, Gemfibrozol, and then Itraconazole plus Gembfibrozol. So you can see the plasma levels are increasing. Itraconazole is a CYP3A4 inhibitor and also a P-gp inhibitor.

Gemfibrozole is a CYP2CA inhibitor, and an OATP1B1 inhibitor, and this combination of the two together you get an even more increases in the plasma concentration.

People have tried to dissect away and this is again

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gets to be very complicated and I don't know that it's worth their while to do it, but tried to dissect away what is the mechanism for Gemfibrozole. Is it a OATP1B1 inhibition that's occurring or is it the CYP2CA inhibition, and shows they've done some studies with Trimethaprine, which does not inhibit OATP1B1. It does inhibit specifically CYP2C8, and they don't see as big an effect, so then they, therefore, extrapolate or have extrapolated and said well, then, therefore, this interaction that we're seeing with Gemfibrozole is largely OATP1B1. That may be pushing it a little bit too far, but that's the kind of thing you have to do when you have these dirty inhibitors.

So, chemical inhibition is not compelling. It's suggestive. It's not definitive. Genetic studies with these polymorphisms, increasing numbers of genetic studies showing the same trend in replication of class drugs with the in vitro data suggests to me that OATP1B1 was playing an important role in the disposition of some of these drugs.

And, therefore, interactions at OATP1B1 could be potentially drug-drug interactions; could be potentially very important.

What would you might want -- what might you want to

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consider?

Well, it's too early at this point to really have a long discussion on this, especially since we're really focused on P-gp today, but these are the kinds of studies.

Again, you might want to consider thinking about in vitro studies in cell lines, and they're already doing them,

as we learned, in a number of different pharmaceutical companies, assessing if your NME is a substrate or an inhibitor.

If the in vitro data show evidence of interaction, again there are possibly some drug-drug interaction studies that you could perform. If it's a substrate, Gemfibrozole may be a good compound to inhibit. Rifampin is also another one of these inhibitors that could be used in a clinical drug-drug interaction study.

If your compound is an inhibitor, it could possibly -- use that inhibit Fexofenadine or one of the statins, for example, Prevastatin.

So these are the kinds of things that I think the Committee should take up at some point, and probably not for today. But OATP1B1 being where it is in the hepatocyte, right there on the sinusoidal membrane, controlling access

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to those enzymes is very important.

I should say one other thing, and that is that they did also in a number of studies find pharmacodynamic changes as well. So when Prevastatin doesn't get into the liver because you have a polymorphism in OATP1B1 that translates to a reduced pharmacologic effect on cholesterol lowering, and that was shown, the pharmacodynamics that went along it, as well as some of the guinons [ph.], so the same kind of thing was shown.

So the polymorphism was PK polymorphism, but also had corresponding pharmocodynamic changes which make them, you know, drug-drug interactions potentially even more important.

Okay. Let me say a word about the OX and the OATS. These are transporters, and I'm going to talk specifically about the OX and the OATS in the kidney. This is a review article by Lee et al., and it just appeared in 2006, and it's a run on renal drug-drug interactions. So that's where you might find an update on some of the different clinical drug-drug interactions, and I would suggest people look at that and see what goes on there, but on the blood side are a number of different transporters.

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I'm going to talk specifically about OCT2, and you'll see that that's quite abundant on the blood side of the proximal tubule cell. And then OAT1 and 3 are also very important over here.

On the apical side, there are some transporters. I'm not going to talk too much about it. I'll say a little bit about OCTN1. I keep my eye on MATE-1 and OCTN-1 and OCTN-2, because there's increasing data that those transporters may play a role in moving drugs from the kidney cell into the tubule lumen for secretions.

So let's see. Let's talk first about OCT-2 and then OAT-1 and 3.

So this is a micro-array data just showing relative abundance of MRNA transcripts, which, as you know, may or may not correspond to protein levels that sometimes reflects it. And you can see in the kidney -- this is from the kidneys -- OCT-2 is the most abundant of the OCTs. This is

OCT-1 and OCT-3, so it's the most abundant OCT.

OCTN-2 is also quite abundant, but it's a quarantine [ph.] transporter and generally doesn't interact with a lot of different drugs.

The organic anion transporters you can see OAT-3 is in

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huge abundance, and this is a very different scale, so even OAT-1, which looks like it's not so impressive is expressed at a much higher MRNA level than all the OCTs.

So OAT-3 and OAT-1 are the two anion transporters on the basal lateral membrane that appear to mediate uptake of a variety of different organic anions.

One of the take-home messages that I have about these kidney transporters is there are charge-specific inhibitors and charge-specific interactions. So cation transporters interacting with cations. Anion transporters interacting with anions, and there's some overlap, but not too much.

So the inhibitors that we'll be discussing tend to be charge-specific inhibitors.

Okay. So what's the evidence that OCT-2, OAT-1, and OAT-3 play a role in drug-drug interactions?

Well, in vitro again there are numerous studies. I think Shiew-Mei showed us even more studies in which you see an isolated cell, and usually these are in heterologous expression systems where they've over expressed these transporters so they know it is OAT-1 or OAT-2 -- I mean OAT-3 or OCT-2, and you can see there are a number of different substrates.

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There is overlap between OAT-1 and OAT-3 in terms of some of the substrates that they take up. However, there's also specific OAT-1 substrates. So, for example, Cidovovir and Adefovir and Tenoclovir [ph.] interact with OAT-1, and you cannot find them interacting with OAT-3. So there could be better probes for OAT-1 and OAT-3 in terms of substrates.

OCT-2 is really right now we think is one of the lone transporters for kidney uptake of a lot of different organic cations, so there are a number of that I'm listing there as substrates for organic cations.

So, again, what we have is we've got in vitro data suggesting that structurally diverse molecules are interacting with these anion and cation transporters. They don't mean anything in vivo until we do the in vivo studies, so let's look at what we've got there.

Here, transgenic mice -- and excuse me, knockout mice in particular have been very helpful for in vivo studies, to know whether a transporter is important. There are species orthologues of OAT-1, OAT-3, and OCT-2 in between rodents and humans, and those species orthologues do have some specificity differences, but they generally recapitulate the

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transport activity in humans.

There are some recent polymorphism studies which I'll present, and then there are drug-drug interaction studies, and I'll show you those, clinical drug-drug interaction studies there.

So knockout mice, this just shows that there is an OAT-1 knockout mouse, an OAT-3 knockout mouse, and an OCT-1,2 knockout mouse. For OCT-2 -- in the human, OCT-2 is the transporter in the kidneys. In the rodents, OCT-1 and 2 are both in the kidneys, so you have to knock them both out. So for OCT-2, there's a complete loss. OCT-1, 2 knockout mice there's a complete loss of active tubular excretion of a model compound, tetraethylammonium. For OAT-1 knockout mice and OAT-3 mice using model compounds, they see very clear evidence that these transporters are involved in the disposition of anion substrates for the particular transporters, and those references are there.

In terms of polymorphisms, we've got some recent data in my laboratory, and this is not published data, but we've recruited people who have polymorphisms of OAT-3. This is one particular polymorphism, and we've measured the renal clearance of Cefotaxime. We're seeing a small, but

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significant, difference in the renal clearance of Cefotaxime with genetic variance of OAT-3. I think we'll see more and more studies on the genetic variance of renal transporters and what they're doing to drug disposition.

Remember that renal clearance will go down when you inhibit tubular secretion, but only to GFR. You're not going to inhibit the GFR component.

So in this particular, we feel that people who are carrying this particular variant of OAT-3 have all probably most of their secretory renal clearance has been inhibited in the people with the variance of OAT-3.

This is an interesting study with OCT-N1. We allele -- star one allele of OCT-N1, which takes up Gabapentin very nicely, and then we have a star two allele, which doesn't take up Gabapentin very well. This is empty vector transfected cells.

So clinically, when you look at the renal clearance of Gabapentin, which isn't so highly renal we cleared, but those individuals who are homozygous for star one have a certain renal clearance and that's significantly higher than the ones that are homozygous for star two.

If we subtract GFR from each one of these individuals,

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what we find is the net secretory clearance for the individuals with star one is, you know, something positive, maybe 40 mils per minute. And all of the secretory clearance appears, net secretory clearance, is abolished in the individuals who are homozygous for the star two allele.

So we're going to start to see genetic studies on these organic cation and anion transporters in humans, and polymorphism studies in humans to me are the most compelling, because knockout mice somebody can always argue that you have species differences and what you're seeing in the mouse will not correspond to what's going on in the human.

When you have a polymorphism and you show something, and you have in vitro data, you have fairly compelling evidence that that particular protein is involved in the disposition of the drug.

So, again, the knockout mice are available. Polymorphisms are just beginning to come. Chemical inhibition studies, selective but not specific inhibitors are available.

So I'm just showing you some examples here of -- so this classical inhibitor for anion transport in the kidney

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is Probenecid. And there are numbers and numbers of Probenecid interaction studies, and if you look at that review paper that I showed you, they've given you some examples of clinical interactions where Probenecid inhibits the renal clearance of a whole number of anionic compounds, Cephalosporin, Fisplatin [ph.], Gancyclovir, et cetera.

So there's a lot of interaction studies there. Again, it will depend upon the magnitude of the renal clearance of the drugs, because all you can get for inhibition is down to glomerular filtration rate in the kidneys.

For organic cation transporters, again, looking at that review article, there are least 14 different drug-drug interactions usually using Cimetidine as an inhibitor of different cations and you can clearly see that Cimedtidine is inhibiting the renal clearance of in this case maybe some 14 different organic cation substrates. And again, that magnitude is dependent upon the magnitude of the renal clearance.

This just tells you that the inhibitors tend to be charge-specific Cimetidine and Trimethoprim tend to be used for inhibitors of cation transport in the kidney. Probenecid in general is the inhibitor for anion transport

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in the kidney.

This is something again that this Committee may want to consider: perform in vitro studies in cellular assays, assess if your compound is a substrate of OAT-1, OAT-3, or OCT-2. If your data show it's a substrate for OCT-2, a Cimetidine inhibition clinical study will tell you whether it inhibits -- whether Cimetidine is going to inhibit the renal clearance of the compound. If your compound is an inhibitor of OCT-2, you may want to try it inhibiting the renal clearance of Metformin, and for the OATS -- Probenecid and in the case of this -- you can look at it with Cefazolin.

So my feeling right now overall is there's a good compelling evidence that OATP is involved in drug disposition. Drug-drug interaction studies we can do them forever. They will tend to be dirty, and we will not know whether, in fact, it's really related to OATP; we'll just have good data that suggests that it may be related to OATP.

In the kidney, I feel these interactions are pretty clear. Cimetidine is inhibiting probably OCT-2, since we don't know of another transporter there on the basal lateral

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membrane, which is being inhibited, and the OATs have -- Probenecid has been shown for many years as an inhibitor of the OATs, so those are also clinical drug-drug interaction studies that the Committee may want to consider. Okay.

CHAIRMAN VENITZ: Thank you, Kathleen. Any questions for Dr. Giacomini?

DR. JUSKO: Hi, Kathy. A very nice presentation. When I hear all about the transporters and drug interactions, I feel like that Dennis the Menace cartoon, where I think my brain is going to explode with all this information.

I wonder is there any generalization possible for the renal transporters in terms of structure-function, log P, PKA, and some way of anticipating whether a transporter will handle a particular substrate or interact with a particular inhibitor?

DR. GIACOMINI: So, of course, as you know, you'd probably have to try it out, but, of course, they tend to be small molecular weight hydrophilic organic cations that are interacting with kidney transporters OCTs and OATs. They're not the hydrophobic cations and anions, but for me to tell you what the log P or the PKA. You know Cimetidine has a PK

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-- it's renally secreted -- and it has a PKA of right around seven. You know, so 50/50 at physiologic pHs, so it's somewhere there. You know, somewhere there or greater for the PKAs for bases and acids tend to have PKAs of 3.5, something like that.

No real thing. Hydrophilic molecules tend to be substrates. Hydrophobic molecules tend to be more -- can be inhibitors. Okay.

DR. LESKO: I think a similar question related to the generalization, but maybe -- now that Bill asked it formally, I'll ask it another way.

With the CYP enzymes, we ended up in place where we are today, namely if we do a certain cytochrome, say, 3A4, inhibitory interaction, we then extrapolate the results of that study and say we do some class labeling, and we do that on the basis of things being substrates for 3A4 and being strongly, moderately, or weakly inhibited.

In the case of the transporters, and you talked about it, it seems like we're struggling to find substrates that are both specific in terms of inhibiting new molecular entities and in terms of being inhibited by new molecular entities so that each experiment seems to be a one-off

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experiment; that is is to say if somebody were to do these kinds of studies in drug development, how could we extrapolate that result beyond the study that was done to become more generalized like we currently are with the CYP enzymes and if we're not there, sort of how do we -- will we get there or is this not a big enough problem to think about it in the way of extrapolating?

DR. GIACOMINI: That's the second question. So to answer your first question first.

DR. LESKO: Sorry.

DR. GIACOMINI: So in terms of kidney transporter, pretty much, if your compound is a substrate and it has a substantial renal clearance so that you're concerned with the drug interaction in the kidney, if it's an anion, and it's interacting with an anion transporter, Probenecid is a

lovely compound to use in drug-drug interaction probes. If you don't see an inhibition in drug-drug interaction renal clearance.

So I think that's pretty standard. Almost every study I see looks at Probenecid as an anion transporter inhibitor.

For cations, Cimetidine is used pretty standardly

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across the board. So I think can you extrapolate it? You can certainly, if your compound is a substrate, you know the clinical studies to do.

Can you extrapolate it to it being an inhibitor? That's a little harder. You know usually substrates like Metformin is a perfect example. It's a great substrate for OCTs. It rarely will inhibit OCT transport. You know, the substrates go through very quickly and are not so hydrophobic, so they're not clinging onto anything and inhibiting it.

So there is extrapolation to inhibition studies that need to be done. But not -- I don't know what you mean by class? You certainly can't refute -- you could certainly say that if it's interacting with this transporter, if the renal clearance is important, and it's a cation, do it with Cimetidine. You could say that.

So it's pretty -- I think it's pretty clear.

For OATPs, that's less clear, because there you've got those three OATPs in the liver on the sinusoidal membrane, so you even have OATP1A2, which I didn't speak about. And there's all this overlapping specificity there, so I don't know about extrapolating that.

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DR. HUANG: So the question is if your Cimetidine study is negative, then can you put in a labeling on the other OCT inhibitors?

DR. GIACOMINI: I don't know about that. Cimetidine is the most potent inhibitor that we've seen, you know, but there are some others that people don't tend to test which makes me wonder about them.

But Cimetidine, its plasma concentrations in a therapeutic window are good inhibitory concentrations for organic cation transport; whereas, some of the more potent inhibitors of OCT, their plasma concentrations don't get up that high, especially if you consider the unbound concentration, which is really what's inhibiting the drug.

I doubt -- so I guess I say yes if you don't see something with Cimetidine, I wouldn't worry too much about the others, but --

DR. HUANG: So to state the question of Metformin is used in a lot of interaction studies that we have seen the interactions done with Metformin.

DR. GIACOMINI: As an inhibitor or as a substrate?

DR. HUANG: As a substrate.

DR. GIACOMINI: Okay. And there -- as far as

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inhibitors --

DR. HUANG: So if you don't see any direction then you might say while this may not affect --

DR. GIACOMINI: Right.
DR. HUANG: Other --
DR. GIACOMINI: You might.
DR. HUANG: Okay. This pair is like Ketoconazole and Madazoline [ph.]?
DR. GIACOMINI: In a way. Yes. And there could be some exceptions, but it's pretty good to try. That's what I would think.
DR. HUANG: Okay. And I have a question about OATPs -- one more.
What do you think about the use of whether the clinical effect of using Gemfibrozole and Cyclosporine? You listed that there are no specific chemical inhibitors?
DR. GIACOMINI: Yeah. And so because Gemfibrozole will inhibit enzymes as well, but I do like Gemfibrizole myself and I like Rifampin as inhibitors as opposed -- will pick up OATP1B1 for sure, but you may pick up some CYP as well, but you'll at least get that. So it would be a good inhibitor clinically.

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DR. HUANG: Yeah, I mean, so most of this chemical -- I mean we see the submission, this cytochrome is substrate or inhibitor and certainly have been captured, so --
DR. GIACOMINI: Right.
DR. HUANG: So if we know their and entity then I guess we could use that.
DR. GIACOMINI: Right.
DR. HUANG: Separate out for that?
DR. GIACOMINI: Right, exactly.
CHAIRMAN VENITZ: Any other questions? Thank you again, Kathy.
Now, let me ask Shiew-Mei to present the questions to the Committee so we can start to deliberate.
COMMITTEE DISCUSSION AND QUESTIONS
DR. HUANG: So our first question was -- well, the first two questions are the decision trees that we have presented earlier about the how to evaluate a new drug's effect as an inhibitor of P-gp is the first question -- as substrate.
So I have presented a chart in which I showed it like this, and so the question for the Committee is are the criteria for determining whether an investigational drug is

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an inhibitor of P-gp and whether an in vivo drug interaction study is needed, as described in this decision tree appropriately.
CHAIRMAN VENITZ: Any comments? Discussion?
DR. MCLEOD: Has there been any attempt to put through a database of pas examples to see what this -- how this would fare? You're going to be flagging eight out of 10 compounds or one out of 10 compounds? Is this -- are there any positive controls?
DR. HUANG: Yeah. One of the inhibitors-a lot of recent submissions, I can talk about the recent submissions because we have more detailed in vitro studies that we reviewed and so, therefore, they're in our system.
Quite a few we have that may compare I over IC50, and

they're lower than .1, but these sponsors did the study anyway, and the results are negative. And I don't think we have too many cases where I over IC50 is more than .1. And we don't have the data for that.

DR. GIACOMINI: So I mean a good example that's -- the .1, as you point out, is arbitrary. I mean you have to pick something, and not be colored by -- and what might be out there, and what people have shown good.

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But I would -- I mean we have a lot of compounds that inhibit our transporters in cellular assays, but -- and then in clinical their concentrations are right in that same range, but they're unbound; they're highly protein bound, these inhibitors, and their unbound concentrations are way off. We don't get the inhibition in vivo.

So do you ever think about using the unbound concentration ratio being -- you know, the I being the unbound concentration or would that -- or would you miss?

DR. HUANG: Well, I guess the argument goes back to when we were discussing what criteria to use, when we were discussing whether it's an inhibitor of CYP enzymes, and then at that time, I mean we could use the equation that Dr. Greenblatt showed: One plus I over KI, which is a very simplistic view of a ratio of AUC with inhibitor or without inhibitor.

So you have up to .1. You have about 10 percent increase. That is that -- that's a very simple equation. But we have to consider the gut concentrations, the liver concentrations, and a lot of times we're thinking about the systemic plasma concentration may not be representative of the liver concentration. And one of the reasons in our

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guidance we say we use the total, which is bound, plus and bound, is just try to ensure that we use a more conservative approach so that that's how we set this point.

One comment that we got is actually that I may be too low, because considering the concentration at GI, especially P-gp does not affect the intestine, and the concentration was much higher.

So our numbers will tend to be more conservative.

So we did see quite a few where you wouldn't recommend a study, and it was shown that the allele study in -- there is no interaction. But we do not have a lot of labels to these compounds.

DR. THANG: Well, in the literature, all the information we have is we suggest in the probe substrate. They did who that Cyclosporine and Quinidine, and if you did calculate I over KI, there were much more. So they do meet that criteria.

DR. GIACOMINI: But do they fall above .2? I mean are they all heavy -- and so you've picked a very conservative, and that's fine.

DR. THANG: Yeah, we don't have enough data to say where that cut off should be, but that's -- I think as to

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say that generating we can P modify this, and, as Dr. Greenblatt just proposed, we should focus on the highly

potent region of OATP -- you know, because I'm not sure what his definition of highly potent is.

Do you have a proposal?

CHAIRMAN VENITZ: I'm asking Dr. Greenblatt to join us.

DR. GREENBLATT: Well, I'm not sure I do, but I think it depends on again going back to the exposure response relationship for the substrate. But just to get back to this, what -- we have to remember the limitations of Digoxin as a probe for enteric P-gp. Net wise, it's the efflux -- the enteric efflux transport is not that great. The F, the next F, the absolute bioavailability of Digoxin in the uninhibited state is around 60 to 70 percent, so there's not that much efflux transport.

If you go up to a hundred percent with complete inhibition of efflux transport that's not much of a change. So Digoxin is not going to be that sensitive for inhibition. You can detect it, but it's not that sensitive.

It will be very sensitive for induction, because it can go down a lot; if you greatly up regulate enteric P-gp,