FOOD AND DRUG ADMINISTRATION

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FDA GENETIC TOXICOLOGY WORKSHOP

How many doses of an Ames-positive/Mutagenic (DNA Reactive) Drug can be safely administered to Healthy Subjects?

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PRESENTATIONS & PANEL DISCUSSION

DATE: Monday, November 4, 2019

TIME: 8:30 a.m.

LOCATION: FDA White Oak Campus
Building 2 (CSU), Room 2031
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Silver Spring, MD 20993

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P A R T I C I P A N T S

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Dr. Bob Dorsam, CDER, Office of Generics, FDA

Dr. Dayton Petibone, NCTR, FDA

Dr. Jennifer Shemansky, NCTR, FDA

Dr. Douglas Brash, Yale University

Dr. Kenny Crump, Louisiana Tech University

Panelists:

Dr. Aisar Atrakchi, CDER, FDA, Moderator

Dr. Alan Boobis, Imperial College London

Dr. Douglas Brash, Yale University

Dr. Kenny Crump, Louisiana Tech University

Dr. Robert Heflich, National Center for Toxicologic Research, FDA

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Dr. Kevin A. Prohaska, FDA

Dr. Errol Zeiger, Private Consultant

DR. TIMOTHY ROBISON: Good morning. This is the Genetic Toxicology Workshop. I just have a few sort of general announcements.

Just to note that this is a public workshop. Both members of the public and FDA staff are present. So no proprietary information should be discussed.

The workshop is being recorded and a transcriptionist is present to transcribe everything that is said.

The workshop serves as an educational event for FDA staff and the public. Further, it's a forum to seek advice from an expert panel on several questions. We have a panel discussion in the afternoon. There will be open question periods during the meeting, both FDA staff as well as the public are invited to ask questions.

I would just like to extend thank-you's to the Genetic Toxicology Subcommittee, the National Center for Toxological Research, the Office of Generic Drugs, and Division of Hematology, Oncology, and Toxicology, the Pharm-Tox Coordinating Committee, and

Pharm-Tox Coordinating Committee Educational Subcommittee, who all helped with this workshop.

The overarching question of this workshop is how many doses of an Ames-positive, mutagenic, or DNA-reactive drug can be safely administered in healthy subjects.

Healthy subjects are commonly enrolled in human Phase I clinical trials of new drug candidates under an IMD. So these studies are typically short. They can range from two days to a few weeks. The treatment may be continuous or intermittent. And since intermittent, there could be a washout period between each dose. Typically this washout period might be up to five half-lives.

Just to note as an important consideration is that these healthy subjects received no benefit and are potentially exposed to significant health risks in the proposed trial. Patients will be enrolled in longer Phase 2 and Phase 3 clinical trials. Our focus is primarily on Phase 1 where healthy subjects are enrolled.

Advantages of conducting trials in

healthy subjects include investigations of pharmacokinetics, bioavailability, and the absence of other potentially confounding drugs, data not confounded by disease, identification of maximum tolerated dose, reduction in patient exposure to ineffective drugs or doses, and rapid accrual into a study.

Just from personal experience, there are a number of disease indications where patients are fully subscribed and it's very difficult to find -- it's a very slow process to find patients with -- in some ways it's much easier to recruit healthy subjects.

Our new IND, the supporting (indiscernible) receive a fairly extensive non-clinical data package that lists the studies here. I'd like to focus on the toxicology studies, which typically range in duration from 14 to 28 days and they are conducted in both rodent and non-rodent. Also there is a standard battery in genetic toxicology studies. This includes an Ames Bacterial Reverse Mutation Assay. That could mean for a single dose.

And then for a repeat dose, it would also be an invitro mammalian cell gene-tox assay.

And prior to the start of Phase II trials would receive an invitro micronucleus study. Primarily in Phase I trials you primarily would have the Ames assay and an invitro mammalian cell assay.

The toxicology studies are used primarily to set clinical doses using -- based on the findings of animal study in terms of dose (indiscernible) toxicity and target (indiscernible) or toxicity. This information is also used to assist with clinical monitoring.

The genetic toxicology studies are used for hazard identification to get a general sense of -- if it's a mutagen or a clastogen. Cancer drugs are often presumed to be genotoxic. The genetic toxicology (indiscernible) are not generally required for clinical trials in cancer patients.

Generally most drugs that are found to be positive for mutagenicity, i.e. Ames positive, outside of oncology indications are not developed and generally drop from development.

The ICH M3(R2) guidance provides follow-up for a positive invitro mammalian cell chromosomal aberration assay in the sense of conducting two in vivo mammalian assays typically in in vivo micronucleus tests and in vivo liver common assay, two in vivo assays with two endpoints.

However, the Ames test results are thought to indicate (indiscernible) reactivity and extensive follow-up testing to assess in vivo mutagenetic and carcinogenic potential would be warranted to assess the potential risk for treatment that's justified by appropriate risk-benefit analysis. I think it's our general thought that a positive Ames assay has a high correlation to tumor findings in a two-year rodent bioassay.

However, in the U.S., a drug with a positive in vitro Ames bacterial mutagenicity assay may still be administered in healthy subjects and are enrolled in the single dose clinical trial if they are not made aware of the study results in the informed consent.

One of the dilemmas that has come up is

that pharmacokinetic studies typically require at least two to four doses, such as a crossover design. Dr. Dorsam will go a little more into detail of this later. This has sort of led to questions about the risk with a small number of doses beyond a single dose.

And just to note that there can be -- still these can be separated by washout period or that could be continuously dosed by a number of daily doses.

This sort of leads to the question of how many doses of a reactive drug can be safely administered to healthy subjects. Does this concern for an Ames-positive drug only applied to chronic administration or does it extend to a small number of doses, i.e. one, two, three, or four doses? The worst-case scenario might be 14 daily doses.

There is a acknowledge of published literature or guidance, documents directed towards trying to understand the cancer risk or other potential health concerns associated with a small number of doses of an Ames-positive drug in healthy

subjects.

Just to note that results of rodent carcinogenicity studies with a new drug candidate are typically not available until late in drug development. We normally wouldn't receive them until the sponsor files a new drug application. Applications will come in very late in Phase III trials. During the IND development stage, we are principally reliant on the standard battery of genetic toxicology studies to sort of inform the potential for cancer. Several review divisions do allow a single dose of an Ames-Positive drug in healthy subjects. However, others do not allow any dosing. And yet some others may allow more than one dose. Several review divisions have raised questions regarding the number of doses of an Ames-positive drug they can safely administer to healthy subjects.

Today CDER is seeking advice of a panel of experts. Again, the overarching question for the workshop is how many doses of an Ames-Positive drug can be safely administered to healthy subjects.

We did reach out to Health Canada,

Japan, and EMA to get some understanding of their practice for dealing with an Ames-positive drug. Health Canada replied that a clinical trial in healthy subjects with an Ames-positive drug would not be allowed to proceed without substantial follow-up testing to demonstrate that the drug is not mutagenic in vivo. In Japan, they did not allow administration of a clearly Ames-positive drug in healthy subjects. They do note in the ICH M3(R2) guidance that it's permissible to allow a small number of microdoses. This is a dose of approximately 100 microgram per day. It's not a pharmacologically active dose, but potentially it could allow up to five doses at a dose of 100 microgram per day. Again, this is not a pharmacologically active dose. It's more than ten to a thousand fold lower than a pharmacologically active dose that would be commonly used in a clinical trial.

Also, they note that they would have less concern if the drugs has no structural alerts and that there is less concern where it will be Ames-positive if it does not possess structural alerts when considering mutagenicity.

Bay Pharma in Germany noted they have not dealt yet with this issue with applications for a first human trials with Ames-positive drug candidates outside of the microdosing scenario.

MHRA UK said they would consider scientific justification as to why a sponsor thought it was acceptable to dose healthy volunteers with a product that was genotoxic, e.g. positive in the Ames test. The sponsor would need to provide specific justification as to why to conduct further -- evaluate -- further studies to evaluate the genotoxic potential of the product and the associated clinical relevance.

Examples are given in ICH S2(R1). A single dose first in human trial with an Ames-positive drug may be acceptable providing that there is adequate justification listed and a number of things based on the threshold of toxological concern, the half-life, the proposed clinical dose, the strains in which the drugs was positive in the Ames Assay. They sort of would be very negative about a dosing out to a week with an Ames-positive drug.

So today we have a series of

presentations. I'm providing an introduction. I will be followed by Dr. Kevin Prohaska, who will speak about the FDA requirements for protection of healthy subjects in Phase I clinical trials. This will be followed by Dr. Bob Dorsam from the Office of Generic Drugs, looking at the Considerations for a Genotoxic API in Clinical Trials: Healthy Subjects or Patients?

Doctors Dayton Petibone and Jennifer Shemansky at NCTR have conducted an extensive literature review. And Dr. Petibone will present as well as this literature sort of to try to get a bit of -- Dr. Petibone will explain more about trying to understand the risks of a small number of doses of Ames-positive drug, to see what's available in the literature. They conducted a search more or less for the past year, going through thousands of articles to glean out information that might be helpful.

This will be followed by a presentation by Dr. Douglas Brash from Yale University, Do the Steps between Genotoxin and Cancer Create Thresholds of Dose or Time?

I have to note that Dr. Crump wasn't

able to make it today due to a death in the family. I'm going to try to give a very brief presentation that highlights his talk. He did send a video, and I'll see if it's possible to send that video at a later date. We weren't able to -- we just received it last night and we weren't able to have it ready today.

In the afternoon, we're having a panel discussion. We brought together a panel of international experts. If you look at the more extended agenda, there is a bio sketch for each of these individuals. They will also introduce themselves at the start of a panel discussion this afternoon.

For today's discussion of DNA-reactive drugs, we have chosen to principally focus on Ames-positive drugs since there is a high correlation between chemicals found in positive assay and the positive Tumorigenicity findings in the two year rodent bioassay. We're assuming that the drug has the potential to be DNA reactive and form DNA adducts that can induce strand breaks of intercalate in between the DNA bases.

We note that the many potent genotoxic carcinogens can be positive for both mutation and Ames assay as well as positive for clastogenicity, wouldn't necessarily be that uncommon if they were positive (indiscernible) more than one assay of the standard battery. Dr. Petibone will provide some supportive information.

I'm going to briefly talk about how mutations may cause cancer. And this is sort of going through the literature to sort of provide a hypothesis on how this might occur. It's not to try to exclude other mechanisms or other potential paths, just to sort of give a general overview.

DNA damage is an important first step in the carcinogenesis process. Chemical carcinogens can cause the formation of DNA adducts. They can induce other modifications to DNA, such as oxidative damage and alterations to DNA, alter structure.

In the cartoon I'm showing benzopyrene. It can undergo metabolic (indiscernible) to form a reactive epoxide. It also could be potentially be detoxified by enzymes in the cell or it can go on to

interact with DNA and form a DNA adduct. There is a possibility for DNA repair to remove the adduct. But what we're focusing on today is that it forms -- will react with DNA to form an adduct. And you can see the (indiscernible) the DNA structure. Potentially these changes in DNA could potentially go on and lead to the generation of the tumor.

Cells do (indiscernible) mechanisms to repair many times with DNA damage. However, these are not always completely effective. The majority of mutations may be largely neutral in that they're passenger mutations. However, mutations in an oncogene, tumor suppressor gene, or a gene that controls cell cycle can result in a (indiscernible) cell population that the proliferation or survival advantage. These mutations are known as drivers, and driver genes are defined as genes containing driver mutations. Oncogenes are defined as driver genes in which the driver mutations are activating or result in new functions. Tumor suppressors are driver genes in which the driver mutations are inactivating. Oncogenes tend to be affected by vocal amplifications

or (indiscernible) mutations (indiscernible) limited number of codons. Tumor suppressors tend to be affected by (indiscernible), frameshift, or splice-site mutations (indiscernible).

The specific types of activating or inactivating mutations can be specific to a driver gene. Gene rearrangements almost exclusively activate the oncogene in non-Hodgkin's lymphoma. The (indiscernible) the cause of (indiscernible) oncogene activation.

There are always some pre-existing mutations, some of which amplify in the tissue because of their driver genes. Mutagens induce additional mutations (indiscernible) so the passenger mutations generally greatly outnumber driver mutations. Some driver mutations occur in DNA repair or replication of genes and induce a mutator phenotype which results in additional driver and passenger mutations with each cell division. Transformation from a normal to a tumor requires accumulation of five to eight driver mutations in the same cell. I have seen publications where it was noted that it might be as few as three

driver mutations.

If most have accumulated already, an unlocking mutation induced by a single exposure has a small but measurable chance of initiating the tumor. And single mutation cancers are known -- single rearrangement to form. The Philadelphia chromosome is probably all that is needed to cause a chronic myeloid leukemia.

The gold standard of evidence that a mutation is a driver is that the mutation produces a cellular phenotype introduced to a selective advantage to the cells harboring it. And such phenotypes may be directly or indirectly to the survival and proliferation and clonal expansion of mutant daughter cells. Each create a pair of mutant daughters, exponentially increasing the prevalence of mutant cells.

From initiation you can have a polyclonal expansion, you can have clonal cooperativity. There is some shown in the diagram. Dr. Brash is going to go on to this in more detail. But I think it's well-known that epithelial cells,

(indiscernible) cells, immune cells can potentially cooperate and lead to expansion of the tumor.

Just sort of moving on, there is quite a bit of literature that a single exposure to a carcinogen can lead to cancer. The is a published study with 426 agents. You can see there is a diverse set of chemicals, polyaromatic hydrocarbons, (indiscernible). So there is quite a bit of literature that a single exposure can lead to cancer.

I just wanted to briefly note, just sort of moving on to intermittent exposure to pharmacokinetic study with the drug administered to healthy subjects. Doses are typically separated by a washout period. We mentioned up to five half-lives were created in 95 percent of the drug, (indiscernible) be cleared. Considering the number of days of treatment, there are intermittent exposures where there is a washout period between treatment. There is a greater probability that the drug could never ready study's date. There's sort of -- just looking at the mode of action, there is a recovery time. This could decrease the potential risk of a

genotoxic adverse health outcome. Continuous exposure could saturate DNA repair capacity or other physiological processes, whereas intermittent exposures would allow for DNA repair or other adaptive or invisible physiological responses. Therefore approach for intermittent exposures is to consider the potential for repeated exposure during a lifetime and we combine these into an equivalent short-term scenario. And finally, apply the same approach for that of the short-term exposure.

I just wanted to get to our concern to the single dose. Which is sort of how I see (indiscernible) might deal with this. Use of drugs that are genotoxic based on this battery of tests in healthy subjects. These subjects are exposed to all risk and no benefit. The minimized risk to healthy subjects of a virtually safe dose of a genotoxic carcinogen has generally been defined as a dose which after a lifetime exposure would result in one additional cancer case in a population of one million. Assuming for a 70-year life span, there is 25,000 days. The linear extrapolation of a virtually safe

dose to a one or ten-day exposure results in a daily dose level of 25,000 times the virtually safe dose, or 2,500 times the virtually safe dose, at which exposure's lifetime risk is considered acceptable. If sensitive populations can be identified, an additional dose correction factor of ten is applied resulting in a tenfold lower daily dose. At these dose levels, the additional lifetime cancer risk is considered to be negligible since they are set for susceptible cell populations.

And I think I'm going to stop here. I have a few minutes for questions if there are any. We'll move on. If there are no questions, we can just move on to the next presentation. Are there any online questions?

WOMAN: Will the slides be available after the --

DR. TIMOTHY ROBISON: Yes, yes.

Our next speaker is Dr. Kevin Prohaska. He is a bioethicist here at FDA. He is going to speak about the FDA Requirements for the Protection of Healthy Subjects in Phase 1 Clinical Trials.

DR. KEVIN PROHASKA: Good morning, everyone. Can you all hear me? Fantastic.

Well, thank you very much for -- first of all, thank you for inviting me to speak at this meeting. But also I wanted to thank everyone for showing up today. This is fantastic. To my understanding there is about a hundred or so people online in addition to the people here. So this is a great showing. Thank you very much. This is a very important topic, and we certainly want to try to get some good recommendations at the end of the day so that we can help this important research go forward.

What I've been tasked to talk about today is the FDA Requirements for the Protection of Healthy Subjects in Phase 1 Clinical Trials. And I also want to clarify that as far as my ethics consultation works go, my focus is in adult research. We do have two pediatric ethicists who are involved in focusing on pediatric research. There we go.

And then the usual disclosures. The opinions that I am going to present today are my own. Especially later when we're having Q&As, I'm likely to

hopefully spill into some issues that might not necessarily reflect the FDA, although I hope they do. And the objectives are to provide a general overview of the FDA requirements for the protection of healthy subjects and also to give you an overview of the ethical principals that are considered when I look at Phase 1 clinical trials offering no prospect or direct benefit to healthy subjects, which is primarily the focus of today's conversation.

Now, of course most everybody here, especially within the FDA, are familiar with this classic drug development outline if you will. The important point about this is we are going to be focusing on strictly the Phase 1 aspect of this development program. However, as we all know, the phase are starting to blend into each other. And a lot of groups are doing Phase 1 work and Phase 2 and then Phase 3 and so forth and so on. And that's an important point to remember, because sometimes it can complicate the analysis that's necessary for the ethics of all of this. But we're going to confine our conversations to the Phase 1 in the classic sense.

So the concerns that we have from a bioethical point of view are pretty straightforward. Phase 1 clinical trials do not typically offer the product of direct benefit of the subjects. The healthy subject is not likely to benefit from the product development because they don't have the target condition. Now, ideally it would be nice if you actually were to enroll people at risk for the condition, albeit healthy. That would actually facilitate some of the risk discussion that might occur.

Pre-clinical work may be suggestive of safety signal. And that's the focus of today's conversation of course with the Ames studies. And we need to assure that healthy subjects are not unduly influenced in participating in the research. Undue influence is sort of a nebulous phrase, and it's sort of hard to pin down what exactly we mean by that. So it's another one of those areas where we have to use a lot of judgement.

And consent document. Again, the process needs to be clear and balanced and minimize

misunderstandings and undue influence.

So as most everybody here knows, the IND content has a lot of different things that are required. And the point of this slide was that here at the FDA when you submit these IND applications to us, it crosses multiple disciplines, including ethics. And I probably get brought in on a minority of these, but when ethical issues arise from the initial review of these applications, they asked me to come in and give my opinions And that's about 80 percent of the work.

Now, the regulations for the protection of human subjects are primarily confined to two areas, Part 50 and 56. Part 50 are the ones that have to do with the informed consent for the most part, and 56 have to do with the IRB responsibilities.

Now, I raise that as only part of the human subjects protection, because I would argue Part 312 and all the other parts also have embedded in it human subject protection concerns. That's why we asked for all of this safety information, because we want to protect people. So that's very important to

keep in mind.

So there are no specific unique human subject protection regulations that apply to research involving healthy subjects. So that's a point that I wanted to make.

What happens is we have to think about the general recommendations and how it applies to the population that's being enrolled in the study. And it doesn't matter what population it is, whether you're talking about a vulnerable population or say cognitively impaired individuals, or in this case healthy individuals, or maybe the full spectrum in between.

Our regulations are based on the work that was outlined in the Belmont report. And there's three principles that we think about. There's respect for persons, beneficence, and justice. Now, there's a lot of different ways of trying to carve up the bioethics if you will. But this is the foundation on which our regulations are developed. And I should say that that developed in response to the Tuskegee events in the 20th century.

So, respect for persons come from the requirements from the informed consent. The additional protections that we have for vulnerable populations that we have, like say for subpart D for pediatric research is a good example. And then there's a growing concern about privacy and confidentiality, especially in this day and age with big data and whatnot, that it seems to be growing even more lately.

Beneficence. We want to make certain that risks for subjects are reasonable in relationship to anticipated benefit. In this particular case, we don’t' have benefit to the individual, so we have to consider it in relationship to the social value that the research offers to the community, to public health. And then the risk benefit is that it has to be at least as favorable as available alternative approaches. So that's sort of a question I ask; how would this product compare to the other things that were available on the market? You know, if it's just a me-too drug, then why are we exposing people to a lot of different risk when there's 20 other me-too's

in that category. If it's a unique drug and it looks like it's going to offer great promise, then it is an area where we might consider some additional risks that we wouldn't necessarily consider otherwise.

Again, we have to always worry about the qualification, not only of the investigator, but everybody else that's involved with the research. So that's sort of something we consider as well. And then trial design, critically important. If the trial is not adequately designed, if it's not scientifically sound, then I would argue that there's really no good reason to expose anybody to any level of risk, because you're not going to learn something at the end of the day. Very important to consider.

And then justice. We want to make certain that the selection of subjects is equitable, that when the population is not exposing all the risks for the benefit of another group or population. The exclusion criteria is reasonable and the recruitment efforts are appropriate.

I'll go over this quickly. In Part 56 there's a whole bunch of criteria that the IRB uses

for reviewing research. The one that I think is going to be the focus of today's conversation is the second element. This is the paraphrase that the risk to subject is reasonable in relationship to the anticipated benefit. Again, we don't have benefit here, so we're thinking about social value. And the importance of the information that may be expected to result.

So a couple of things related to FDA policies. The FDA does not consider payment to subject as a benefit. So that is not something that we -- say, well, they're getting paid a lot of money to do this research, so they can get the risk. That's off the table. That's not part of the conversation. We do want people to be adequately compensated for the time and effort that they're doing of course. But they're not being paid per se to be exposed to lots of risk.

Risks should be considered in the context of the social value. I've said that a couple of times.

Social value could be thought of as the

goal to have research hypothesis that asks an important question. For Phase 1 trials, the expectations would be that the study contributes in a significant way to the development plan of the investigational product. So we know we're at the beginning of the process, and there's a lot to be done. But you have to get started somewhere. But the Phase 1 trial needs to ask some important hypotheses.

But there are limits as to how much risk can be justified by social value alone. Many ethicists believe that potential harm should not be irreversible, lead to permanent disability, or potentially fatal. Others believe that there is a need to balance paternalism with autonomy. And for me, it's very helpful to codify the risk whenever possible. I know that it's extremely difficult to quantify risk in any substantial way. But when that can be done, it's very helpful to try to help with the analysis. It almost always comes down to a judgment call.

This slide is interesting. The concept of paternalism and autonomy are ethic concepts that

have always been in conflict with each other in some ways. And what I wanted to present here was the evolution over time that's happened since the end of World War II with the Nuremburg Code all the way up to the CIOMS guidance in 2016. There has been a greater understanding that there are sometimes appropriate to move forward with doing some research when there's no prospect of direct benefit. Unfortunately, none of these ethical codes sort of quantify what's the acceptable level of risk, while they do sort of support the use of some research in this population. Except for the Nuremburg Code. And the Nuremburg Code was funny in the sense that it sort of suggested that they start experimenting on the investigator, which is interesting. But I think that that was certainly an outcome of the atrocities of World War II.

So the general requirements for informed consent. Most everybody here knows all this stuff. The investigator shall seek consent only under circumstances that provides prospective subjects or their representatives, their legally-authorized representatives, sufficient opportunity to consider

whether or not to participate and that minimize the possibility of coercion or undue influence. The reason I read that is because it's a critically important sentence to unpack.

Coercion I'm going to take off the table. And what that basically means is that there is a threat going on. And obviously there should be no research going to the FDA in which there is a sense of threat. But undue influence is a nuanced phrase that requires some unpacking and some thinking about to determine whether or not it's occurring.

What this sentence reminds me of is that not only are the elements of constant important -- and there are currently eight of them in the FDA regulations -- but that the context in which consent is obtained and how it's obtained is critically important. People ought to be given time when it's appropriate, be given time to think about it. If there is a very high level of risk, we want to make certain people understand what that risk is. And there's ways of doing that for the consent process by test backing and things of that sort to make certain

they understand.

So then there's other things you want to do. Then there's the issue of compensation for injuries is one of those controversial things that we sometimes have to deal with. The right to withdraw at any time must always be respected. Now of course there are some research protocols in which it may not be safe for people to abruptly stop, in which case that's part of the consent process. So making them understand that they need to table off the drug or be followed by a short period of time for their own safety. But at the end of the day, people should be allowed or must be allowed to withdraw at any time.

I'm not going to go over these, but these are the consent elements. They're under 50.25, the required basic elements under 50.15(a). The additional elements are the ones that IRBs consider including when the conditions are appropriate. And then the applicable clinical trials is 25 (indiscernible). And that's that verbatim statement that has to be in the consent document for any applicable clinical trial.

As far as the consent document goes, when I look at them, I want to make certain that the risk discussion is very clear, that significant risks should be emphasized, that you might want to consider a test to assess people's understanding of the risk when they are substantial or difficult to understand. Statements about lack of benefit must be clear. Do not conflate the payments of other healthcare services provided during a trial. That's important. The social value can be scribed, but it must be balanced. You want to avoid overly-optimistic or misleading statements when you do craft these consent documents. And the amount and process for paying subjects should be described of when creating undue influence.

Here one of the things that can happen is if all the payment is weighted until the end and you must finish the (indiscernible) if you're going to get anything. Well, that may not be fair if it's a multi-visit study. So perhaps the payments should be staggered so that they won't feel compelled to finish the research if they don't want to.

Grounds for clinical holds. You all

know this. But the one that I point out every once in a while. We all think about safety, but we also have to keep in mind that scientific soundness is also a ground for clinical hold. So, unreasonable significant risk is the one that I sometimes get pulled into, and it's a judgment call.

So what do I look for when I'm asked to look at these things? I want to know are the risks reasonable, minimized, and justified by the potential social value of the trial.

Two, are vulnerable populations protected, meaning what sort of efforts are done to mitigate (indiscernible). Are people being allowed enough time to look at the consent document? If they're cognitively impaired, are their families involved? Is there a robust informed consent document in process? So that goes along with what I said about number three.

Is the level of compensation adequate and not undue? Is their right to withdraw respected? Have independent experts reviewed and conducted -- I did check this. I don't know what happened. Have

independent experts reviewed and conducted -- this is sometimes very helpful and something I look for in applications and protocols. In trials that are likely to be controversial, it's very helpful to have independent experts look at it and give their opinion and may sway judgements internally. Is there a system for compensation for an injury? Is there adequate safety monitoring, both short-term and long-term? And in this we give a case where there is concern about cancer potential, long-term monitoring may be appropriate.

Are there alternative approaches or populations that would be more justifiable? And this is actually a big area where I spend some time thinking about it. The question really has to be asked; must it be done in people who have no prospective direct benefit? Can it be done in people who have the condition when possible? Because that can justify more risk because you would hopefully benefit from the potential administration of the drug. If not the people who have the condition, potentially how about the people who are at risk for the

condition? Maybe not as much risk, but more risk than a purely healthy population. So that's something to think about. There may be scientific reasons for doing it in healthy volunteers, and that ought to be explained so that we understand what the rationale is for exposing this population to these potentially dangerous drugs.

And I put that one there to remind you that this is all judgement and it's a balancing sort of act that you have to weigh all of these things together. And reasonable people can disagree. And so risk is an area where we all have different risk thresholds of what we think is tolerable. And I respect that, and I understand that. And it's important to just hear people out and to understand where they're coming from. But at the end of the day what we have to do is we have to assure that we're moving forward in a reasonable way that respects the individuals that are involved in this research.

So, thank you. I think I have time for questions. Yes, I do.

At the end of my slides, and I think

you all have my slides, I put out a link to a number of different resources that you might find helpful, ones that I looked at while I was creating this slide deck for this.

Yes, sir.

AUDIENCE: I found your Nuremburg Code thing interesting, because as a researcher I'm forced to think about that. There's a whole lot of, like, subliminal considerations that might actually not be on my consent forms that it forces me to think about. So is there any experiences (indiscernible) research (indiscernible) participating in these studies?

DR. KEVIN PROHASKA: That's an interesting question. To my knowledge, the FDA doesn't have a ton of experience in that. There is one experience -- and I've not prepared to think about the guy's name. But the guy from Australia that developed -- who had the hypothesis of H. pylori and peptic ulcer diseases. He self-inoculated himself. That was widely criticized by a lot of bioethicists, but ultimately I think he got an award for it. He got an award.

So attitudes change over time. But in general I would not necessarily support a researcher doing research on themselves or their family members because of the concern for undue influence.

AUDIENCE: I probably should have asked this question before when we were preparing for this. But I was curious, have you ever encountered the specific question we're dealing with previously where a genotoxic drug was proposed and given to healthy volunteers?

DR. KEVIN PROHASKA: You know, I've been the agency's bioethicist now for about six or seven years. And to date I have not been brought into this conversation. So the answer is no. But I've been brought into analogous conversations in other disciplines and in other areas where Phase 1 trials involving potentially risky drugs were going to be given to healthy volunteers. And the analysis that I outlined on my last slide there is sort of the majority of what I do. You know, I want to as much as possible understand why they're selecting the population, could it be done in somebody who has the

condition? If they could possibly quantify the research, that would be very helpful.

I should add that I've looked at the literature on this in preparation for today's conversation, but also for previous consultations that I've done. And the literature is all over the place as far as the bioethics literature as to what is the level of acceptable risk. And there's no real definitive answer to that question I'm afraid.

There's one study, and I think I gave the resource for it. A gentleman by the name of Resnik who in a way sort of compared it to the risk of highly-risky professions like a policeman or a fireman. And they, for one reason or another, choose to do that type of profession, for compensation of course. And we allow that as a society. So the question is, why shouldn't we allow a certain amount of risk in clinical research. It's an interesting -- he presents -- I don't know if it's his own, but he presents it in his paper that I cite. And I thought that was very interesting and worth considering.

But the physician (indiscernible) goes

back to the first rule of medicine is first, do no harm. You know? And so I said, well, that's nice, but we have to also be a little bit paternalistic and make certain that we offer reasonableness.

AUDIENCE: So I assume these kinds of issues have been discussed among ethicists before.

DR. KEVIN PROHASKA: Oh, absolutely.

AUDIENCE: And it sounds like there's no consensus of opinion. It's all a matter of judgement.

DR. KEVIN PROHASKA: It's judgement. You know, what's interesting -- and maybe I shouldn't say this in the public venue. But a concept or understanding of risk, and even privacy now, which is a big risk issue, evolves over time. And our understanding changes over time. And so right now I think the pendulum is swinging towards -- it's somewhere in the middle I would argue, but it's swinging towards respecting people's individual autonomy and allowing them to choose for themselves the type of research they want to be in. And that's fine, and I respect that. But we also have an

obligation to make certain they understand what they're going into. And you can go through the literature yourself and see that people sometimes don't understand what doctors are telling them. So it's important that we evaluate that (indiscernible).

MAN: So it sounds like if this question came to you, you'd set the bar pretty high.

DR. KEVIN PROHASKA: Not necessarily. It depends on -- I apologize, you know, for the vagueness of my response, because it really -- it's the context. You know, what are the alternative therapies that are available for cancer meds being sought after? If there are 20 other therapies and they all seem to be doing just fine, I wouldn't necessarily be in favor of exposing highly risky drugs to healthy people. But if it was a novel drug that was going to potentially have an incredible promise, then that might justify more risk. There's no one factor to consider; there are other factors to consider, too.

MAN: So one last question. Genetic toxicologists love case studies.

DR. KEVIN PROHASKA: Right.

MAN: Are you aware of a collection of case studies dealing with risky situations like this in Phase 1?

DR. KEVIN PROHASKA: I'm aware of them, but I've not --

MAN: Are they published?

DR. KEVIN PROHASKA: I'm sorry?

MAN: Are they published as case studies? I can imagine health [OVERLAPPING SPEAKERS] might have it. Completely different view of this than the FDA.

DR. KEVIN PROHASKA: Well, actually, I was looking at the report that was done -- Health Canada seem to have a very moderate approach to this. I think that they were advocating microdosing. What was the presentation that preceded me? There was a suggestion as to which regulatory body and what they allow. You know? And I was thinking, oh, that's sort of important. Certainly I would advocate for microdosing wherever possible. You want to expose people to the lowest dose possible and so forth.

MAN: My understanding is we don't like microdosing for (indiscernible).

WOMAN: It's not really commonly used.

MAN: Not commonly.

DR. KEVIN PROHASKA: Yeah. Well, fair enough. If there's a sound scientific reason for not doing it, then that would be appropriate not to do it. But where it can be done, it should be done.

DR. DAN LEVY: Hi, I'm Dan Levy from the FDA Center for Foods. So I have two questions. I'm going to ask them and then -- because I think they relate to one another. The first is many of our adulteration standards for food talk about significant and unreasonable risk of illness and injury. So I'm wondering, is there a relationship between the food adulteration standard and your standard for reasonable risk for healthy volunteers.

And the second question -- and this sort of gets to what Bob Heflich was talking about later, is do we need to develop information now when we're considering the policy and what you need to collect to make a decision knowing that the ultimate

decision is in the hands of informed consent by the patients. That is, does that mean that we need to look at this in a div way than we would for another kind of risk? Say for a prescription drugs where the decision is made by a learned intermediary?

DR. KEVIN PROHASKA: I'm going to tackle your first question first. But keep the thing because I want clarification on your second question.

The first one as far as the standards that are used within (indiscernible) standard, I've not ever been brought into any conversations within (indiscernible), but I would imagine that the context is quite different as far as food and what's available out in the market. And so the threshold that we might use for what's reasonable, likely to be different in that context I would assume. So as it is, whether you're talking about cancer treatments versus hair loss treatment, the threshold that we use in the drug environment varies depending on the indication.

And if you could please clarify the question number two?

DR. DAN LEVY: So we have to think

about risk as we develop the science behind whether we're going to do this or not. So does the fact that it's going to healthy volunteers who are going to have to give informed consent, does that change the kind of information we need to develop so that the healthy volunteer will be able to give an informed consent?

DR. KEVIN PROHASKA: Okay. Fair enough. Well, each division, if you will, there are certain standards of what's expected to be done before going into first in humans (indiscernible). And it may differ by indication and the acuity of the illness. Whether you're going into healthy subjects as a Phase 1 study or people with a condition Phase 1 study, there may be some differences as to what's required. I would probably turn to the Review Division and ask for their opinion on that. But at the end of the day, we want to have some basic understanding of the safety profile or at least some understanding of what sort of signals we might need to look for during the Phase 1 studies so that way we can adequately inform the subjects no matter what population it is, whether it's healthy people or

people with a condition. And as you go through Phase 2 and Phase 3 and so forth, you're learning more and more about the drug. And so the consent document is likely to change considerably.

In the Phase 1 environment, I would expect the informed consent document to probably have a lot of open-ended statements about not knowing what the risk profile looks like, but that we think it's this based on whatever. You know? Hopefully that answers your question, sir.

MAN: Is there any research to study how well the volunteers understand the information in the informed consent about (indiscernible)?

DR. KEVIN PROHASKA: Understanding. Whether or not they understand the consent document, I think that was the question. That's critically important, especially if there are some serious risks. And how well do I think that they understand it? I don't know. But if you look at the literature, frequently you find that people don't understand. And so I think if there are very serious risks, consideration might be given to testing their

understanding. And there's one common method for testing the understanding, is the test back. You present the list, perhaps, and you ask people to explain it to you. You know, what did you hear? Please tell me what sort of understanding of the risk. And if they can't explain it within a reasonable way, then maybe you haven't done a good job of explaining what the risks are, and you go back to scratch and explain again that these are the risks, and do you understand and so forth.

Does that help? Okay, good. There are other ways (indiscernible).

MAN: Do you think there's any difference in the way healthy subjects and patients decide to be in clinical trial? It seems like a healthy subject could decide, well, to avoid this risk completely, all I have to do is not be in the trial, whereas a patient might decide, well, I feel sort of obligated to be in the trial because I have this and maybe I could get (indiscernible) or maybe I could help more?

DR. KEVIN PROHASKA: Yeah, very good

question. Thank you very much. With people with the condition, a big part of the conversation is not just the risk profile of the drug, but also a discussion about the alternative available therapies. You need to make certain or hopefully make certain that they understand what else is out there, that there are other -- if there are other -- if there are not, then maybe they may not have other options. And again, you have to be careful that they understand the risks, the focus of the research, and so forth and so on.

All right. Glad there are questions.

WOMAN: I think we have to move on.

DR. KEVIN PROHASKA: Okay, fantastic. Thank you very much, everyone.

DR. TIMOTHY ROBISON: Our next speaker is Dr. Bob Dorsam. He is in the Office of Generic Drugs. He is the Pharm-tox division director. The title of his presentation is entitled Considerations for a Genotoxic API in Clinical Trials: Healthy Subjects or Patients?

DR. BOB DORSAM: Good morning and thank you very much to the organizers, Dr. Tim Robison, Dr.

Aisar Atrakchi, for organizing a session a very important topic. Also I'd like to start by thanking in advance our expert panel for providing their insight on what we have, some complex but very good questions.

I'm coming to you today as a member of the Pharm-tox discipline. I very much intend today to present a high-level view of what information from a genotoxic perspective we have available at certain points in clinical development. So in part I'll paint a picture of what information we have prior to Phase 1, because that's one of the focuses of our talk today, of our session. But then secondly, I'll also present a second frame, which is after that initial approval, there is subsequent applications that are also (indiscernible) to develop similar products, similar API. There may be more information available. And also we are interested in using that more information to the best of its value.

My intent is to present two frames, one at the Phase 1, and then secondly paint that portrait of when we're developing generic drugs, for example,

what information do we have. And then I'm simply going to pose questions that exist at each of those stages.

Okay, so starting at the high level. Clinical trials are integral to support the development of new drug applications, biologics, as well as ANDA submissions. ANDA for those of you who don't know, stands for Abbreviated New Drug Applications. That's a generic drug submission. All of those submission types have trials underneath them. And the safety of clinical trial subjects in all of those submissions is critical. It's a multidisciplinary issue.

Now, as pharm-tox, it is assessing the genotoxic risk of the active ingredient. And to support that assessment, we have both invitro and in vivo studies as is described in ICH guidance, ICH S2(R1). And we are assessing this to inform safety in clinical trials. And we have several things going for us, but we also have several -- in terms of standardized studies and information on how to interpret data. But there are also several areas that

warrant some further consideration. And that's one of our purposes for coming here today.

So clinical trial safety is a complex review issue. There are many different sorts of toxicities that we aim to minimize or mitigate the risk of. Today we're strictly focused on genetic toxicology. Now, as I said, for the protocols for many of the gene-tox studies are standardized. For example, according to OECD protocols. And we have guidance such as S2(R1) and N3(R2), which provide guidance on how we interpret some of these results as well as the timing of their submissions.

Okay, so the question that we're here today to question is how do we interpret some of these study results to translate it to safety for clinical trial subjects? And as you've heard before, one of the question is whether to involved healthy subjects in these trials where there are some results to suggest an Ames-positive API, active pharmaceutical ingredient. Should we involve healthy subjects, or rather are patients really the appropriate subjects?

Also, as you've heard, there are

several mitigation strategies in a trial that also help to ensure safety. And so we look forward to panel's input on how to appropriately mitigate some of the risk using those strategies.

So as I mentioned, ICH S2(R1) presents us with a gene-tox battery. And that will inform several mechanisms of gene-tox risk. And if an applicant chooses to take Option 1 in the guidance, they would provide a study which informs invitro mutagenicity or the Ames assay. They would also present in their submission invitro chromosomal damage information. There is also an option to provide mutagenicity in Option 2, study on mutagenicity, as well as an in vivo gene-tox assay.

So to put this a little bit more clearly, prior to the IND, we have this Option 1 and 2 from ICH S2(R1) that will inform trial subjects in clinical trials. And then as clinical trial development continues, and perhaps that's several years go by, by the time the new drug application is submitted, all of the information from the clinical trials would be submitted for assessment, whether this

product is actually safe and effective.

And during that long development, there may be follow-up assays and perhaps carcinogenicity studies if in fact they're needed for that (indiscernible).

So pharm-tox assessors are really relying on that gen-tox battery from S2(R1) prior to Phase 1 trials and (indiscernible). This is the primary focus of today's talk.

Any questions as you've heard remain about how individual results from these studies inform patient or healthy subjects?

So let's put ourselves for a moment in the place of a pharm-tox reviewer or a pharm-tox assessor, as we call them. At the IND stage, we have invitro mutagenicity information, we have invitro clastogenicity information. And if we can just imagine a hypothetical compound A, after reviewing the studies, we find that there is a positive signal for mutagenicity and perhaps there is a negative signal for clastogenicity.

So at that point a pharm-tox reviewer

is faced with a question. And the quandary that they're in has been from (indiscernible). But one of the questions that we have is if a drug is genotoxic, and in this case mutagenic, is there a follow-up study to assess potential risk that should be done prior to conducting studies in healthy subjects? So the appropriate follow-up is a question.

Secondly -- and this is a little bit aside the primary focus of the session today. But if we look at compound B, we have a compound that's negative for mutagenicity but positive for clastogenicity. Okay? So again, a little bit different from the primary focus. When accepting questions from various people in preparation for this session, one of the questions was for a case like this, certain drugs may be clastogenic but mutagenic. Should consideration be given to the mechanism of action of gene toxicity in designing studies with healthy subjects? So effectively does it matter if it's mutagenic or clastogenic? Should they be considered similarly? This is a lasting issue that came up in preparation. So we thought we would pose

that to the expert panel as well.

As Dr. Robison mentioned, there is considerations on trial design. So does it matter if a healthy volunteer is exposed up to a single dose or up to four doses of an active ingredient? And I'll speak in a moment about why four doses of an active ingredient is notable or why I picked four.

In most cases is continuous daily dosing acceptable, and if so, for how long? Or otherwise, is intermittent dosing preferable, and if so, how many doses would be acceptable in those cases?

So dosing in clinical trials can take many different forms. The does level, the frequency of dosing, and the dosing interval could all be adjusted for safety. And certainly those are considerations that we pose to the expert panel for consideration and providing our feedback.

I'm providing one example of a typical clinical trial. So in this case, it's called a single dose two-way crossover trial design. This is a case where some test product is looking to establish a bridge to a reference product. So to establish that

bridge, they're going to dose test article and the reference product in a patient or in a healthy volunteer and establish some similarity between pharmacokinetic characteristics. Okay?

The two-way aspect of it is if you have the test dose followed by washout and then reference -- so that would be A and then B, we would then later have B, then A. Again, comparing the pharmacokinetic (indiscernible) to establish similarity. So the question is, does it matter if a healthy volunteer is exposed to a single dose for up to four doses?

And as we've seen and heard, microdosing is of course another consideration. As we've heard, it's used relatively rarely. But altering the dose may be one option. In M3(R2) we see that a dose of 100 micrograms is reasonable, or up to five of these doses may be used at a microdose trial if that suits clinical development.

So that's at the IND stage where we have mutagenicity and clastogenicity. One of the things that I would like to show you today is that later in the lifecycle there are applications, namely

505(b)(2) and 505(j). 505(j)is a generic drug solution. And these applications often are looking to bridge back to the safety and efficacy of an original submission. Okay? So a 505(b)(2) may rely on some information that the applicant doesn't know and maybe something that was published, it may be a more abbreviated clinical development program, as a generic drug is aiming to establish prior equivalence. Okay? So they're not showing safety and efficacy; they're really showing prior equivalence to what we call the reference listed drug, or the innovator product. Okay? So therein a pharmacokinetic bridge is pivotal to their drug development program.

Now, the benefit that we have in these sorts of applications is that the genetic toxicology and carcinogenicity information are stated in the drugs labels that's been approved. So that's a bonus. However, we do need clinical trials in these application types to develop those drugs. So we have more information. There is a need to develop these drugs. Questions still remain about a way to resolve all of these studies for clinical trials supporting

these applications.

And so we too are faced on the generic side with the question of should we involve patients or healthy subjects in these trials and should we adopt certain risk mitigation strategies to protect their safety?

I say this because a large number of 505(b)(2) applications, as well as generic drug applications are submitted to CDER. Ninety percent of prescriptions are generic drugs. And we certainly want to ensure that the trials that are supporting them involve similar sorts of safety principles so that subjects in those trials are not put at greater risk than those of the original innovator application.

So I've told you (indiscernible) generic drugs. We do have more information. When generic drug has a clinical trial, okay, they need to demonstrate bioequivalence to the reference-listed drug. And that will involve either healthy subjects or patients. So input from today's session is very much important to me in this regard.

To demonstrate bioequivalence, they'll

dose a test article, a dose of reference product. There will be a trial under fasting conditions, a trial under fed conditions to ensure that there is similar bioequivalence across both conditions.

And a safety review is done to consider prior use of healthy subjects in the innovative program. So currently generics are using healthy volunteers when, for example, healthy volunteers have been used in the past.

Also in collaboration with our colleagues from the Office of New Drugs, Office of Generic Drugs folks are sometimes reaching out to ensure that healthy subjects (indiscernible) for this trial for generics.

But I do note that genetic toxicology information and carcinogenicity information have been reviewed, it's in the (indiscernible) label, and we're looking to use that information to its greatest value to inform this healthy or patients topic. And we certainly look to the expert panel for some assistance in how to weigh this information. And I say that because we surveyed FDA-approved drugs labels for APIs

that have positive results in gene-tox or carcinogenicity studies.

Within our team, we've used a tool called FDA Label. Now, FDA Label is developed by our colleagues in NCTR. FDA Label draws from the structured product label resource. And there are about 35,000 prescription drug labels in this archive. We merely did a keyword search for the non-clinical section of drug labels using the term positive. Because oftentimes when a new study is positive, that's the way it's going to be on the drug label. So we just search all of the available labels for the word positive in this one section of the label. And out of the 35,000 or so labels, you'll get about 3,200 results. But there are many duplicate applications and many duplicate drug labels. So if you remove all duplicates, you've still kind of got 250 non-duplicate APIs that have the word positive in the drug label. And we just took a subset of that. We took about 30 applications, 30 drug labels, and then just calculated what the results are.

My goal is not to show you the results

here, but merely to say when looking at those results, it does stand out that some APIs have a positive result in either neutral or in vivo assays. And there are APIs and antivirals, antihypertensives. There are some therapies for migraine, acid reflux, high cholesterol, arrythmia, or inflammation. There are positives from individual assays.

What we want to do is use this information to the best of its abilities so that trial subjects for these studies are put at no greater risk than in other trials. So we have more info.

And our question is, now what? So if we go back to that Compound A that is positive for mutagenicity, later on after the drug was approved, we find out that it's negative for carcinogenicity. So perhaps we can (indiscernible) perhaps maybe healthy volunteers may not be a problem. Perhaps the positive clastogen also shows to be positive in carcinogenicity. Okay? So either of these compounds are anonymized, but there are examples that fit into this sort of a case. So these could be said in, you know, real cases.

My question to the panel is should a weighted evidence approach be used to decide whether a compound should be tested in bioequivalence studies with healthy subjects. If yes, which test results should receive the greatest consideration in the weight of evidence assessment? Also, are there any other factors relating to genetic toxicology that should be considered when determining if a study should include healthy subjects in these bioequivalence studies?

So what I've done is gone over somewhat quickly some of the questions that the panel will be encountering later in the afternoon. My hope is that I've provided some look at the sort of data that was presented to our pharm-tox assessors at the Phase 1 stage. But also later on when trials are being conducted for 505(b)(2) as well as generics.

The questions that we pose are regarding dosing, and specifically how many doses of a positive drug can be safely administered to healthy subjects. Is it one, two, three, or four doses? Is continuous dosing acceptable? So for how long? Or

rather if the dosing is intermittent, how many doses would be acceptable? These are questions that are remaining for us.

Secondly, we've heard a question about follow-up assays. Are there appropriate follow-up studies that should be conducted prior to studies in healthy volunteers if an API is Ames-positive? We also pose a question regarding mechanism. Certain drugs may be clastogenic, but not mutagenic. Should consideration be given to the mechanism of actual genotoxicity and designating studies with healthy volunteers?

And then a question about weight of evidence. Should a weight of evidence approach be used to decide whether a compound should be tested in bioequivalent studies with healthy subjects? If yes, which studies should we give the greatest consideration to, and then are there any other factors that we should consider when doing this assessment?

So with that I'll just finish with a quick summary of saying clinical trial safety is of paramount importance and genotoxic risk is very much

important and underlying some of the safety that we wish to uphold for our trial subjects. We have different information prior to first in human studies. We have more information available at later stages when clinical trials are also conducted.

Our question, healthy subjects with patients is a key consideration for risk management for (indiscernible), biologics, as well as for the generic drug applications.

Not only healthy subjects or patients are a consideration, but also those trial design elements that might make a risk. And you can certainly appreciate the expert panel's insight on how to best translate available information and the appropriate safety recommendations for participants in these clinical trials.

With that I'll just briefly acknowledge my colleagues in the Office of Generic Drugs who assisted me in the formation of the slides, as well as the FDA Label exercise that we went through. My collaborators in the Office of New Drugs who were helpful in also creating the slides, as well as

formulating the questions that we are posing here today. And my colleagues at NCTR who developed a great tool to get information within FDA labels in a way that I think is pretty unique and pretty powerful. So with that, I'll say thank you. And happy to answer any questions.

DR. TIMOHY ROBISON: We have time for one quick question.

MAN: So in your slides, you mentioned about geno-tox, and you mentioned (indiscernible). But to my knowledge, none of the standard battery actually (indiscernible). So, I mean, are you recommending we recommend screening with (indiscernible)? And for drugs that we said no pharmacology on the genetic regulation, do we consider actually patients instead of healthy volunteers in Phase 1 study?

DR. BOB DORSAM: Thank you for a very good question. So I'm not proposing necessarily that there should be something added to the standard battery. I'll leave that to my colleagues who are on the ICH S2 to consider whether to expand the battery.

The question is good. Where there is a named pharmacologic effect towards (indiscernible), I think that that is an important consideration. That question will be addressed a little bit more so with some slides and further discussion this afternoon by the expert panel. So I think you raised a good question, and it's one that I think we're going to (indiscernible) a little bit later. Okay, thank you.

DR. TIMOTHY ROBISON: Mike, I was just asked to announce that people can please mute their phones. And when you're speaking into the microphone to speak -- to identify yourself and speak clearly.

Our next talk, Doctors Petibone and Shemansky, spent over a year investigating the literature in terms of exposure to a small number of doses of a genotoxic drug and the potential health risks. Dr. Petibone is here today to present the results of this literature review. Thank you.

DR. DAYTON PETIBONE: Thank you. Great, thanks. So yes, in order to prepare for this event, we have conducted a review of the literature to identify studies that could inform the risks

associated with administering one or a few doses of DNA-reactive drugs, particularly Ames-positive drugs, to help these subjects during Phase 1 clinical trials.

So here are presented some of the most informative findings from this ongoing effort. So the approach to this was fairly simple. We reviewed basic databases and search engines such as PubMed, Google Scholar, and the (indiscernible), as well as some other search engines and database to a lesser extent.

The search terms that were queried concerned the mutagenicity and related search terms to that and the Ames test, as well as exposures that were at milligram doses, which is the dose range in which APIs would be administered to healthy subjects during Phase 1 clinical trials.

We also looked for exposures that were done in animal model studies as well as reviewing the literature for any data that were present for exposures in human subjects with cancer endpoints.

We looked for data modeling of the threshold of toxological concern and the threshold of regulation for modeling of less than lifetime

exposures, and then we looked at a number of different dose levels.

So today we've summarized the findings from over a hundred relevant and supporting documents and subject areas such as data modeling and database analysis, animal studies with single, few, or short duration exposures to DNA-reactive endpoints that also had -- or DNA-reactive agents that also had tumor endpoints. And cancer epidemiology studies for environmental, occupational, and medical exposures to DNA-reactive agents.

In addition to that, we've compiled over 1,300 manuscripts that contain single exposures to test articles in animal models that have tumor endpoints.

So to ask the question, can we use the existing data to assess the risk of administering an Ames-positive drug to healthy subjects during Phase 1 clinical trials, I have chosen two subject areas to review. And that's the data modeling and database analysis of Ames-positive chemicals. And also the second section deals with cancer data from single,

few, or short duration exposures to Ames-positive chemicals in animal models and in humans.

So for Part 1, the first study, I've chosen to analyze the EPA GENE-TOX database to provide an assessment of the Ames assay's ability to predict carcinogenesis. The second study that we looked at reviewed the NTP Database for studies with short-term exposures as well as chronic lifetime exposures to see if the chronic exposures could predict the carcinogenic effects of the short-term exposures.

So because chemical-induced carcinogenesis can often involve both genotoxic and non-genotoxic events, it's difficult to predict how well a specific gene-tox assay would predict its carcinogenic potential. So a retrospective study was performed of the EPA GENE-TOX Database of over 3,500 chemicals with GENE-TOX data. Of those, over 1,600 had Ames-positive data, and 988 of those also had rodent cancer bioassay data.

So the investigators in this study, Matthews and colleagues, sorted the data according to their outcomes of the Ames assay, whether those were

positive or negative. And they then classified those events by their findings in the rodent cancer bioassay. So in this instance, there are 275 chemicals that were positive for both mutagenicity and for carcinogenicity, as well as 282 chemicals that were positive for carcinogenicity, but negative for the mutagenicity. And then for the compounds, they were negative for carcinogenicity. There were 85 that were positive for mutagenicity in the Ames assay as well as 346 that were negative for both carcinogenicity and mutagenicity.

So when they evaluated the Ames assay for its ability to predict carcinogenicity, they looked at the causative predictive value. And for the Ames assay there was a 76.4 percent prediction that -- predictability to identify the fraction of mutants which are carcinogens. And then for the specificity, there was a 80.3 percent ability to identify the fraction of non-carcinogens which are not mutagens. So they also looked at a correlation indicator which is an indicator of a positive finding of an aerobic cancer bioassay. And they found good agreement with

the Ames assay and the ability to predict carcinogenesis with a 78.3 percent return on that. So this indicates that the Ames test is a reliable indicator of a positive finding of the aerobic cancer bioassay.

Next we looked at a study that evaluated chronic lifetime exposures as well as stop exposures. Stop exposures refer to studies in rodents which are initiated and then terminated several months following exposure.

So cancer risk assessments assume that excess risk increase as a linear function of a cumulative carcinogen dose administered at a given rate, also known as Haber's Law. So for instance, Haber's Law would predict that an exposure for two years to a carcinogen would pose one-tenth of the risk of an exposure for 20 years to a carcinogen.

So to test this assumption, 11 carcinogens were identified in the NTP Database that had both combined lifetime and stop exposure data. The data were then modeled to determine the maximum likelihood that corresponded to a one percent increase

in cancer risk.

So it was identified that tumor incidence was significantly higher for six of 11 chemicals in this database, the 11 chemicals that are shown in the table to the left here. And one thing that most of them have in common are that five of the six are positive for findings in the Ames test. So ADBAQ and ortho nitro anisole were positive in two Ames assays. And then BBMP, 1,3-Butadiene, and coumarin were positive in at least one Ames assay. Methyl eugenol was the only carcinogen that produced a positive response that was negative for the Ames assay.

So what they found was that most of the carcinogens in the stop exposure studies had significantly higher -- a greater than twofold response in cancer potencies than the chronic lifetime exposures for at least one tumor site.

So as an example, in addition to having a twofold greater response, BBMP and 1,3-Butadiene and ortho nitro anisole were positive for increased tumors (indiscernible) only when the stop exposure data were

included.

The findings from the stop exposure modeling suggest that the short-term exposures could pose cancer risks not identified in a continuous exposure studies. An example of that is 1,3-butadiene exposures for 13, 26, 40, or 52 weeks produced a much higher tumor response in heart hemangiocarciomas as compared with the continuous lifetime exposures for the same dosing rate.

So to summarize the findings of Part 1, the GENE-TOX Database analysis suggests that the Ames test is a reliable indicator of positive finding in rodent carcinogenicity bioassays for a mutagenetic agent. The majority, five out of six chemicals in stop exposure studies gave a greater than twofold response in cancer potencies. Were also positive in at least one Ames mutagenicity assay. And also the findings from the stop exposure modeling suggests that short-term exposures could pose cancer risks not identified in the continuous exposure studies.

So in the second part we looked at exposures to a single, few, or short duration of

exposure to Ames-Positive chemicals. The data that I will go over today are the Single Exposure Carcinogen Database, which is an accumulation of animal studies that have a single exposure to a chemical. And then I will go over some examples of epidemiological data where subjects were either exposed occupationally or to a medical exposure for a short time. And a short duration in this context refers to a time less than or up to a year.

So the chemicals for the occupational exposures are beryllium, which are in a wide range of products. And the cohort are the beryllium production workers. We then looked at the aromatic amine benzamidine and the cohort of workers involved in the manufacture, use, and purification of those compounds.

For the medical exposures, we looked at phenacetin, which is an OTC analgesic. Its use was discontinued in the U.S., Canada, and the U.K. Chloral Hydrate, which is a prescription sleep aid, and then finally we looked at Thorotrast, which is a contrasting agent. It's a little different from the other exposures, because it is an alpha-emitting

particle. And it was used from the 1920s until it was discontinued in the 1950s.

So as it turns out, there are numerous studies that have data for a single exposure in animal models. And these have been compiled by Calabrese and Blain into a database of tumor incidence following a single exposure in order to estimate the less than lifetime exposures. This database contains over 5,500 studies for 800 chemicals from 2,000 articles that address the single exposure carcinogenesis.

So in order to be included in the database, the agent could be administered only once with no other treatments administered. And the tumors were examined as the endpoint. The database compiles a number of metrics such as; the number of citations each study has accumulated; chemical details such as the (indiscernible) number, synonyms, and chemical class; the study design, which includes the controls, treatment groups; and other principal aspects of those studies.

So as Tim mentioned earlier, there are 426 chemicals with doses that were administered as a

single dose. And those spread across 17 chemical classes, with many of those containing mutagenic compounds such as polyaromatic hydrocarbons, nitrosamines, hydrazine, and nitrosourea classes.

When we do a comparison of the studies that were positive for tumor induction as well as those that were negative for tumor induction, there were over 4,200 studies that were positive for tumor induction following the single exposure to a chemical, as well as nearly 1,300 studies that were negative for induction of tumors following exposure to the chemicals.

So both the studies that were positive and the studies that were negative were similar in several key aspects, some of their principal aspects. For instance, the studies that were positive and were negative, both used a similar percentage of both male and female sexes and incorporated both sexes into those studies. In addition, both positive and negative studies used histology as a endpoint.

They also were similar in their number of subjects per group in that a number of them used

ten or more subjects per treatment group, and a similar percentage of them also used greater than 50 subjects per treatment group. They also varied somewhat in some of the principal aspects of their study design, such as mammary tissues being looked at as an endpoint for tumors and respiratory tissues.

In addition, the studies that were positive used a higher percentage of rats as compared to the studies that were negative. And those studies that were positive particularly used the Sprague Dawley rat, which might explain some of the positive findings in the mammary tumors. And the studies that were negative used a larger percentage of mice.

So what the study found was that a single dose of many agents produced tumors in both males and females and in all age groups, whether it was fetal, neonate, or adult stages. And the findings were positive in numerous animal models. You can see the table on the left that there is a diverse set of species that were positive following a single exposure to a chemical.

The doses that resulted in tumors were

generally low proportion of the LD50, between 0.1 and up to the LD50 itself, and not acutely life-threatening. The tumorigenic responses observed for a single exposure to the DNA-reactive chemicals had wide structural diversity and were in all principal animal models and several other animal models, implies that humans are likely to exhibit qualitatively similar responses.

Next, they look at a single dose versus a fractionated dose. So the single dose was administered once and the fractionated dose was administered over the lifetime of the animal for an equal and cumulative dose.

So when they compared the single dose and the fractionated dose, they found that there were some chemicals where the single dose caused fewer tumors than the fractionated dose. That's shown on the left here.

There are also chemicals where a single dose caused more tumors than the fractionated dose for a given tumor site. And then there were chemicals where the single dose and the fractionated dose

produced similar results in tumor response.

Also, some of the chemicals produced a mixed result, such as DMBA, benzoate pyrene, 3-Hydroxy xanthine and procarbazine, where depending on the tumor sites, they were either fewer tumors, more tumors, or an equivalent number of tumors produced in both the single and the fractionated dose. So this suggests that there are chemical-specific carcinogenic responses or varied responses to a single versus fractionated dose and that the single dose can have carcinogenic effects that aren't always observed in lifetime exposures.

So because cancer epidemiology studies usually address the results of prolonged exposures, the short duration exposures or single exposures to a carcinogenic compound can sometimes be overlooked. And it is also difficult to associate an exposure event that might have happened 20 or more years ago with cancer. Therefore, there are limited data for DNA-reactive or mutagenic exposures in humans.

For the analysis of exposure in humans, we considered a short duration exposure to be that of

less than one year or exposures to one or a few doses. And human subjects exclude those that have cancer or terminally ill patients. And the treatment includes drugs or chemicals with positive mutagenicity data and exclude antineoplastic drugs.

So for short duration occupational exposures, we looked at beryllium and the aromatic amine benzidine. Beryllium was negative in the Ames assay, but it was positive for HPRT mutations in CHO cells and in Chinese Hamster B79 cells. The cohorts that were investigated were white males that entered into the beryllium case registry and that were involved in the manufacturing of beryllium.

So what the investigators of these studies found is that employment of a year or less to beryllium resulted in significant increases in the incidence of lung cancer.

Then for the aromatic amines benzidine, the subjects that were involved in this study were involved in manufactured use and purification of the AABs. What this study found is that the overall risk from dying from a bladder tumor is approximately 30

times greater than that of the general population if exposed for at least six months and exposed up to one year.

The table shows the findings of the short duration occupational exposure of beryllium in humans. So there are seven cases of lung cancer that were reported. All reported acute chemical bronchitis upon entry into the beryllium case registry. They were primarily involved in the extraction and smelting of beryllium. And of the seven cases, five of those had exposures that were for less than one year. Three workers had exposures for one month, one worker had an exposure for two months. And then there was another worker with an exposure for six months.

In a similar study, there were two lung cancer deaths reported 20 years after their last exposure. One was employed for six months and the other was employed for 21 months, which is longer than a year. So the data on smoking history was not collected as part of either one of these studies.

So then we looked at short duration exposures to the aromatic amines benzidine. This

table shows the different chemical classes that were investigated. Benzidine, alpha-Naphthylamine, beta-naphthylamine, and then mixed exposures.

What the table shows is the percentage of the observed number of bladder tumors as compared to the expected number of bladder tumors. And then measured the effect of time and the incidence of cancer risk.

So we can see even with one year for benzidine, beta-naphthylamine, and for mixed exposures, there are increased risk for development of bladder cancer. And you can see that that increases for those same chemical classes up to one year and that with increasing time there is an increasing risk for developing bladder cancer before that risk drops off at 20 years.

But all of the subjects that went into these data developed tumors. So after developing tumors, they were removed from the study, which explains the shape of these data in the graph.

So what they found was that even with less than a year exposure, that there were increased

risk for developing bladder cancer. And also, again, as I note, the data for smoking history was not collected as part of this study.

So we then went to look at short duration medical exposures. Phenacetin, which was an OTC analgesic, had mixed results in the Ames test and was positive only in the presence of S9. It also was positive for (indiscernible) mutations in the kidneys of transgenic rodents. The dosage of phenacetin is generally 300 milligrams four to six times a day and not to exceed two grams.

One study by Ross and colleagues looked at the consecutive or continuous use of phenacetin and compared that to less frequent use of -- less than 30 days a year -- or greater than 30 days a year, less than 30 days a year, or no use. Chloralhydrate is a sleep aid and it has mixed results in the Ames assay. It's generally prescribed as a 500 milligram dose. And Haselkorn and colleagues looked at the effect of zero, one, two, three, of four doses of chloralhydrate and the incidence of cancer over a four-year period.

And then finally we looked at

thorotrast, which is positive for T-cell receptor mutations. It's negative for GPA mutations and had mixed results in the p53 and KRAS gene mutations assays. There were no data for the Ames assay for thorotrast.

So there have been over 9,000 people injected with thorotrast between 1929 and 1956. It's generally administered acutely at a rate of 500 grams per liter and volumes that can range from one mL all the way up to 100 mLs. The study we looked at today observed that there were dose-dependent increases in the time to tumor formation with increasing volume of the thorotrast that was injected.

So phenacetin is classified as a Group 1 carcinogen, carcinogenic to humans. And it was withdrawn from the U.S. market in 1983. The long-term use of phenacetin has been shown to cause renal and ureter tumors in humans. And in a study by Ross and colleagues, they looked at men and women from the Los Angeles Case Registry and with the matching controls for those cases. The doses of phenacetin that were used were continuous exposure for more than 30

consecutive days in a year or use of phenacetin for more than 30 days per year. And then those were compared to use of phenacetin for less than 30 days per year or no use. They calculated the (indiscernible) of phenacetin use and then adjusted that to the controls that were either no use or fewer than 30 times per year.

And what they found was that there was a slight yet nonsignificant increase in the risk for renal, pelvis, or ureter tumors in those that had 30 days of consecutive use as compared to those that were either greater than 30 days per year or less than 30 days per year or that had no use of phenacetin. So this shows that some risk was involved with exposures to a carcinogen with continuous use.

Next we looked at short duration chloralhydrate administration. So chloralhydrate is a mutagen in salmonella that was positive in four out of six assays using TA100 and was positive for two assays using TA104 strains. It was also carcinogenetic in animal studies. And chloralhydrate is a major metabolite of trichloroethylene, a general anesthetic

that was banned in the U.S. in 1987 due to its ability to induce tumors in rodents.

So Chloralhydrate was used in a 500 milligram form, or dispensing as it's referred to it in the paper. Based on the study, most patients received a few doses of 500 milligrams for short-time use.

When they analyzed the data -- so the table on the left shows the number of cases that developed a cancer versus the other chloralhydrate users that did not develop cancers that were used to calculate the dose response. And what they found is that for all cancers overall, there was no significant increase and no dose response. However, when they looked at prostate cancer, they found that there was a dose response for induction of prostate cancer with increasing numbers of chloralhydrate doses, from three cases for one dose, five cases for two to three doses, and six cases for four or more doses. So this suggests an increased risk of prostate cancer with the increasing number of doses that were administered.

So finally, the last chemical we looked

at for medical exposure in humans was that of thorotrast. And as I mentioned previously, it is a bit different because it an alpha-emitting agent. thorotrast contains thorium, which has a half-life of ten to the tenth years, and it has a biological half-life of 20 years. So once exposed, the subject is internally exposed to the thorium for the remainder of their life. It's typically used for cerebral angiographies to identify arterial venous malformations or to evaluate head injuries.

So the graph on the left shows the cumulative frequencies of liver tumors with time after angiography in relation to the volume of thorotrast that was injected. And the graph on the right shows the cumulative frequency of hematopoietic malignancies amongst subjects injected with thorotrast. And in most of these, you can see that there is a dose-dependent response in the formation of liver tumors that correspond to the dose level that was injected. So the subjects that were injected with 20 mLs or more developed tumors more rapidly than subjects that were injected with 11 to 20 mLs or those that were injected

with one to ten mLs.

A similar pattern is seen for the development of hematopoietic malignancies. And the subjects that were injected with greater than 19.7 mLs developed the malignancies sooner than subjects that were injected with less than 17 mls as compared to those of the control. So there is an increasing risk -- there was an increasing risk for cancer based on the level of exposure to the mutagenic thorotrast injection.

So in summary for Part 2 of the talk, the tumorigenic responses observed for a single exposure to carcinogens with wide structural diversity in all principal animal models imply that humans are also likely to exhibit quantitatively similar results. The cancer epidemiology studies provide suggestive but not conclusive evidence of a causal relationship between short duration exposures to mutagenic compounds and cancer. And of course the examples that I showed you based on the limited data available obviously have their shortcomings and limitations, but they do provide some suggestive data.

And finally, as a (indiscernible) that animal experiments have limited exposures other than that of the doses that they are administered, whereas humans are exposed to additional and environmental exposures.

So this is an ongoing effort and we are continuing to search the literature to find relevant information as well as we are attempting to reconstruct the Single Exposure Carcinogen Database, exposures that were done in animal models. So I'll take any questions that you have now.

MAN: Do we have time for a few questions?

DR. ERROL ZEIGER: Errol Zeiger, a member of the panel. You showed that compilation of Calabrese and Blain, which is very interesting. But the problem is they failed to stratify by exposure route. I know back in the old days, in the 1960s and 50s, there were a lot of cancer experiments done with single subcutaneous injections or intratracheal injections, which are clearly not relevant to anything that we're talking about now. And without removing

these, you'd get a very biased view of it.

And I found that the study with (indiscernible) is very interesting. Beta-naphthylamine seems to be -- alpha-naphthylamine seems to be the most potent carcinogen. And it's beta-naphthylamine that's classified as a carcinogen. Alpha is really -- it's not mutagenic, pure alpha. And it's considered to be non-carcinogenic. But those results show just the opposite.

DR. DAYTON PETIBONE: I'm looking at the --

DR. ERROL ZEIGER: No, I'm sorry. No, the alpha showed the higher response, but the beta is considered to be the carcinogen.

DR. DAYTON PETIBONE: Early on, the beta-naphthylamine showed a larger response, it's the open column here, with increasing time. But as these subjects developed cancer, bladder tumors, they are removed from the study so that the remaining studies -- the remaining tumors that were developed in the time after five years or ten years exposure, it does appear that the alpha-naphthylamine did result in an increase

in (indiscernible). But I'm not sure the explanation for that.

DR. CHARLES THOMPSON: Charles Thompson, CDER. Excuse me if you've touched on this, I missed it. But I'm curious, what's your single animal exposure database that you looked at? Did any of that involve initiation-type, promotion-type modeling?

DR. DAYTON PETIBONE: I would note the only data that we were able to look at was a high-level overview of that database. We were not able to access that database. We are in the process of trying to reconstruct it. That's the 1,300 papers that we have with the single exposure. So we have not been able to do any detailed research into that other than we have gone in and randomly spot-checked some of the studies to see that -- to verify that the findings do match up with those that were found or reported in the Single Exposure Carcinogen Database. But hopefully reconstructing the Single Exposure Carcinogen Database is something that people will think is worthwhile. As of 1999, there were over 2,000 studies that had single

exposures in animals in the past 20 years that could have increased quite significantly. And we'd like to compile those data for analysis.

DR. PETER CULLINS: (indiscernible) Peter Cullins, London. I think it's really interesting to reconstruct that database. But in doing that, I think we would need to look carefully at at least two aspects. One is the age of administration. Because all of those studies were done by (indiscernible), which I think adds an additional biological component, which is the safe rate of cell replication, which would be different from an adult exposure.

And the second is -- and you touched on the thorotrast -- is the difference between a bioaccumulate compound, which although it's a single (indiscernible), it's continuous internal exposure and something which is short-lived but there is a toxicodynamic component. And certainly for the inorganics there will be a significant number of them where bio persistence is a factor.

MAN: (indiscernible), CDER. So

subjects who are taking from this to be (indiscernible), is there any follow-up?

DR. DAYTON PETIBONE: For the short duration exposure to aromatic amines benzidine? I am not sure if there was any follow-up that was conducted with those subjects. It wasn't reported in that study.

DR. ROBERT HEFLICH: Bob Heflich from NCTR. (indiscernible) of business that he analyzed. I did happen to read the Calabrese paper yesterday. I looked at it. I was surprised at the few studies that (indiscernible) on the bioassay studies. It was less than 20 percent I believe. And if you look at the (indiscernible), obviously there's a lot of Sprague Dawley model treatment that produced mammary tumor results. But it's not exclusive. I mean, there's a lot more going on there. And I think one of the criteria for inclusion in that database that there were no other treatments other than the single dose.

DR. DAYTON PETIBONE: Correct, yes.

DR. ROBERT HEFLICH: So there was no promotion (indiscernible) initiation kind of study.

AUDIENCE: Just a comment on the beryllium workers. There is also a type of an immune component to beryllium disease. It's almost like a type of allergy that develops. And that also continues to (indiscernible). So it's not just a simple straightforward story and it was (indiscernible), but it's something that (indiscernible).

DR. TIMOTHY ROBISON: We scheduled a 15-minute break. I think the speakers will be around if you have questions during the break.

(Break)

DR. DOUGLAS BRASH: Okay. So, first question is, can people in the back row can hear me? And Dan, hopefully, people out in cyberspace can hear you.

So, my name is Doug Brash. I'm a molecular biologist who hasn't thought about genetic toxicology since I probably was a graduate student. I think my role in here is to take what we know about biology and see how much of it applies, and what can we expect to see with regard to those four treatment

duration thresholds.

But there'll be a number of things I'm going to say that are going to be obvious to some of you. So, different things will be obvious to different groups of you. But also, I think that probably none of it's obvious to graduate students, post-docs and assistant professors these day, who I think largely don't think about these things.

And a big part of the reason for that is that for the last 40 years there's been, in the cancer world, almost an obsession with genes. And I want to put that in perspective. So, let's see if I can do this here.

So, the outline, I'm going to start with why cancer is not just mutations, and then go on to (indiscernible) affect those thresholds in the literature. I'm not going to talk about tumors because that's a (indiscernible). But for stress signaling survival mutation, I'll show you kind of a zoo of data and tell you what I think it's telling us.

And in my own world, I focus on skin cancer, so I'll tell you a few things on melanoma, if

you don't know, I think that are relevant here. And then in the course of reviewing all of this, I have some opinions on things like linear agent and dose responses and thresholds, and I'll tell you what, as a biologist, the situation looks like to me.

So, ever since I was a grad student or a post-doc, this is the way the cancer world has been looking at cancer, near as I can see. And that's not the whole story.

So, one way to think about it is, if this were the whole story and what a carcinogen did was solely to mutate genes, and you have 10 genes, like five or six, that you had to mutate, well then, the cancer incidence is going to be proportionally (indiscernible) to the end. But that's not actually what happens. It's a little more like it goes to second or first power, and duration of exposure to the fifth or sixth. So, there's some biology going on here. And so, there are other things we'd have to think about.

So, I think of this as the cancer cell loop. And the genes are in the middle, but there's

things that go on before and things that go on after. And people always at least think about the first few steps. But I also want to talk about the last few.

So, we're going to start out with the carcinogen and it's going to make a DNA lesion, and it's going to make a mutation. That's going to happen in some gene. That gene will have a phenotype of this cell. And one cell never killed anybody, so you've got to clonally expand that cell, and now eventually you get to a precancer or a carcinoma.

But particularly for ultraviolet light that I work on, in general, we know it's one of these numbers. And as you (indiscernible) tendonitis through her gene is cell generation. So, if you want to hit five or six genes, or two of them, you also find that six genes is even worse, this is not going to happen in one cell. So, this end step here about the clonal expansion, I think is the ballgame to getting cancer to work at all.

So, yes, you would have to have a mutation, but you also have to think about this clonal expansion step. And in normal skin, we have clones --

I've got a picture of one -- that have one (indiscernible). This happens to be p53, but not the other ones. They're not even just sitting there as single cells. They're growing as clones already, but there's no obvious defect in the skin. I'll talk about this more in a little bit.

And so, we go around and around this cycle. And so you clonally expand. Now you've got a bigger target, and now the carcinogen can (indiscernible) or another carcinogen, you get the second mutation and you keep going around. But now you've even got the multiple (indiscernible) you need to actually have a tumor. And people with sequencing have now looked in normal skin at (indiscernible) genes, and at the bottom is a cartoon of all the (indiscernible) of the genes they did. And you see there's even clones inside of clones. And this is just 74 genes that they looked at.

So, by the time you get to be 60 or 70, your skin is a saran wrap full of mutant clones just waiting for something to go wrong. But it still pretty much works as a skin anyway. So, that's what I

call the cancer cell loop. So, it's not just genes.

But on top of that, it's sitting in the middle of a bunch of modulators. So, on the one hand, you have metabolism, which could either get rid of your carcinogen or activate it and make it worse. Or you have repair, which is going to remove some of the (indiscernible), maybe in time, before DNA replication, maybe not.

Then there's this whole issue of (indiscernible) transregional synthesis. I guess we're (indiscernible). Anyway, so the point where this comes along and it has a decision. Can it bypass this legion? Is it going to make a mistake if it does that? Is it going to block (indiscernible) the cell dies? So, a few things can happen.

With regard to cell phenotypes, one of them is apoptosis, so cells that are abnormal tend to kill themselves. So, in a way, that's good, so that they're not going to become cancer cells. But we'll revisit that.

But (indiscernible) lesions can also induce a re-differentiation of cells. People don't

tend to think about that prospect. I'll say something in a minute. Just doing the (indiscernible) can have a lot of gloss, so you're losing all (indiscernible) for large regions of chromosomes. And that also can be important, not only as a mutation mechanism, but as already mentioned to initiation of promotion earlier.

And so, promotion, as most of you probably know, would have an initiating event which is probably mutagent. Something later could happen, but it doesn't matter unless you already have the initiating event. And one of the things (indiscernible) can be to the (indiscernible) is the tumor promotor would be (indiscernible) if there wasn't something wrong with the other (indiscernible) it might be okay. So, there are multiple reasons why it's important.

Now, these (indiscernible) so, now this is all physiology that's superimposed by your genetics. And many of these things are inducible. So, you've got dose responses for inducing these responses. And so, this is all pretty much homeostasis at work. And it's largely helpful.

I mentioned differentiation. This is a paper -- I thought it was really interesting -- from a long time ago. I couldn't get anybody interested in it, including Leo Sachs, who did it. But he was inducing a differentiation with different chemicals.

And so, here is the list in his nature paper, and what I really like is he even has the carcinogenic and non-carcinogenic analogs in polycyclic aromatic hydrocarbons, and the ones inducing differentiation and the other ones don't. I thought it was a great experiments, like he knew what he was doing. But that's not what he was trying to do. But anyway, it was a differentiation effect.

Then there's selection pressures. So, you can have mutation (indiscernible), just like in evolution, species evolution. We have mutations and we have selection pressures. So in the case of UV and p-53 in skin, what happens is the (indiscernible) of apoptosis. It's great on the first trip to the beach. You kill off the damaged cells before they can go on to make a mutation. What happens on your second trip to the beach?

Well, you've got (indiscernible) mutations last time. You're going to kill off the normal guys. The guy who's going to take their place in the clonase band is the mutant, who is no longer UV-sensitive. So, sunlight becomes a selection pressure favoring the outgrowth of the mutants. And in fact -- well, I'll show you how that plays out in a minute.

Another way that this happens is the UV in the presence of p53 changes the way cells behave as stem cells or not. I won't go through it, but you know, a stem cell can be two stem cells, or a stem cell differentiated with two differentiated. You tip that balance a little bit, and now you're expanding exponentially if you make more of the stem (indiscernible) divisions. And so that also is going to (indiscernible).

Here is an experiment we did. I won't tell you the (indiscernible) because it takes too long. But in scan cancer, there's some stages that depend on more mutations, and others that depend on clonal expansion. So, if we found (indiscernible) to

bias the apoptosis rate, it affects the -- there are too many double negatives, so I'll just tell you without taking you through it.

If you have more apoptosis, that's great we get rid of -- we have less of the mutant requirement steps. But you get bigger clones during the clonal expansion steps, which is just what we would have predicted. But everybody always talks about apoptosis as if it's this great anti-cancer thing. Well, maybe. It depends on when. So, it's not all that simple.

Now, the clonal expansion (indiscernible) is the exponential growth is important, because if you think about it, if you're making mutations, each time you make mutations you make some more, and the number of mutants is going up literally. But if you're doing this clonal expansion thing, you go out and mutant daughters (indiscernible) exponential (indiscernible) really important.

Now, this clonal expansion may not be so important for the present question of what happens if you only get so many (indiscernible) wants. But

these other things like tilting differentiation phase well may matter, if you can do that in one exposure, for example.

Now, everything I showed you is still sitting on the side of other stuff, more physiology. So tumor promotors, or the self-proliferation state. You know, if you've got a virus in your liver, it matters whether you get a carcinogen superimposed on that. And then there are microenvironment issues. Inflammation is another one. So, it matters whether your carcinogen is coming in through an inflammatory environment. (indiscernible) down.

Then at the end of all this, you have, hopefully, some immune surveillance that's trying to get rid of these (indiscernible). And at the very beginning, it's coming around to -- I think (indiscernible) is ahead of everybody. There's some messages the world does not want to hear. And I think this is one of them, that cancers are polyclonal, and there's not simply a single cell that's putting out. There's more than just the one cell that matter. And so, all these things factor in.

And so then we step back as a biologist and say, do I even expect any kind of linear dose response out of this? You would think that the actual dose response has to look like this and it's going to different from one person to another. And (indiscernible) affect all this (indiscernible) stuff.

So, sorry I had to simplify things. So, then, the lessons, I would say, for this part of the talk, are the biology (indiscernible) no real reason to expect a simple dose response is linear or even monotonic. And then, if you think about why this is, it goes back to (indiscernible) homeostasis (indiscernible).

So, the cell is in many, many ways trying to maintain a particular state. And on low doses, the cell is doing that. And then at some point, you're out of its operating range, and now what you're doing, you're breaking the cell. And so, whatever goes on when you're breaking cells is different from whatever is going on when you're doing homeostasis.

So, it seems to be (indiscernible).

There is no reason to expect you're going to be able to take data from high doses where you're breaking cells and extrapolate down what's going on in homeostasis. There's just too many (indiscernible).

And then, how can homeostasis play out? Well, it'd be partial, in which case you have not quite as much effect of the carcinogen (indiscernible) levels. Or it could be complete, and that could look like a threshold. Or it could even overshoot and look beneficial. And (indiscernible) I know in a couple papers, that once you induce things (indiscernible), you clear out other damage that's been accumulating for other reasons just over the past 10 years or six months in an experiment. And so that's really great.

And then what exactly happens, you don't know in your case with your carcinogen and your model, and probably your human volunteer, which is probably more (indiscernible) until you do the experiment. So, then you have this policy issue of, well, if you don't know, what do you decide? And (indiscernible) people (indiscernible).

Oh, and homeostasis depends on age.

So, the number one characteristic of aging is not that your basal (indiscernible) go down a lot. It's just that you can't depart very much without dying. So, this is just one example of how much blood loss does it take to kill a rat? Well, it decreases with age, because they can't do homeostasis. But there are similar experiments with (indiscernible) and so forth. So, that also is going to factor into the (indiscernible).

Now, so then the question is, well, if I don't think should be a threshold, are there (indiscernible) thresholds? Well, so let's go through the data. And I'm not going to talk about cancer. I'm just going to show you some data for signaling survival in mutations.

So, on the left is an apoptosis. This (indiscernible) experiment we did in apoptosis. But I just want to point out (indiscernible). On the far left one -- so, there's a (indiscernible) which is a reparative (indiscernible) knockout mouse. Increasing UV doses, how much apoptosis do I get? Well, so there is a (indiscernible) increase. Looks like for the

wild type, there's a little curve at the bottom.

So, there is a little curve down here. So, you could say, okay, great, there might be a threshold. And then if you look here, there is not (indiscernible). Here's p53 induction. Now you've got different (indiscernible). MDM2 induction is really interesting. You see it goes up and comes down. Over here we have (indiscernible) this is a repaired (indiscernible). And so it goes up and comes down; so not linear.

And the (indiscernible) biology we think about these things is we're not looking at how things change from the y-axis. You need to look at how things have changed on the x-axis, (indiscernible) modification factor. What repair has essentially done is scrunch everything to the left and it thinks it's at a higher dose than it is, because it wasn't comparing anything.

Now, why should it be that complicated? Well, here's the biological circuit, so you (indiscernible) it's supposed to be in charge of apoptosis in this case. This is regulated by

(indiscernible) two, which is regulated by some other guys, who form a loop with p53, and the notion is that this all keeps p53 from turning on too much.

For UV, actually, the apoptosis done by E2F1, and this is the modulator up here. And then (indiscernible) loops here you've dose responses and we don't know what they are until we actually do the experiment. Again, that's, I think, a general problem with what we mean by causality in biology. What do we want to know here? You know, who (indiscernible). So, that's why (indiscernible).

Survival dosage process. See, it's the same sort of theme. This is the classic. So, if the repair defect of this -- repair defect goes in a straight line, increasing those, goes straight down (indiscernible) kill it. If you can do repair, there's (indiscernible) here which kind of looks like a threshold, and now eventually you get to something like when we do loglinear (indiscernible).

Loglinear, what that means is that each (indiscernible) increase kills the same percent it killed in the (indiscernible). So, you kill a certain

percentage in the first (indiscernible) dose. Whatever is left, you kill the same percentage (indiscernible) that's going to get a straight line no matter what occurs. And it's almost like the (indiscernible) hypothesis (indiscernible) kill something (indiscernible) there.

And these other guys would just show you that, well, this is the (indiscernible) of the thing, and it doesn't really matter. You see it with other kinds of agents and other kinds of (indiscernible). So, over here is your (indiscernible). The shoulder is your homeostasis part, and here is (indiscernible) cell part.

Now, mutations. So, this is HPRP for alpha rays which are considered non-repairable (indiscernible) on a straight line. You see the other guys are mostly kind of curvy and (indiscernible) different (indiscernible), while being resistance is a similar sort of thing.

It doesn't depend on drug selections, so here is something (indiscernible) whether you're just (indiscernible). You can see these guys are --

the ones at the bottom are wild type and these are (indiscernible) where it goes. So, great.

(indiscernible) graph (indiscernible) are almost always not (indiscernible) on (indiscernible).

MAN: We can't hear you over here.

DR. DOUGLAS BRASH: Oh, okay. So, the mutation plots are not usually plotted to the model. Oh, yeah, before I go there, I want to show you one other cell. Here's an example where you see -- so this is a mutation (indiscernible) still plotted in the ordinary way.

You see this increase here has a little bit of a curve down here. What they did in this experiment (indiscernible) they (indiscernible) cells and let them get different lengths of time before DNA replication. How close can you get to DNA replication? And if you get really close in DNA replication, you start seeing this little curve. These guys are linear, but that just means you had enough time to do repair.

So, this little curve down here, you

notice it's sloped; it's the same as this other one.

MAN: (indiscernible) closer to this microphone.

DR. DOUGLAS BRASH: So, it's not really so much that there was a threshold dose. It's just that you didn't have this catastrophic effect of UV radiating the cell right next to DNA replication.

Now, the other thing I want to talk about. So, (indiscernible) plot mutations on a log scale, right? So, there are some papers that do that. And now, what does it look like now? Well, you get -- you're not really seeing anything that looks like a threshold. In fact, it's quite steep near zero. So, why was this (indiscernible) highest (indiscernible). It's in the first finger of the (indiscernible). Oh, well, okay. (indiscernible) more survivors. But this is mutations per survivor. So, that's not it. So, what's going on here?

Here's a paper from (indiscernible) where they actually brought in the exact same data, but (indiscernible) if you look at it on a non-log plot, you'd say, oh great, a threshold (indiscernible)

down (indiscernible) it goes. And if you plot it on a log plot, you'd say, oh wow, most of the action is down here at (indiscernible). So, what's going on? Why is this?

And it dawned on me as I was thinking about this that, oh, this is actually just algebra, although it's going to be misleading algebra. What happens is that mutation frequency, mutations per survivor, so that's mutations per initial cell divided by surviving cells per initial cell.

Surviving cells per initial cell is not just survival. Mutant cells per initial cell is something nobody ever talks about. You know, it's not a thing. But if you do that, you see what you're going to have is one line is an exponential divided by an exponential, and no wonder you get these funny curves. And so, if you plot that on the log, you get the shape that I was telling you about. But if you plot this same thing (indiscernible) ordinary way, non-log, well, it looks like we have a threshold.

So, I'm now really suspicious of thresholds, and I think it'd be important to be really

clear on what our definition is on that threshold. And I'll come back to that briefly again.

So, conclusions, two. Survival seems to show a real threshold, as reflected in homeostasis, unless the cells are repaired efficient, which is going to matter when you're looking for volunteers (indiscernible) homeostasis (indiscernible). Mutations can show a pseudo-threshold, if you're not plotting it as a log, in one of these two ways. That absence of the catastrophe curve or this (indiscernible) consequence.

So, lessons from (indiscernible) about thresholds and single exposures. These are more or less just (indiscernible), but they're indicative of things that we saw a lot of in the last talk. So, there's evidence of melanoma cells, or cells derived in tissue culture that came from a melanoma tumor are in fact deficient in post-replication repair.

Then there is something that -- we've got a paper coming out on in a few weeks that I call attention to this -- but there are recurrent (indiscernible) mutations in (indiscernible). The

mutation (indiscernible) are 100 percent (indiscernible), which is the UV signature. And it shouldn't be 100 percent. It should be 60, 70, 75 percent, maybe, and a repair effect of like (indiscernible) pigmentosum patient, this may be 90 percent. So why are they 100 percent? So does that also tell us that the patient or the cell became a melanoma was (indiscernible).

So, Dr. (indiscernible) your volunteers have a (indiscernible) dose. And so that would be just for survival.

Then there's single UV exposure story in melanoma. There's a couple of them. So, the famous one, (indiscernible), is that epidemiologically, there's a correlation predicting risk. Sunburns in childhood are a strong risk factor. And so the notion is that after you get a strong sunburn in childhood, you did something and that predisposes you to a melanoma (indiscernible) years later.

There are some caveats. Sunburns in middle age could also increase your risk. And sunburn

could just be a (indiscernible) of skin type (indiscernible) like fair skins. Oh, I should say that you may or may not know that product sunlight exposure, which is to say farmers and fishermen get basal squamous carcinoma through (indiscernible) cancer, and that seems to be a little bit protective with melanoma.

And then there is this whole other story about melanoma being correlated with childhood sunburns. So there is this notion in the melanoma field of acute exposures. How solid or flimsy that is, I don't know.

But motivating that, people looked in mice and they gave mice a (indiscernible) on UV exposure, then you can get melanomas. And those melanomas have UV signature mutations. There's a caveat. These are transgenic mice. First of all, mice don't have melanocytes in the epidermis, so you break from (indiscernible). And then there are other genes to knock out. And then the notion is that, well, okay, (indiscernible) some of the steps, but we can at least do an experiment in a reasonable time.

You could ask whether, okay, waiting for those other steps is what took a long time and why this is not working (indiscernible).

Then there is a paper that just came out from the (indiscernible) lab. They did four UPV plus UVA exposures and they got melanomas sooner than when they did clonic exposure. Which, again, reiterated some of what you heard in the last talk.

So, what are the mechanisms for the single exposure effect? So short answer is we don't know, but there are a few things proposed. The classic one, and the quite reasonable one, is that single UV exposure does make melanocyte proliferate. So the notion would be, okay, now you're stimulating the cells from (indiscernible). And then that's now written into your genome.

There's a more central story, which if anybody gets interested, I wrote a "News and Views" on it, so you can find it on (indiscernible) on somebody else's experiments. But there's a feedback (indiscernible). One is you can induce melanomas in tissue cultures with just growth factors and no

mutagens at all. And you get the melanoma anyway. It's reversible.

Then what the Walker lab did, (indiscernible) is they used a (indiscernible) cross to ask, okay, if we make all these mice with different genotypes, can we find polymorphisms that will accelerate the neonatal UV phenotype, neonatal (indiscernible) phenotype.

So, they went to all this work, and what they found was that there were just a couple of genes that do this. And what they do is there are (indiscernible) related genes that are UV inducible. So it looks again like they're doing something with physiology. UV is changing the physiology of a cell and that somehow accelerated (indiscernible).

And in this paper, we've shown that your gene (indiscernible) single basis for UV (indiscernible) which we cite (indiscernible) that are a hundredfold more likely to get a (indiscernible) from elsewhere. These are sitting in regular (indiscernible) genes. They're sitting in some of these recurrent mutation sites. And they're so

frequent, the mutation frequency is now on the order of one percent. And so, every -- oh, and (indiscernible) certain metabolic pathway.

So, if you go to the beach, every one of your cells is going to get hit in every one of these pathways at least once, given (indiscernible). So, it's almost like an epigenome mark now. And whether this is required for cancer to be able to (indiscernible).

Another (indiscernible) from a paper from Jimmy Cleaver, which I think got kind of buried in the (indiscernible), but I think it's really important. What they have, they were trying to do deep sequencing of UV-induced mutations. And they started out with (indiscernible) cells, but you don't want to have to keep using different cells strains. Well, that turned out to be a bad idea because (indiscernible) cells have 10 to hundredfold higher background mutations (indiscernible) it's just a spontaneous mutation.

Then they went and (indiscernible) radiated them and the mutation frequency went down,

not up. So, that's just kind of (indiscernible). So what is happening here? And so the bottom panel (indiscernible) look more closely at what these were. And so, what they have in the spontaneous (indiscernible) cells were (indiscernible), subclones -- you get these big clones, little clones -- and what happened was that the UV exposure was killing off the little UV clones and sort of favoring the large (indiscernible) clones. And so, they were thinking about this (indiscernible) on one hand is it (indiscernible), or on the other hand, just a little bit like things that happen in a species' evolution, where you constrict the number of genetically different individuals in the population.

So, again, there is something that can happen. It's sort of like purifying selection in evolution. And it could happen with a single UV exposure. So, the conclusion here is that melanoma may involve some no-threshold and single-threshold (indiscernible).

And then last, I'm going to go out of my area of expertise and just make some comments on

some things that I noticed as I was going through some of this literature for this meeting. Although it's sort of relevant to things that we have to deal with, like the question is, oh, is it bad to get a sunburn, is it bad to get a tan? And how does that affect your risk?

So, the number one thing that amazed me -- I have to say, I started reading some of the (indiscernible) in particular, with the hope of identifying what the errors were. And I came away more impressed than with a list of errors. However, I did find a few things. So, this is a graph from a paper that was (indiscernible) is officially a part of the (indiscernible) journal issue around -- with different people commenting on it.

So, they're talking about -- so this is an example of a non-linear threshold. Okay. Well, what was the equation? Well, it's r=ADQ. And r=ADQ, if I follow it on the log, it's going to be a straight line. If I magnify this part of it, it's going to just look like that. So, it's a terrible example of the threshold. It's just not really there.

And so, if this the kind of thing people are dealing with in threshold, then you've really got to get a handle on (indiscernible) of the question.

Now, they have a linear model that had its own sketchy origins, going back -- this is mostly taken from Calabrese's paper. First, I understand it was (indiscernible) present. We're going to have to trust him on the history digging that we do. A bit of a character, but I think we (indiscernible).

So anyway, initially, a lot of this started with just target theory. Talking about a physicist. This would be before we knew about repair and any of these other homeostasis things. And so the idea was we would just have like numbers (indiscernible) exponential (indiscernible). So there at the time, except they never published the calculations, so it's a little bit scandalous, I think. (indiscernible) calculations (indiscernible) hit on that.

Then the next generation, you had the (indiscernible) mouse experiments, which were an

attempt to actually major frequency (indiscernible). But I guess there was -- or I'm gathering there were statistical errors in the control population.

Then there came the models. Too bad Kenny couldn't be here, because he could explain all this better than I. But you have these various mathematical models and then you ask how they can extrapolate down to a (indiscernible)?

Well, one of them was the idea that the mechanism must be the same for spontaneous and genotoxin-induced cancer. But we know that can't be right, because we see different (indiscernible) mutations in spontaneous cancers and reduced ones. So, they are different things.

But all of these models -- and this is important (indiscernible) -- for everybody to keep in mind that I have to deal with daily whenever I'm dealing with (indiscernible). Whenever you've got an equation or a model, there is some assumption underway. You've got to find out what those are, because you don't what the guys assuming. And particularly if he's pulling off programs off the

shelf. And are these models assuming (indiscernible), just to be (indiscernible) or something, for example. Are they assuming this, that or the other (indiscernible)?

So, I've listed here some of the assumptions that go into these models. I'm in no way competent to judge any of these models. But these are the things we should all be asking your bio (indiscernible) and model makers, hey, what about this?

So a lot of the assumptions in those early models -- and I haven't even looked at the main ones; I got up to about like 1980-something -- thought that there would be a chronic exposure, assumed the tumors were (indiscernible) clonal. But there's no growth advantage until all the hits occur, so that, you know, each driver is not contributing, in those early models, anyway. Nothing happens between here to the end.

And now there's a concept of backseat drivers, which are the strong (indiscernible) drivers, but I think that's a little closer to the

(indiscernible) truth.

Then also the assumptions that cancer increases monotonically (indiscernible). Not an experimental observation. An assumption that went into the equation. Okay? And things like no repair or cell death.

And I was looking at a recent review of Kenny (indiscernible), and he was kind of -- seemed to me, despairing of actually having a biology-based model, because it's too complicated. And I have to sort of sympathize with that. And I have some thoughts. We'll save it for discussion, as to what may be an alternative to coming up with theoretical dose responses. But I can save that for later.

So, here then are (indiscernible) which I guess the terminology has moved to (indiscernible) responses is just a way better idea, because (indiscernible) is so (indiscernible). And I see didn't people see different percentages in this. But it seems to me it happens. It seems to be what you'd expect homeostasis. There have been a few things (indiscernible) there, because you haven't broken the

cell. The cell is trying whatever this is you're doing to it.

And so, it seems to me a reasonable phenomenon. You don't know whether it's happening to you in your system and your carcinogen or not until you do the experiment. But I think there are some conclusions, nevertheless, that you can draw. So, here are my overall conclusions.

One is that biology offers no reason to expect a linear or monotonic dose response or expect a fresh (indiscernible). You might find one (indiscernible). Homeostasis implies that the (indiscernible) within the system's operating range, a genotoxin will have a smaller impact, for some reason or another, at that low dose, because your cell is trying.

Yet -- and this is not my analogy; it's something else having to do with the skin cancer -- even if that's true, it's not a good idea, just because your office has a fire department, doesn't mean you set fire to your wastebasket to turn on the fire sprinkler to prevent a fire. And so, say we did

have low levels of radiation everywhere. And say we even have a homeostatic (indiscernible) protecting us. That's not a good reason to say to say we should have our fire sprinklers on all the time, I don't think. That's a little different from today's question where you're talking about just one or two doses. But still, I don't think we would want to (indiscernible) on (indiscernible) as an operational living principal.

Then if you see toxicity, that means you're outside the operating range, so you're breaking the cell. And that (indiscernible) is different from breaking homeostasis (indiscernible) extrapolate one from the other.

For survival, there do seem to be thresholds if the killing is due to reparable lesion and the cell is repaired proficiently. (indiscernible) So that gives you some hope, but the question is, okay, do we know which does range we're in (indiscernible) percentages.

And for mutation, there is a no obvious threshold dose that I can see. Because the low dose has had a larger mutation frequency per dose. Now,

you could ask yourself, do I care about the mutation increase per dose, the steepness of the curve, or do I care about the absolute value?

And that's a little bit like asking do I care how much the interest rate is on my money, or do I only care about the amount of money I have? And I could be getting six percent as a post-doc and not have very much money in my bank account, I still get six percent when I'm about to retire, but it's a little more money by then. But it's been six percent the whole time.

So then the question is which do you care about, the total mutation prevalence or the rate at which you may (indiscernible). And then, that pretty much covers that.

And then we have these dilemmas, which I will only spend one minute on because it becomes apparent. So, do we have thresholds? Do we (indiscernible) on a threshold if we can't measure it? Single exposures, do we (indiscernible) that they're harmless if we haven't done the experiment?

And then this ethical question of like

how close to the railroad tracks do we want to let our kids play? You know, if it's a gray area where we can't measure it, what kind of decision do we make? And then the question of who decides on (indiscernible) add other (indiscernible). This might stuff might be on the end, but okay.

And if I can answer questions...

DR. AISAR ATRAKCHI: Are there any questions?

MAN: Well, thanks a lot for that talk. That was great.

DR. DOUGLAS BRASH: Uh huh.

MAN: I haven't seen some of that data for (indiscernible). Thank you, thank you.

MAN: (indiscernible)

DR. DOUGLAS BRASH: The person who should really be here is (indiscernible).

MAN: So, you really sit a spell with someone who (indiscernible). Unfortunately, most of the people associated with it are either dead or retired.

MAN: (indiscernible)

MAN: I was wondering if you have a (indiscernible) the EMS that was generated with the (indiscernible) if you have an (indiscernible) issue where they claim to have a threshold or a practical threshold permutation that was used to develop a (indiscernible) decision that there was no risk associated with the EMS examination that was experienced by some patients.

DR. DOUGLAS BRASH: I haven't seen it. I'd be interested --

MAN: (indiscernible)

DR. DOUGLAS BRASH: Yeah. Did they have (indiscernible) basis for why there was such a (indiscernible)?

MAN: DNA repair (indiscernible) was reducing the (indiscernible). But it was a situation where there was a really high -- this is a transgenic rodent (indiscernible) where they showed a shoulder and then an increase in (indiscernible). But there was a really big background to this assay, with a big range, depending on how many animals you used in it, as far as the standard deviation.

So, you mentioned the mega mouse experiment. It looks for the world that the bladder tumor incidents looks like a threshold with response, but would you really look at the statistical data if you have any kind of background at all with an error associated with it? It's almost impossible to distinguish at a low level of exposure whether or not you're dealing with a true threshold or just a shadow of dose response, because you're always within the (indiscernible) at low doses.

I was wondering how you feel about using benchmark dose rather than (indiscernible) for accepting (indiscernible) acceptable limits.

DR. DOUGLAS BRASH: So, you might not be -- so, since I'm an amateur in this --

MAN: Okay. Maybe I'm getting into the wrong (indiscernible). All right. I'll (indiscernible). But I wouldn't hear about it, but okay.

MAN: Maybe (indiscernible).

DR. DOUGLAS BRASH: I guess the answer is I don't have a current feeling, but I may have to

fill you in.

WOMAN: So in the mega mouse study, (indiscernible) on a parallel study (indiscernible). And in the bladder, the adducts were (indiscernible) but the idea was that the threshold was caused by an event (indiscernible) this (indiscernible) profile. So, at a certain dose, you increase (indiscernible), and that's when the tumors (indiscernible)? But you had adducts (indiscernible) adducts plus (indiscernible)?

DR. DOUGLAS BRASH: So, that would be a nice mechanistic reason.

WOMAN: Yeah.

DR. DOUGLAS BRASH: Yeah. And then the question is how do you ever know in each particular case if that was going on.

WOMAN: Well, you don't.

DR. DOUGLAS BRASH: Yeah.

WOMAN: Obviously.

MAN: I'll admit it's (indiscernible). Well, in addition to the mega mice study, of course, there's the mega rat study (indiscernible), and

there's the (indiscernible) study on (indiscernible), which were intended for higher numbers. And we vandalized all that data as well for the (indiscernible) agency in the U.K., in trying to justify the numbers they've used to permit a genotoxic substance in food.

And as you'd expect, the answer is you cannot say with confidence in any of those studies that there is a threshold, based on the empirical observation, because you're not at the range of the acceptable risk in humans. And so you need diagnostic studies, and they don't exist for the majority of those data.

DR. DOUGLAS BRASH: It's almost like we're in position with the military. You have to make that decision, but you don't have enough information, and it's serious, but what do you do?

WOMAN: Thank you for the presentation. I really enjoyed it. Seems from the one conclusion that no (indiscernible) cannot be (indiscernible) from the high (indiscernible). And this also (indiscernible). So, in the clinical situation, the

(indiscernible) is very (indiscernible) almost on the (indiscernible) him speaking. Therefore, it's very hard to make call whether we can do the (indiscernible) test (indiscernible) for that when you find at very high dose the animal model and you see the (indiscernible). So I did not see from today's discussion (indiscernible).

So, my question is how to (indiscernible) a rat, whether we are allowed (indiscernible) we know the (indiscernible) low, much lower compared to the observed (indiscernible) animal model.

MAN: Yeah. I agree --

DR. DOUGLAS BRASH: I'll just say then one word about what I was going to say and decided to postpone until the discussion. Getting a dose response is more than any kind of equation, you're taking -- what made me really think about this was you raised, I think, the issue of weight -- somebody had mentioned the issue of weight or reference is that relating to. And what does that even mean?

And if you get any kind of equation,

you've got -- okay, so it's Ames-positive, micronucleus-minus. Do those cancel each other? Do they add? Or you know, what do they do? And so there's two general kinds of computations. One's called a blending computation, where you just kind of mix things up and out comes the numbers.

The other sort of thing, there's a thing called a particular principle, and this is the way genes (indiscernible) is you've a gene for red, a gene for white. You mix them, you don't get a pink gene, you get a pink cloud. Okay? And you retain at the beginning the identity of the individual compounds at the lower level. But it requires this hierarchal computation. So, what I'm wondering is whether the solution to this -- well, it would be nice to get better vision so you could make (indiscernible).

But the other stuff, we've essentially got a whole bunch of carcinogens that we know a lot about. Can you come up with basically a clustering scheme where you find out, okay, there's 30 different groups of known carcinogens. I've got a new chemical. Which of the 30 does it (indiscernible) based on all

of the tumors that we know about? And then there would be a way of getting the information that we don't really have. And then this only works, of course, if you're on clusters.

WOMAN: Thank you.

DR. AISAR ATRAKCHI: As I mentioned earlier, Dr. Crump wasn't able to be with us today due to a death in the family. We're going to try to get some of the high points of his presentation. We had originally reached out to Dr. Crump as a mathematician and might be able to provide some estimate of the cancer risk, the low number of doses of a Ames-positive drug. It was sort of our original motivation for reaching out to him.

And I'm restating the question for the workshop here. And an acceptable answer to this question was somehow take into account some measure of the (indiscernible) genic or injected carcinogenic potency of the drug. Even though we have a full suite of typical data on the drug candidate, making credible estimates of cancer risk would be very difficult. Most of these data would be typically from chronic

exposures (indiscernible) short-term level exposures would require (indiscernible) data on chronic exposures, making (indiscernible) of this (indiscernible) impossible to extrapolate from chronic exposures to the (indiscernible) doses.

He's premising to some control of human inhalation exposure studies. I'm going to leave this to you to look at. I will try to forward this audio presentation in the next few days.

On (indiscernible), rather than estimating a risk, he sort of came up with a model that we might be able to use, using a (indiscernible) chemical. For this exercise he chose a nitrite as the comparative chemical. And that nitrate seems to be unique and then it's an Ames-positive chemical that (indiscernible) present in (indiscernible) small amounts. And it's positive in TA-100. And he's stating here that nitrate has not been shown to be carcinogenic. And yet he did two-year carcinogen studies in mice and rats that seem to be negative. However, IR has -- I don't know if it's listed -- nitrate is probably a carcinogen, based on use in food

as preservative. Maybe the panel could discuss this, if they choose to go down and talk further about this.

He notes that (indiscernible) allows a maximum consumption of some (indiscernible) containing 200 parts of (indiscernible) nitrite, and this would be equivalent to 17 milligrams of nitrate per three-ounce serving of fish. The WHO maximum daily dose is .13 kilogram of nitrite. This would be equivalent 8.9 milligrams for a 150-pound person. And the WHO maximum recommends daily intake of .05 milligrams or kilograms of nitrite. It's equivalent to 3.4 milligrams.

More or less, he's wanting to use nitrate to employ this as a comparator in the Ames assay. I can't really speak to his math. More or less, he wants to use nitrate as a comparator in the Ames assay with a chemical of interest. He's making reference to (indiscernible) with 1997 paper, where protecting (indiscernible) on the Ames assay.

He suggests that nitrate may be tested concurrently with a candidate drug using the same experimental protocol, same salmonella strains,

(indiscernible) protocol, et cetera. He suggests that the lower bound be used as a maximum (indiscernible) exposure rather than a (indiscernible) estimate.

Decisions would need to be made regarding how to use the data from those strains (indiscernible) protocols, use them to find maximum (indiscernible) exposure. This approach could not place any restriction on the number of days a volunteer could be exposed. This is in keeping with the fact that based on the maximum daily exposure limit, or nitrate, which also do not have such restrictions. Prudence would dictate that exposures should only last for the minimum number of days (indiscernible) answer the scientific question.

Exposure to the drug candidates' maximum daily exposure will entail some mutagenic potential, as exposure to an amount of nitrite allowable by U.S. FDA (indiscernible) positive chemical (indiscernible) found to be capable of causing cancer.

Here he is showing the positive for sodium nitrate and ta-100. He's also comparing it to

some other known carcinogens. Here he is (indiscernible) the potency of Allyl Urea versus sodium nitrite. Here sodium nitrite is supposed to have a higher potency. Here there is (indiscernible) amino azobenzene, here (indiscernible) aminozobenzene is a higher potency. The idea would be to use the slope of the line to slope the dose response to sort of a maximum daily exposure.

And aminoazotoluene, again, has a higher (indiscernible) count than sodium nitrate, which is the comparator. Similarly, for another carcinogenic, it also has a higher potency than sodium nitrate.

This is all in his essay. (indiscernible) of this approach is straightforward and easily implemented. Takes into account the mutagenic potency of a drug candidate. Does not restrict the number of days that the (indiscernible) exposure per day. It's based on the precedent sent by U.S. FDA. (indiscernible) positive chemical.

This approach ensures that maximum daily exposure for a candidate, drug has an equal

(indiscernible) FDA's maximum daily exposure for nitrite. And then he's basically using the slope of the dose response (indiscernible) it's curved to (indiscernible) maximum daily exposure.

And I guess the panel could address this this afternoon. Thank you.

MAN: If you're compelled to respond, because my name was flashed all over the place. But one point that hasn't been made is this all assumes that there is a correlation that goes -- a potency correlation between Ames-test mutagenicity and rodent carcinogenicity. And if there's going to -- this afternoon, I have a slide that shows that the correlation is zero. Approaching zero.

The thing is, the Ames-test potency doesn't even correlate with other in vitro endpoint tests of (indiscernible). The Ames-test potency and (indiscernible) potency do not correlate. So why would you expect it to correlate with carcinogen (indiscernible). And it assumes he doesn't. And essentially, everything that these graphs show assumes a correlation.

WOMAN: Yes. And this is the math that he suggested -- he couldn't really answer our direct questions.

MAN: (indiscernible)

WOMAN: He also (indiscernible) critical. What is the threshold (indiscernible)?

WOMAN: (indiscernible) the last question because he's not here. But what slope is critical? So at what point do we say this is not safe and not given (indiscernible). And what slope is okay to give? And if it's okay to give, how many doses do you give? So, I mean, this might be a start, but there are lots of steps I think that needs to be filled.

WOMAN: (indiscernible) the assumption that nitrite is a model for all chemicals. I mean, that's really a problem. Carcinogens do a lot of different things, so it's gene (indiscernible). And chemicals, you just can't (indiscernible) them (indiscernible) that they won't (indiscernible) and that's (indiscernible), which otherwise is a reason why (indiscernible).

MAN: I do want to call out the concepts here, because I think there is merit to the concept. And that is it's trying to -- it's talking about quantity of risk against something that I think we understand, in this case something that is sodium nitrate, at which level we think is safe. So, it's (indiscernible) to compare that, an unknown to a known. And we could also compare where that unknown to a known hazard as well. That's not something we typically do.

I think even in a (indiscernible) consent, going back to the bio (indiscernible) talk, if there's an (indiscernible) that, you know, your risk in this is in the ballpark of getting a dental x-ray. It's an experience shared by everybody. That would resonate. Maybe they would have better communication that way.

I think the flaw in this particular approach, of course, is that the metabolic (indiscernible) is sodium nitrite is going to be different, very likely different, from whatever chemical it is that you're testing. (indiscernible)

contrast agent, which is 10 to the 10th years. Is that a half life or is that a (indiscernible) life?

You know, I wonder what the slopes would be between sodium nitrite and something like that. I think that explains that the lack of correlation in terms of potency. So, this is flawed, but I do want to call out that concept because I think there's merit in there.

MAN: Just try to show you (indiscernible) doses he was proposing (indiscernible) at the bottom row, the lower bound, like for (indiscernible) he's saying, according to (indiscernible), 58 were -- I guess the lower bound, he was proposing to administer the lower bound. And I'm not trying to endorse his proposal. I think that was what he was trying to get at.

I think we're scheduled to have lunch from 12:00 to 1:00. The panel discussion will begin at 1:00 P.M.

(Break)

DR. AISAR ATRAKCHI: Okay. Good afternoon, everyone, and welcome back to the second

session of this important workshop. I am Aisar Atrakchi. I'm a pharmacology toxicology supervisor in the Division of Psychiatry at CDER and I am a co-organizer of this workshop.

In the morning session, you heard Dr. Robison's introduction and background for why we are holding this workshop with the emphasis on a few key words: Healthy subjects, number of doses, mutagenic DNA reactive drug, and cancer risk estimate.

We heard why this information is of importance and the roll it's played in the development of generic drugs, discussed by Dr. Dorsam, where clinical bioequivalents, clinical study, enrolled healthy subjects relying on the information of the listed referenced drug as stated in the drug label, and also the importance of this for the beginning -- the early stages of drug development Phase 1.

This paucity of the information on the topic of this workshop was clearly presented by Dr. Petibone through the results of the extensive and exhaustive literature search that he and Dr. Shemansky carried out over the last year for many months, and

continue to do so.

We also heard from our two experts in the field, Dr. Brash explaining the process and steps encountered from a mutation to tumor induction and the role of pills, dose rate, and duration of exposure and the presentation by Dr. Crump, at least the slides, using mathematical and statistical approach to cancer risk prediction.

We also heard from Dr. Prohaska addressing the ethical issues and concerns enrolling healthy subjects in clinical trials. For this afternoon's session, we have assembled some of the best experts in the relevant scientific fields and have prepared a number of questions to engage and stimulate the discussion, including the points made in the morning session.

At the end of today, we hope to gather information from the panel discussion that will advise and assist the agency to better understand the current scientific thinking of allowing safe dosing of a mutagenic DNA reactive drug to healthy subjects without increasing their cancer risk.

So with that, I'm going to go through the first question, but some of those questions are also repetitive, more or less, but -- and I tried to gather, to put some of them together to address at the same time. So question one, how many doses of an Ames-positive drug, DNA reactive drug that can be safely administered to healthy subjects?

Can it be administered at all? One dose, two, or up to four doses? And if it is okay to administer these one or more doses to healthy subjects are acceptable with a mutagenic, how should the study be designed? I think let's go to first through the first question and then we'll go -- move on to the next one. If it's okay, then we'll move to the next question.

So what I would like to do is, if possible, starting from my left, introduce the panel, introduce yourself with -- and your affiliation and very brief background.

DR. ROBERT HEFLICH: Hi. My name is Bob Heflich. I'm from the FDA and NCTR, which is in Arkansas. We're a research center for the FDA. We

have no regulatory role. We're strictly here to advise and help the product centers. I've been FDA employee since 1979, coming out of Veronica Maher's ranch -- slides were given earlier in a talk.

And for the last six-plus years, I've been director of the Division of Genetic and Molecular Toxicology. Over the hears, I've been involved in a lot of in vivo mutagenesis-type studies seeking to develop methods that could be used for -- to compliment the in vivo (indiscernible) assays that are generally used for in vivo assessment of gene tox using a gene mutation influence.

The most commonly used today is the transgenic rodent assay which appeared in the late '80s and has developed into an assay over the '90s and 2013, I think, is the last OECD test guideline version. More recently, I've been involved with developing the PIG-A gene mutation assay and we're currently engaged in trying to get a OECD test guideline for that approved.

The other -- the major research initiatives in the division are to explore the use of

error-corrected next generation sequencing for evaluating sequence changes. I think that's -- this has a great potential to revolutionize the practice of genetic toxicology and perhaps we'll find usefulness in regulatory applications.

The other thing we do a lot of is in vitro tissue models, is a more of a risk characterization tool for genetic toxicology, so I think I'll stop there. Doug?

DR. DOUGLAS BRASH: Okay, thanks. My name is Doug Brash. I'm basically a biophysicist -- I'm at Yale -- basically a biophysicist who ended up working on how sunlight causes skin cancer. We started out in some of the biophysical events looking for the mutagenic photo products, back in the days when we were just able to look -- use DNA sequencing-like technologies to locate them and found out which ones are mutagenic.

We found out that mutations aren't just coming randomly from (indiscernible), and then we started -- said somewhat foolishly, well, gee, can we find the genes that are hit by sunlight causing -- in

order to cause skin cancer. And in retrospect, amazingly enough, that worked and we found what came to be called UV signature mutations (indiscernible) of other genes in skin cancer.

And then, we started to worry about (indiscernible) anyway and that got us into the apoptosis story and (indiscernible) expansion that I alluded to a little bit earlier. Lately, we've gone into some unusual chemistry where UV (indiscernible) will cause DNA damage, even in the dark for hours after leaving the beach, so we're trying to follow that up. It involved melanin.

We think it may be involved in other diseases besides skin cancer, like you have melanin in your brain, for example, and also we're trying to use detection -- sequencing-based methods toward detecting mutations in, right now, DNA photo products (indiscernible) skin to get an objective measure of what your past sunlight exposure is -- was, so that we can maybe devise a measure of risk so we can tell people, you should go see your dermatologist once a year.

DR. ALAN BOOBIS: Good afternoon. I'm Alan Boobis. I'm emeritus professor of toxicology, Imperial College London.

I retired from my fulltime position a year or so ago where I was at the postgraduate research department of Imperial for 40 years as a academic research worker and over that time, my research has involved a variety of different activities including mechanisms of toxicity and carcinogenicity and the genetic toxicology of polycyclic aromatic hydrocarbons (indiscernible) the toxin both looking at in vitro, in vivo assays as a means to an end, to try to understand the mechanisms of activity and experimental models in humans.

The department I was in was a department of experimental medicine in the medical faculty, and as such, I have been exposed to medical research for my entire academic career, which includes conducting and participating in Phase 1 trials of new drugs and also doing exposure of human volunteers to radiation for experimental purposes.

In parallel, for the last 25 years or

so, I have been a member of national and international scientific advisory committee, assessing the risk to humans or potential risk to humans of a variety of chemicals including (indiscernible) drugs, pesticides, contaminants, and food additives and that has included having to look at the toxicology, genetic toxicity, and carcinogenicity of those compounds in both data-rich and data-poor situations.

DR. TIMOTHY MCGOVERN: Good afternoon. My name is Tim McGovern. I'm an associate director for pharmacology, toxicology, (indiscernible) new drugs in CDER. I'm not a genetic toxicologist by training, by I know many people who are. Part of my role, I sit on the Executive Carcinogenicity Assessment Committee in CDER.

Also a member of the gene tox subcommittee. I'm also a member of the ICH M7 working group which is for DNA reactive impurities as well as the S1 group looking at carcinogenicity assessment.

And also part of my work is working with review divisions (indiscernible) when these issues come up, where we get positive findings or

questionable findings in gene tox assays and making that determination whether those findings rise up to the level of warranting a clinical hold or not and what possible followup studies may need to be conducted to further evaluate the issue.

DR. MIRIAM POIRIER: Hi, I'm Miriam Poirier. I started out my career doing animal (indiscernible) studies in the laboratory of James and Elizabeth Miller at the University of Wisconsin. For something like 48 years, I was a paid employee of the National Cancer Institute. I'm now an emeritus employee -- that means I don't get paid.

But what -- the major part of my career, we developed methodologies to measure DNA adduct in human tissues and then we applied those methods to look at the parameters of that information in humans and to try and understand the mechanisms and the consequences of DNA adduct formation in humans.

And -- oh, and I'm past president of the Environmental Mutagenesis and Genomics Society.

DR. KEVIN PROHASKA: Thank you. I do get paid. My name is Kevin Prohaska. I was

introduced earlier, obviously. Currently, I'm serving as the FDA's senior bioethicist for adult research. I'm the only ethicist for adult research, so that, by default, makes me the senior guy, which is fine. There are two other individuals who do the pediatric research and as you can well imagine, more issues sort of arise in research involving pediatrics, so there is a need for two.

My background is -- I'm also board certified in family medicine. I'm ex-U.S. military, Army, and while I was with the Army I (indiscernible) a lot of emergency medicine and so I was probably more of an emergency room physician that a primary care doctor, family practice doctor. Let the military. Went into private practice.

Decided that it wasn't for me and then I decided to come to the FDA where I started as a primary reviewer in one of the review divisions in neuropsych, which is now two different divisions, neurology and psych department, and while I was there, I was working on an awful lot of products including ones related to bioterrorism which was right around

the time of 9/11 in 2001, which sort of stoked an interest in (indiscernible) protections, so I started getting involved in that, in some of the policy work around that area and decided to pursue some experience in bioethics and so I became over time the agency's bioethicist.

In between all of that, I moved over to the Office of Compliance where I was the director of the Division of Safety and Investigation which I was responsible for (indiscernible) oversight, radioactive drug research committees, the post-marketing pharmacovigilance program, and I'm forgetting the (indiscernible) program.

So I have quite a few programs and I'm doing (indiscernible) stuff. Thank you.

DR. ERROL ZEIGER: Hello. I'm Errol Zeiger. I'm currently an independent consultant. I started working in mutagenesis or genetic toxicology at the FDA in 1969, before there was an Ames test, but I was working with Ames' bacterial strains and doing mostly testing and playing around with techniques using bacteria, using yeast as good test organisms.

In 1976, I was recruited to go down to the NIEHS to run a research lab and also to start a testing program. Interestingly, in 1975, Bruce Ames published a paper that, “Hair Dyes Are mutagenic.” For some reason, Congress picked up on it and in 1975, ’76, held budget hearings and directed NIEHS to start a mutagenicity testing program to identify potential carcinogens in the environment.

I was asked to design and develop it and started up -- we started it up in 1979, the same year that the National Toxicology Program started and without my consent, against my wishes, we were taken into the National Toxicology Program. So any NTP detox studies you see now all came out of that congressional hearing -- budget hearing, and initially it was very well funded.

I asked for 12 slots; I got 12 slots. So, but most of my career at NIEHS, from ’76 until two -- the end of 2000 was evaluating data, running the test program, and publishing quite a few papers, presenting the data, because this was before the internet so we were actually presenting it in hard

paperback publications, and also evaluating the effectiveness of different tests, alone and in combination, for detecting carcinogens and for complementarity.

I also spent over a year working with OECD in Paris to look at, not so much gene tox but other toxicology end points. And since then, since 2001, I've been an independent consultant in Chapel Hill. Most of my consultations are related to genetic toxicology (indiscernible) carcinogenicity.

DR. AISAR ATRAKCHI: Okay, thank you very much for -- everyone. I appreciate you guys coming back this afternoon as well as the start of a very, hopefully, interesting discussion. So I would like to start with the first question, which is really the topic of this workshop and anyone who would like to start addressing how many doses of an Ames-positive DNA reactive drug can be safely administered to healthy subjects, and in the sense that not -- these are healthy subjects, so we should not, preferably, change the cancer risk from one in a million as opposed to in patients, we could accept 1 in 100,000.

DR. TIMOTHY MCGOVERN: I'll start out. The -- one thing I wanted to bring up that was not brought up this morning regarding scenarios where the agency as well as other regulatory bodies already allow exposure to mutagens by, in this particular scenario, both patients as well as healthy volunteers because under the ICH M7 guidance for DNA reactive impurities and a threshold approach was developed under that guidance, with a lot of work going on before then where, essentially, for a long-term exposure and a daily dose of up to 1.5 micrograms per day is allowed without any further qualification data, with exceptions of very high potency compounds like nitrosamines.

And the guidance also works in a stepwise approach as duration of exposure degreases that that threshold will then increase to the durations we're talking, possibly zero to five, maybe up to 14-day exposure. ICH M7 has a threshold limit of 120 micrograms per day for a one-month duration of exposure.

And that level is associated with a one

in a million increased cancer risk. You could continue that calculation (indiscernible) to even shorter durations. If you're talking about a one-day exposure, that would be the equivalent of 3.8 milligrams per day, still associated with one in a million cancer risk.

So I just wanted to point out that we do have scenarios in place. It's slightly different than first in human, healthy volunteer trial, but for impurities, yeah, we assume for the most part that there's no inherent benefit being gained by the presence of an impurity, the same way we're talking about for a healthy volunteer trial, that there's no inherent -- for the most part, inherent benefit for healthy volunteers to be exposed to a mutagenic drug.

So you can make the argument, anyway, that through surgery we've already made a case that healthy volunteers can be exposed to mutagens and questions really -- what level of that exposure do we find acceptable. If we go with the 30-day cutoff for M7, we're saying 120 micrograms per day.

Then obviously we'll put a fairly

limiting cap on those drug development programs without further evaluation being done, but you can also push that argument further and say, you have 3.8 milligrams per day for a single dose could also be acceptable, except for very extreme cases.

So I just wanted to add that to the case that was brought up earlier regarding microdosing scenarios which allows up to 100 micrograms per day as well.

DR. ERROL ZEIGER: Well, I would answer that simply to say, it depends. It depends on the -- I would be interested in, not only, was it a mutagen in vivo or in vitro, but what was the structure? Is it something you expect to be highly DNA reactive? Is it something that would be much less DNA reactive -- much less reactive?

And I can't separate out the concept that we just had -- all of us had doses of mutagens at lunch today: The soup, the broth -- chicken broth, coffee for people that drink it. We all are doing it in that background. And that's, obviously, acceptable. But I just like keeping that in mind.

But as far as the rest goes, I think we need to know more if it's just Ames positive, to take -- to come up with a dose, have to know a lot more about the chemical, maybe even something about the volunteer.

DR. AISAR ATRAKCHI: I think we have to make the distinction between what we eat and what can be present in drugs. Eating, it's a personal choice. You can eat as many smoked meats or coffees or roasted things, but I think the risk is different for drugs. The drugs, we have to take them. We have no choice. So I think, in my mind, we have to be more protective of the patients.

In this case, what we're discussing is healthy volunteers, so clearly, we all know that on a daily, on an hourly basis, we do inhale or drink or eat these type of mutagens. But I think, really, the emphasis is more the quality of a drug as well as, in this case, the drug itself. Is it okay for people who -- again, these are healthy volunteers?

They have absolutely no benefit of taking that drug except to serve, perhaps, the PK --

to determine the PK of that drug in order to give it next to patients. It's well taken, the point, but again, I think your point earlier for the structure, it's not just mutagenicity. You indicated that we do also need to know little more information.

MAN 1: I was going to agree with that, yes. You know, hopefully we all got something out of our lunch including some nutrition, so there was some benefit there. The numbers that Dr. McGovern just mentioned are very helpful to understand, very useful putting the risk in perspective.

But when you're evaluating the risk of research, you have to take all the risks involved and so that includes the risk that might be inherent in the structure, unique things, but all the other things that might occur during that clinical trial, so all of that needs to be considered.

DR. ALAN BOOBIS: So I'm assuming when we're trying to move away from a de minimis approach like the threshold of toxicological concern, because that is established. It's based on a large database of genotoxic carcinogens, which is currently

undergoing refinement, but it's an approach that could be used to find what is the maximum dose that's associated, at worst, with (indiscernible). It's not associated with one in a million. That's the worst case because that's the extreme of the distribution.

But if we want to try and move the dose up, it does depend, but I'm assuming that in the Phase 1 trial you don't have a in vivo tox followup on a gene mutation assay.

DR. AISAR ATRAKCHI: Sometimes, some companies will do that entire battery, but in general, based on the guidance, we do not need to do that. You only need the gene mutation to make the Ames test and in vitro (indiscernible), generally.

DR. ALAN BOOBIS: Because if I had a good in vivo gene mutation followup, not a micronutrients test but I mean, actually looking at a proper gene mutation assay in vivo and it was a (indiscernible), my conclusion would be somewhat different in -- even if (indiscernible) in vitro, than if it was just an in vitro positive with no in vivo followup.

That’s the first thing. Second thing is that not all adducts which cause mutations are equal, so that we’ve got to think about the kinetics and the dynamics, how reparable is the lesion which is information we generally don’t get. But you might be able to (indiscernible) from the structure because we know something about the reparability of seven types of adults.

And the persistence of the compound will determine how many doses or what duration. So all that information would go into a weighted evidence conclusion. I do not think there’s a single answer to this question.

DR. AISAR ATRAKCHI: I think -- so we don’t -- I’m loud. So we don’t have the structural similarity. These are -- so (indiscernible) a trial that there’s new molecular entities, we don’t have similarities to other structures for the point that was initially discussed, so what happens when we don’t have any structural similarities that we can compare it to something else because we have (indiscernible) entity.

We don't have another data, either, so we are just relying on Ames positive. That's all we know. And there's no carcinogenicity data, either, because we are so early on in the development that such data are not available.

DR. ALAN BOOBIS: But you do know the structure of the chemical.

DR. AISAR ATRAKCHI: We know the structure --

DR. ALAN BOOBIS: So I'm not a computational chemist, but I know the computational chemist can tell us quite a bit about what a chemical might do based on structure. And it's not based on the entirety of the structure. It's based on structural motifs. Which motif is -- confers on that chemical Ames positivity?

And how reparable is that motif? A compound that contains that motif, if I get an adduct of a similar compound with the same (indiscernible).

DR. AISAR ATRAKCHI: Certainly, we get this with M7. The M7, you do get the structure of the QSAR (indiscernible), but I'm not sure we can or

should do this for an API, for a drug that is NME that's coming in and it's already, we have a test.

DR. ALAN BOOBIS: No, I'm just --

DR. AISAR ATRAKCHI: The test is telling us it's an Ames positive.

DR. ALAN BOOBIS: No, I'm not suggesting you place the -- I would certainly never suggest a computational approach should override a biological test.

DR. AISAR ATRAKCHI: Okay.

DR. ALAN BOOBIS: I'm saying it adds to the overall weight of evidence.

DR. AISAR ATRAKCHI: I see.

DR. ALAN BOOBIS: It helps you in your interpretation of what might happen.

DR. AISAR ATRAKCHI: And that could be very muddy, too, because you get on page back that it could be this, it might be this, it might be that.

MAN 2: (indiscernible) can I just pursue that for a moment? So Errol told us earlier about a lack of correlation between mutagenic potency and cancer potency, but now, it sounds like you're

going back to that and so the question is, has anybody looked at that kind of QSAR or the kinds of adducts and correlated that with cancer potency? Is there data on that?

DR. ALAN BOOBIS: I'm not sure that we've gone all the way out to cancer potency. I think what they've done is looked at the persistence of mutations in vivo.

DR. AISAR ATRAKCHI: Because, again, I mean, we have to remember these are gene (indiscernible), we use them as a (indiscernible), so -- and they're mutagenicity (indiscernible). To take them as far as predicting, extrapolating the potency of a mutagenic to a carcinogenicity, it seems inappropriate at this time. I don't think we have -- certainly as I hear you saying that it's a weight of evidence and maybe that's what it comes down to.

MAN 3: I want to push back a little bit and also be a little bit of a devil's advocate, which I'm very good at sometimes, about this first question about healthy subjects. It kind of defies that. We're okay if it's patients. When, in fact,

one, two, three, or four doses is probably going to have no beneficial effect on that patient (indiscernible). Right?

So what makes the difference? Why do we accept it for patients but not healthies because of this -- neither one are going to be (indiscernible) the efficacy of the drug? Is it just purely the social aspect in that the patient, if this drug were to be successful, will ultimately maybe benefit more personally because they have the disease that would be treated by that drug in the future?

Is that what makes the difference? And is that enough to make that difference?

DR. KEVIN PROHASKA: I'm going to have the review division helping me answering this, but in part, I think part of it has to do with the fact that there is a potential for benefit in some of these circumstances when you have the condition, albeit, it may be small, but the other thing to keep in mind is you might be able to roll in from a Phase 1 right into a Phase 2 with that same individual if there's -- some good basic work has been done to be able to do that.

So there is some value in that approach.

DR. ERROL ZEIGER: And you're also going on the assumption that the patient with the syndrome will handle, will mechanicalize the drug, the drug will be similar response to a healthy person because the drugs are not going to be given to a healthy person. They'll be given to a person with the syndrome, whatever you're trying to cure. And that's the assumption that they will both respond similarly to the drug.

DR. MIRIAM POIRIER: I don't think you can answer this question the way it's written. It's written, what can be given safely, one, two, three, or four doses. I don't think there's any way of knowing that. For most new compounds that you see that (indiscernible) and you have some basic information, but if you look at the (indiscernible) paper and then what you all have done and then (indiscernible) to follow up on that, I mean, I don't think that we can, in good conscience, ignore 60, 70 years' worth of tumor studies.

And even if some -- you can make arguments that some of those studies weren't perfect, the weight of evidence, I think, with your paper and with what came before you is that there is -- there are many, many cases in which one dose or a low dose or a single dose has produced cancer.

So we can go ahead and give these drugs and make our best estimates of what's going to be safe, but I honestly think in the light of the evidence that's out there, you can never really say that something is going to be safe. And, bottom line is, you don't have the end of that experiment because that person is probably going to be a young person.

They're going to go on and reach 70 or 80 and maybe they're going to get cancer, maybe they aren't, but you're never going to know where that cancer came from. And so there is no way of answering this question as it's written, I believe.

DR. ROBERT HEFLICH: So I get the impression that you're hesitant as for additional information when this comes up.

DR. AISAR ATRAKCHI: No, it's not the

hesitation. We get the implication from a sponsor with a -- they just want to do the (indiscernible) and a single dose, maybe up to 14 days of an NME, new molecular entity. They've done -- all the guidances that we have out there in terms of the mutagenicity. They have -- sometimes, they have 14-day tox study, (indiscernible) toxicology study (indiscernible), maybe up to a month, maybe, and that's all we have, and some other information in clinical. And the non-clinical will determine what is the first dose that potentially is safe that can be administered. So we really -- that's all we have and that -- and they're following what we're telling them to do.

DR. ROBERT HEFLICH: Can you just say stop, we can't go any further with this?

DR. AISAR ATRAKCHI: We can.

DR. ROBERT HEFLICH: Because it's a mutagen and you need to give us X, Y, Z before you can go --

DR. AISAR ATRAKCHI: Yes, we can, for -- I mean, again, I think most of you know, or if you don't know, it is rare that we get an Ames positive

drug from Phase 1. I've been here 27-plus years. Only once I've seen that happen and we put that drug on hold. So reality is we don't -- we're not faced with this all the time, but we do -- you could get an equivocal response, but it's still slightly positive in Ames.

DR. ROBERT HEFLICH: Well, maybe if you had a path forward, you would see more of this.

DR. AISAR ATRAKCHI: Meaning -- path forward what?

DR. ROBERT HEFLICH: If, you know, the drug turned out to be Ames positive and you had a followup that you could recommend to the company, the company might be more willing to put such a drug forward as an IND.

DR. AISAR ATRAKCHI: Well, but I think what the issues is some of us who, if we see such a drug, we will say, but it's only -- they're doing one dose in the healthy volunteers. I think it's okay because, let's say, the (indiscernible) was negative. Others will say, no, this is a positive Ames. I'm not going to let you go, but you need to give me more

information.

Others will say, but two or three doses, it's okay. So this is why we're asking this question and we -- I mean, that's why we're trying to get from you --

DR. ROBERT HEFLICH: I think every --

DR. AISAR ATRAKCHI: (indiscernible).

DR. ROBERT HEFLICH: Everyone here would say it's unknowable.

DR. AISAR ATRAKCHI: Is what?

DR. ROBERT HEFLICH: It's unknowable. You can't give an answer to that based on what we know. It requires more information. I mean, there's lots of examples where single doses produce tumors, in both humans and animals.

DR. AISAR ATRAKCHI: And we could see that today, we have -- from the presentation --

DR. ROBERT HEFLICH: So in other words, if you had a followup test in vivo gene mutations test that was negative by statistical criteria that you can set, would that be sufficient to say the Ames test is not significant for in vivo effects?

DR. AISAR ATRAKCHI: That is a question later on, so we're starting here, then we can move --

DR. ROBERT HEFLICH: Well, maybe --

DR. AISAR ATRAKCHI: Because we want -- so I think what I'm -- Todd, would you like -- let's hear your question and then we can...

TODD: It was just a comment that the examples that we saw today, those are pretty well recognized mutagenic genotoxic agents, and to your point, what we're most likely to deal with is equivocal things where we have a mixed profile with a standard battery.

It's not like the things that we were seeing earlier today, so one, two, or three, four doses may be if we reword it to say, it can be reasonably safely administered, is probably a more accurate way of saying it. If you really think about it, I mean, at least in our division, we do healthy subject (indiscernible) trials all the time.

With -- and there are toxicities, minimal amount reversible and monitorable, that we allow them to go to in healthy people. Is that risk

any different than if this was an (indiscernible) toxic drug, when, let's say it's a renal toxicant, right? You can monitor for that, you let them come up to a certain level but not higher to avoid brain toxicity.

That's a lot more of immediate potential toxicity than cancer 40 years after that single dose of (indiscernible) toxic drug. So it seems like, I don't know, maybe our priorities there in terms of assessing that risk is a little backwards. The difference, though, is we monitor for these non-genotoxic sort of toxicity, so we're able to do that, typically. And error correct the sequence and maybe reassert potential there for us to do, take blood tests and look for mutational frequency in order [OVERLAPPING SPEAKERS]. I'm sorry?

DR. AISAR ATRAKCHI: And people?

TODD: Yeah, and people. I know companies will not like to do that.

DR. AISAR ATRAKCHI: They can't, you know [OVERLAPPING SPEAKERS] but, ethically, we cannot do that. That's a lot of reasons that companies would

not do that.

TODD: I think we can make that -- we can argue the evidence of it, but let's forget people. What about (indiscernible).

DR. ERROL ZEIGER: Okay, if there's one thing we know about Ames and in vitro mammalian cell (indiscernible). They are tremendously imperfect predictors of potency in cancer. So, say we got something equivocal in the Ames test really is not necessarily put your mind at ease that this is only marginal.

Of course, it could be -- because it may be part of particular activation pathway that's not represented in the S9 that's used in the standard Ames test. It could be a lot of reasons why you get a weak response. So I don't think you can necessarily equate an equivocal in vitro assay with a lack of risk.

TODD: Well, when I say equivocal, it's (indiscernible) that with more than just the one test. We have several tests and it might give us marginal positives, one, maybe (indiscernible) another -- but I

understand.

DR. AISAR ATRAKCHI: So, I think -- well, Dr. Brach, you didn't say -- I'd like to hear from every person on the panel, so would you like to add to this in any way?

DR. ROBERT HEFLICH: (indiscernible). Yeah, I do have a couple of thoughts. So one is, basically, you don't know and at some point have to say, oh, I don't know. And another one is -- a thought that had occurred to me (indiscernible) other times is, okay, so if you gave low doses over several times so that you had this washout period, not just for the drug but time for repair, that maybe you could talk me into it, but you don't know that until you (indiscernible).

And, that brings me to the third point which is, who's the normal volunteer here and the -- an exam question when I was at school was what's wrong with the phrase, "You have the wrong number," and the answer is, "the."

So there's lots of wrong numbers and there's lots of normal volunteers and so you have all

(indiscernible) to deal with and even -- if the patient has an inflammatory condition or something you didn't know about or is the drug going to be hitting at the same time the liver cells decided to divide? You don't know any of this.

And so the only, I guess, coherent thought to put all this together is that only (indiscernible) test in light of the presentations about single doses, low doses, is that the existing data isn't enough to reassure me that a small number of low doses (indiscernible).

DR. AISAR ATRAKCHI: I think if I -- to summarize for question one is that there is no one answer, which we kind of knew that, but weight of evidence and also you -- if you saw a clear dose responsive, positive Ames of a drug, you would not or would you still say, for a single dose in humans I would give it? I think that's really what we're trying to understand.

Now, again, we -- like was mentioned, you do have chromosomal aberration test and it's negative. This structure -- I mean, I think what I've

heard is that it's the weight of evidence. Everything about that particular molecule. But if you have an Ames -- clear Ames positive dose response and the sponsor repeated it and it's positive, maybe their dose -- the concentration, they narrowed it down. It was too large and they narrowed it down and it's still positive.

We don't see the (indiscernible) there. But just if we do and we're not talking about equivocal, would you allow such drug to be tested in healthy people?

DR. ROBERT HEFLICH: You're turning this into, certainly, an Ames test interpretation.

DR. AISAR ATRAKCHI: I am, because that's what we get.

DR. ROBERT HEFLICH: We know from a number of different compilations that a positive Ames test, if you're looking just across chemical structures, there's about 70 to 80 percent predictive of rodent carcinogenicity as either ra or mouse, not necessarily both. Whether it's a strong Ames test positive or a weak Ames test positive, it's positive

and the date showed -- you look at the data, the TD50s and the Ames test, the potencies, there is absolutely no correlation.

We did a study -- I did a study about 10 years ago using 100 chemicals that were Ames test positive in the NTP. They're looking at the slopes and lowest (indiscernible) TD50 values for the same chemicals. The correlation was 0.04-something. Not the P value, the correlation. It looked like a shotgun pattern from about 10 paces.

So where there are some -- where the Ames potency is 1 microgram -- mutation per microgram or 10,000 mutations per microgram, it's not going to make any difference as to whether or not you should be more or less concerned about that response.

DR. ALAN BOOBIS: I think that's right. I think it's a positive ID and I would really fell very uncomfortable giving a clear Ames positive unless I had some reason to strongly think that this is just an artifact of the assay and there would be a dispositive indicator. There are compounds that we know will give a (indiscernible) positive.

We also know they're going to be (indiscernible) because it's very difficult to make that case in the absence of the (indiscernible). So in general, the question -- the answer would be not unless there's really good reason to come to a different conclusion.

DR. TIMOTHY MCGOVERN: So, I mean, you can get a reproducible dose response for -- in TA100 for phenobarbital, which is a little carcinogen but almost certainly not a mutagenic (indiscernible) carcinogen. So perhaps the way to ask question one is not, can you give it for one, two, three, or four doses, but what would you need to know about a drug to feel comfortable giving it for a small number of doses to a healthy volunteer.

And the other thing to keep in mind is that you're really making two decisions here, not just one. Because after you finish your Phase 1 trial, you're probably not going to have a whole lot of more pre-clinical information about the drug and you're going to have to start deciding, are you going to give this drug to patient volunteers in Phase 2.

And so you're going to have to inform them of their risk of cancer from this mutagenic drug and so unless you're thinking ahead to that and what you're going to need to know to answer that question, because presumably most drugs make it through Phase 1, there's really a bar -- that way if the problems come up, problems come up in Phase 2 so you're also going to be giving more doses of that drug to patient volunteers.

DR. ROBERT HEFLICH: I think as part of that calculation, what we typically -- this narrative came up with the person (indiscernible) clinical trial being proposed, the division might say one dose okay, two sometimes four okay, but then it becomes a partial clinical, before you go any further you're going to need to provide this additional information to clarify or to address our concern regarding the positive Ames finding.

DR. TIMOTHY MCGOVERN: And maybe the answer is if you do that, work as one, you can go into it as one. So if you've already worked out that scenario for going into Phase 2, then maybe those are

the kinds of -- who was it, Bob, talked about case studies -- case studies. You know, maybe you can look at some of those case studies of drugs that were Ames positive and you had to make that decision for Phase 2 and what made you feel comfortable about the safety in Phase 2.

In some ways, I would say, okay, it's two. Patient is more vulnerable than a healthy volunteer because the Phase 2 patient is being motivated by getting a drug that may or may not benefit them.

DR. AISAR ATRAKCHI: Okay, any other --

DR. ERROL ZEIGER: Another thing about this is we're talking as if the Ames test data is written into law. Regulations can change. There's nothing to stop FDA from looking at the situation saying before you -- if you have a positive Ames, we want this additional information before we'll approve any clinical trials. That's the function of FDA's ability, not Congress or anybody else.

DR. AISAR ATRAKCHI: No, absolutely, but I mean you don't want to -- it hasn't been even

administered to, (indiscernible) first to anyone, so potentially that drug that was developed by the company, presumably, will have some benefit down the road. So we don't want to stop that and ask for a lot more information if -- unless, of course, we feel the need for that, we're not convinced with the information.

We think this is real finding, real toxicity. I mean, certainly, like Todd was mentioning, it's not only mutagenicity. If we have, in my division, if we have a variety of causes, brain lesions or cause -- brain lesion in the rat in a 14-day study, we are not allowing that rat to unless we have one mechanistic understanding. They need to do a lot more to show us that that finding either is reversible or it's not (indiscernible). So it's not just mutagenetic.

DR. ERROL ZEIGER: Yeah, but the discussion, as it's being directed, is strictly towards mutagenicity now. We're being asked to address the question, if we only have a positive Ames test. So, granted, you're talking about 14-day

studies. Ideal. You're not going to know from an Ames test if you're going to get kidney lesions or brain lesions or anything else, unless you've got a structural similarity to known bad actor drugs. So you just changed the question on us.

DR. TIMOTHY MCGOVERN: No, but I think what you're -- obviously, it's not written -- I agree, it's not written in stone. We can ask for additional information. But what we're looking to get input on is, when do we need to get that additional information? Can we wait until -- can we say, okay, single dose does not represent a significant risk to the patient, and get that information later or do we need it before we go and do healthy volunteer dosing at all?

DR. ERROL ZEIGER: I think part of the answer to the question is, so you're saying that maybe we shouldn't consider four, three, or two doses, only a single dose and then make a secondary decision.

DR. TIMOTHY MCGOVERN: I mean, it could be -- I mean, that's what was presented earlier is that some divisions allow the single dose and then ask

for additional information. Some divisions have been asking or allowing two doses and then in some more limited scenarios allowing up to four doses. That's where one, two, and four are coming from.

So it's really trying to get input from the panel as to, does one represent a significant risk or some number larger than one represent that risk and at what point should we really be asking for that followup information before making that decision --

DR. ERROL ZEIGER: And maybe the answer is, no dose [OVERLAPPING SPEAKERS] decide what significant risk is. If four gives you a risk, significant risk, does one give you one quarter of that risk?

TODD: I'm trying to remember what I was going to say. There was a time when I first joined FDA about eight years ago that if a drug came in (indiscernible) drugs that we look at or oversee are -- obviously, diabetes, endocrine related indications.

If there was any question, there was -- it was Ames positive, whether it's a blip or extreme

positive or just a mixed picture from the (indiscernible) profile, it'll go on hold without dosing, pending the results of the six-month transgenic study. And then it would be released from hold if there are no tumors and they go on with their lives.

It's very uncommon that we would see that situation anyway, but I'm wondering, does (indiscernible) to go back to that sort of paradigm or is the relationship between a positive outcome and a transgenic and a mutagenic or something that's clinically genotoxic or truly genotoxic? Is the connection that strong where we're not going to get falsely assured by a negative transgenic?

I'm always worried about that, so if it's a negative transgenic, go ahead. But is that really speaking to the cancer risk of whatever mechanism we're worried about in the beginning? Should we move back to that? I mean, the other element, of course, is from a very practical point of view, some -- if a sponsor comes in for developing a diabetes drug, if we have a question about its

genotoxicity, no, we're probably not going to let it go anywhere because we're just going to tell them to start over.

We don't need -- it's not exactly an unmet medical need, so I assume that the cases, scenarios are being discussed here, there's a clinical need for testing these drugs because otherwise why even bother? It's a non-starter.

DR. AISAR ATRAKCHI: Right, I mean, certainly we will -- as you know, we will weigh the risk versus the benefit. If this is -- like you said, if this is just another diabetes drug or it's another sleep drug but it shows this equivocal or even a positive Ames, we certainly -- likely we will ask for followup. Go tell us why this is positive.

But the question about what would you follow it up with, whether it's in vivo transgenic or something else, that's going to come up in the third or fourth question.

TODD: Okay.

DR. AISAR ATRAKCHI: But right now, I think since it looks like it's a weight of evidence

more or less, if it's -- we can summarize this, maybe, a little later, but that's why I said, the second question relates to the first one which is, if we are to allow this mutagenic DNA reactive drug to be administered to healthy subjects, would you allow this drug to be given continuously, meaning daily, or -- if so, for how long, how many days? Is 14 days, 10 days? Or if not, then would you allow to give it intermittently, let's say, with a washout period of, as mentioned earlier, you have five half-lives, so that the drug will be cleared and then you get a second dose.

DR. BOB BRASH: On the intermittent issue, I'd like to see Paul Brown come up here.

DR. AISAR ATRAKCHI: Well, we're going to hear --

DR. BOB BRASH: -- put you on the spot.

DR. AISAR ATRAKCHI: -- from the panel to see what they think and we'll move on to your questions.

DR. MIRIAM POIRIER: So I wanted to talk about dosimetry. Phase 1 clinical trials are for

determining toxicity, right? And they're often escalated, so mean, the dose makes the poison, so, I mean, we talked about 1.5 micrograms or 3 milligrams. What -- different doses -- different drugs probably need different doses for efficacy and to hit the toxic limit, so seems to me that the dosimetry should be somewhere in the calculation.

And I also wanted to bring up the third rail, which is human monitoring. I mean, as -- nobody wants to do it, I guess, but along with -- I mean, the safe harbor that we had in the past and everybody was afraid of looking at transcription that would be very sensitive and it turns out it's not, hey, we could learn and healthy volunteers probably wouldn't mind giving some blood or urine.

We'd find out what -- a lot about what's going on in humans that we can't otherwise determine just by -- we have the technology. It's there an it's improving, so I think we should at least consider it in the context of understanding what a new drug might be doing in humans, and in different humans with different metabolic capabilities.

DR. AISAR ATRAKCHI: So, can we move on to the second question?

DR. MIRIAM POIRIER: Oh, you wanted (indiscernible).

DR. AISAR ATRAKCHI: Sure.

DR. ROBERT HEFLICH: I like what you said, (indiscernible), because I think there is a role for human monitoring because we're going to make the decisions based on surrogate systems, rodents, and we're going to say okay, this positive Ames is mitigated by a negative in rodents in the cancer or -- transgenic cancer assay or a gene mutation assay.

But the question remains, there's a reason for that and it's probably related to inactivation of the chemical by some pathway or repair or something, but does the same thing happen in humans.

And, of course, this is not necessarily important to the healthy volunteers, but it could be healthy to Phase 2 and Phase 3 individuals if you get a surprise and the same thing doesn't happen in humans and you can, perhaps, determine that by, if you go

ahead with this drug, finding out whether there's a genetic toxicology signal in these volunteers that take this drug. Just a thought.

DR. AISAR ATRAKCHI: So I think we should move on to the second question, which I'm guessing is you may move fast, because we already have discussed some of it and the first question, so would anyone like to start with the Part A of the panel?

Would you give it for, I guess -- it's the same thing as we've been discussing if -- a lot of things to consider in order to give it, but I think it would be helpful if we can hear from you whether, would you give it on a daily basis for a short period of time or would you simply say, no, because of the results of the mutagenicity, let's just -- and maybe some other toxicology findings, that we're going to do it maybe twice or wait -- give a dose and then wait for five half-lives and then give it again?

Yes, please.

DR. ALAN BOOBIS: So assuming that the decisions we take (indiscernible) possible (indiscernible) in these Phase 1 studies --

DR. AISAR ATRAKCHI: Sure.

DR. ALAN BOOBIS: I think a starting point would be simply to fractionate the dose. I would argue Haber's rule and say that worst case -- potential worst case based on information we heard this morning as well, the really -- if you think you can give a single dose of X, then if you're going to give it, basically for multiple doses, it should be the appropriate fraction of those doses, unless they're going to be so widely separated in time that you could think they're separate, single doses.

DR. ROBERT HEFLICH: But for a PK stud, you wouldn't fractionate the dose, would you?

DR. MIRIAM POIRIER: You can have -- in general, it's a single ascending dose or it's once a day for maximum 14 days. That maximum -- Phase 1, more than 14 days, at least not in my division. So it can be on a daily basis for up to 14 days, but it's usually ascending. And it depends on the -- recently, we've been having drugs that are given only once a month, so maybe that is it. It's only one dose because they have a very long half-life, so you're not

going to wait for -- if the half-life is one week, you're not going to wait for four weeks in healthy volunteers to do the other one.

DR. ROBERT HEFLICH: But when you say a fractionated dose, you're comparing one good dose --

DR. ALAN BOOBIS: -- smaller dose.

DR. ROBERT HEFLICH: -- smaller doses that add up to that one big dose.

DR. ALAN BOOBIS: Yeah.

DR. ROBERT HEFLICH: But you'd never do a study like that, would you, for a PK study. Would you do an experiment like that?

DR. AISAR ATRAKCHI: (indiscernible).

DR. ERROL ZEIGER: Going along with this, if you're going along with (indiscernible), my first question would be, why are you doing this study. What questions are you -- specific questions are you asking?

DR. AISAR ATRAKCHI: Oh --

DR. ERROL ZEIGER: Is this going to be looking at clinical side? Are you going to be looking at --

DR. AISAR ATRAKCHI: This is a clinical PK, usually, and to determine what's the maximum tolerated dose in people.

DR. ERROL ZEIGER: In other words --

DR. AISAR ATRAKCHI: So the (indiscernible) dose for the patients.

DR. ERROL ZEIGER: You're looking for -- for a clinical size.

DR. AISAR ATRAKCHI: Yes, and a form of the kinetics and what dose will be the most appropriate dose to use for Phase 2 in patients.

WOMAN 1: (indiscernible) based on (indiscernible).

DR. AISAR ATRAKCHI: I mean, it's the PK.

WOMAN 2: -- not for the human side, but also in Phase 2 (indiscernible) the PK study (indiscernible) somehow we can (indiscernible) multiple doses, so (indiscernible) so it's really not that simple. (indiscernible) in vivo, and we cannot really -- for oncology patients, we cannot draw on that because patient have a lot of comorbidities.

We cannot -- so that's a rarity we have, (indiscernible) level. Even we have (indiscernible) study to provide this information, otherwise the (indiscernible) information. (indiscernible).

DR. ALAN BOOBIS: I think it's going to be easier to do a single dose PK study than to do a full Phase 1 study with -- looking for maximum dose with a (indiscernible).

MAN 4: One of the reasons to do the study is a food effect study under fasted and fed conditions, so that you like to use the same patients and -- the same subjects and give them two doses, maybe spread a couple weeks apart and it reduces the variability of the study.

Another one is the bioequivalent study that Bob Dorsam talked about today. It's better to do these studies in healthy volunteers. You reduce the variability and if you're using patients making a lot of -- the underlying disease may really affect the data that you get. So that's why this one dose versus two doses is important to us, is that impacts the

study design. Do you do -- for, like, the food effect study.

DR. AISAR ATRAKCHI: I don't know, I think you -- I'm sorry, go ahead.

DR. MIRIAM POIRIER: No, I was going to say, it seems to me that sequence, the first thing you need to know is what your PK is because then if you're going to have a washout period, you need to know what that is, so (indiscernible) your PK and then take it from there and decide on your second dose or whatever you're going to do, but I think it has to be in a specific order and sequence if it's going to make sense.

PAUL BROWN: Paul Brown, CDER. Since Bob told me to say something -- this issue about the intermittent versus not intermittent, presumably intermittent means for that space (indiscernible) doses of, whatever, two weeks or something.

I mean, if we believe Dr. Brash's, what is it, cancer cell loop (indiscernible) hypothesis, then I'm not sure intermittent is any safer than doses right next to each other and you could also come up

with a hypothesis that maybe intermittent is actually less safe because now, you're fixing mutations and then you -- in the second dose, you now cause a mutation in that exact same cell.

You get that second hit and you build them up over time rather than killing them all off with your dosage right at once, so I mean, it's an interesting thing to think about. I think we think about it for other toxicities, right, where you do recover from them. Okay, you can separate the doses, recover from that tox, but maybe it's a little different issue here.

The other -- I think the other point that was made about dose is important. I mean, as Tim pointed out, we're already doing this. We already accept mutagenic compounds in health subjects. It's the impurities. Now, they're very low levels. But if we just think it's a hazard (indiscernible), why do we do that? Obviously, we think there is a dose response, that there is -- you know, (indiscernible) with a straight line, whether that's right or not, with risk and dose and so on, so can we come up with a

dose that's low enough that we'd be okay regardless (indiscernible) TTC?

It probably isn't going to be a useful dose in most PK studies. The reason to have the microdose studies is because there are sensitive assays now for looking at PK. Sometimes they can do the microdoses and get those answers.

DR. AISAR ATRAKCHI: But didn't you, Paul, with the impurity doing the QSAR as M7 clearly says, up to 1 milligram, you can do all of these. So once the impurity is above 1 milligram, then they're going to go to (indiscernible) where they have to do general tox study. They have to do the chromosomal abrasion and so forth, with other caveats in there.

So I think, like we have discussed internally, it's really the level of comfort with an impurity versus an API. We're okay with the impurity for a QSAR and not doing the test, but we're not that comfortable when you're getting the drug in milligram -- at least a milligram dosing. So I think there's a little bit of a distinction and that underlines the reason for M7 does not apply to APIs.

PAUL BROWN: Although, if you add the same doses --

DR. AISAR ATRAKCHI: Right.

PAUL BROWN: I mean, if anything, you'd be okay with the API.

DR. AISAR ATRAKCHI: Exactly.

PAUL BROWN: In a higher dose. Yeah.

DR. ALAN BOOBIS: But just to be clear, the (indiscernible) toxicological (indiscernible) of 1.5 is based on cancer data not gene toxicity. In other words, what he says is, if I've got a compound I suspect, I know, is an Ames positive, I -- we will be at this end of the distribution of carcinogens in potency. And so it can permit up to 1.5 micrograms per day with a reasonable assurance that (indiscernible) over one in a million risk.

So it's not based on gene tox potency. And I made that point because several groups, including Health Canada are trying to find a surrogate for the cancer (indiscernible) data in gene tox assays and as Errol has pointed out, what we know is the Ames test is not the answer and they're looking at various

in vitro and in vivo assays.

They think they're (indiscernible) an in vivo assay. There might be a suitable surrogate for that purpose. We don't know what the distribution of the values is, so we don't know where the TDC would be if you base it on a gene tox end point, but it will probably be -- you could probably get away with a more potent compound than for the carcinogens.

DR. TIMOTHY MCGOVERN: Yeah, I think at this point when -- especially first in human trial, we're using the Ames as a (indiscernible) predictor for carcinogens, if we get to a point where we had a marker from an Ames assay to give us a reasonable estimated carcinogenic potency, that would be great.

Then we can go at it in a more educated way, but unfortunately, now we assume it's a carcinogen if it's Ames positive until shown otherwise and it's really, what further -- is that okay for one, two, et cetera dose and, as Paul was saying, at what -- how high a dose can we give, as well.

DR. AISAR ATRAKCHI: So any further discussion on -- I think we got that there is not

plans -- if I can summarize, I don't think there was -- there is any summary of this, whether you can give it continuously, daily, for an Ames positive. I heard that it's better if you break it down, to fractionate the dose, but again, it's similar to the microdosing.

When you give the drug that it's a new molecular entity at a microdose, that's a below the pharmacologic effect. So I'm not sure how valuable that, which may be that's the reason we have not see many companies coming in with microdosing clinical trials. So from a practical point of view, I'm not sure that would work.

So I'm not sure we got an answer to this, but maybe it relates to the first question, that it's all a weight of evidence type of thing. But I did hear from Mr. Boobis that if it's a clear positive, you will not like to see that drug administered.

DR. ALAN BOOBIS: Unless there's a very good argument.

DR. AISAR ATRAKCHI: Unless there is very good argument, which is, actually, the opinion of

Health Canada based on our assessment of how they do things. They do need -- if it's clearly Ames positive, they will not allow that drug to proceed unless it has some followup studies in vivo that shows it's irrelevant, the finding.

So I think let's move on to the third question. So now we're -- for generic drugs. As you may know, they already have -- they usually reference to the drug that's already approved, the innovator drug, which they follow the label. There is a full battery, generally speaking, of the gene mutations as then there's a (indiscernible) study, if the drug is administered chronically.

So they have this information already. So they're -- but they still need, in order to develop a generic drug, they still need to do a bioequivalent study in healthy people to show that the innovator and their -- and the generic drug have similar or comparable (indiscernible) PK data.

So the question becomes, should (indiscernible) evidence a product be used to decide whether a compound should be tested in a bioequivalent

study in health subjects? If yes -- and again, this is assuming that it's in the label, it says it's a mutagen.

Already we know, and presumably we're talking, again, about the gene -- about the mutation, Ames test plus the other tests, the full battery; if yes, which test results should receive greatest consideration and the weight of evidence? Are there any other factors relating to genetic tox that should be considered when determining if that study should include healthy subjects and bioequivalent studies?

And I open it for discussion.

WOMAN 3: How long are the bioequivalent studies?

DR. AISAR ATRAKCHI: Couple weeks, max. same thing.

DR. BOB DORSAM: (indiscernible) say around four doses. It's within that range.

DR. AISAR ATRAKCHI: Okay. So --

DR. BOB DORSAM: There are others where (indiscernible) focus on something (indiscernible).

DR. AISAR ATRAKCHI: So it's a simple -

- it's four doses, 14 days. Yes. Could be continuous or not. Anyone from the panel, please. Dr. Boobis.

DR. ALAN BOOBIS: Yes is the answer to Part A, one, before Part A. Should weighted evidence of course be used? I think yes.

If yes, which test results would receive greatest consideration? I would argue that the appropriate followup for the in vitro positive, so if it's in vitro Ames test positive, you need -- you would like to see an in vivo gene mutation followup, not just a micronucleus test or (indiscernible) test and then I don't know about the determining whether I should healthy subjects. Depending on the answers to the first two.

DR. MIRIAM POIRIER: I just have a question. Do they always analyze the generic? In other words, can you assume that chemically you're talking about two products that are exactly the same? So, somebody comes along with a generic, you already have a brand drug on the market. Do you (indiscernible) analyze the generic that you're given so you know it's the same or not?

DR. BOB BRASH: So by regulation, the generic must have the same API as the (indiscernible) listed drug, must be the same exact chemically.

DR. MIRIAM POIRIER: (indiscernible).

DR. BOB BRASH: The same identity. The same (indiscernible).

DR. MIRIAM POIRIER: Okay.

DR. BOB BRASH: I wanted to ask a followup question to doctor -- so a positive Ames assay would an appropriate followup be the potent carcinogenicity study?

DR. ALAN BOOBIS: No, because there are other effects that can be a consequence of mutation -- gene mutation. So that would be part of the weight of evidence, but I think you'd also want to satisfy yourself that it's not in vivo (indiscernible) as well. Because mutagenicity is an end point and so is in vivo.

DR. BOB BRASH: Okay. In vitro mutagenicity is the first signal?

DR. ALAN BOOBIS: Yeah.

DR. BOB BRASH: Second step, in vivo

mutagenicity?

DR. ALAN BOOBIS: Yes. And if you've got the cancer (indiscernible) assay, that would be very useful confirmatory (indiscernible).

DR. BOB BRASH: (Indiscernible).

DR. ALAN BOOBIS: Yeah.

WOMAN 3: I already have -- right, Bob? We already have the answer -- the results of the cancer, of the (indiscernible), right?

DR. BOB BRASH: Yes.

WOMAN 3: Yeah.

MAN 5: If it's chronically administered.

DR. BOB BRASH: If it's chronically administered.

MAN 5: I mean, there are two things here. One is that technology moves on and we have tests today, like the transgenic rodent assay that we didn't have five or 10 years ago, and so depending on when the innovator drug was approved, you may have less information now than you would, had it been approved more recently.

So that's part of the weight of evidence that Alan is talking about. But one of the things I'm wondering about is at some point, somebody at CDER made a decision that here's a mutagenic API and we are going to allow it in patients, so they have been through a process of thinking through exactly what is the risk.

We think it's okay. Why is that not -- why can't you use that thought process to say, here is the risk for one to 14 doses to a healthy volunteer, it does or does not meet our standard for safety for a healthy volunteer?

What's changed since you approved the NDA for (indiscernible)?

DR. TIMOTHY MCGOVERN: Well, I think part of it is, the drug is approved for a disease indication and so depending on the severity of that indication, so it may be fine for 14 days, may be fine for chronic use in that particular indication, but I would argue it's a different scenario -- if you're going back to healthy volunteers for a bioequivalent study, that's a different risk/benefit assessment.

And then, just going back to that issue of what's -- the scenario if you have a positive mutagen but you have your standard to your carcinogenicity assays could include a transgenic assay as well that was negative.

From the CDER standpoint, we -- I think we would generally say that's sufficient for addressing potential carcinogenicity, but if that were -- we're using the gene tox battery for, I know EPA, other organizations, also use it for heritable changes as well, so, but we do have the reproductive tox battery as well. So I think from -- if I had the positive mutagen and somehow it got all the way to approval and that positive -- carcinogenicity studies were well conducted and negative, that would be my key piece of information I'd be looking at.

DR. TIMOTHY ROBISON: We had a (indiscernible) where it was a positive Ames metabolite. We allowed the drug to go forward in patients for a short period of time, 28 days. And this is several years ago. Prior to exceeding 28 days, they had to have a six-month (indiscernible)

carcinogenicity studies.

DR. AISAR ATRAKCHI: But not -- you didn't allow it in healthy people.

DR. TIMOTHY ROBISON: No. (indiscernible) where there was a (indiscernible). I mean, today, it might look more towards an in vivo (indiscernible).

MAN 6: The policy or practice of allowing some of those studies in healthy volunteers is not really based on any risk/benefit data, that -- this is about 10 to 12 years ago, where (indiscernible) policy assessment and that it was just the belief that, well, there's a threshold of single doses, not likely to do harm, and that we needed to not -- to aid drug development.

So it was more of a practical determination rather than any risk/benefit determination.

MAN 7: Thanks for that, John, because this confuses me a little bit and Dan's point, weight of evidence has already been done with the reference listed drug, whether it's a (indiscernible) we

administered or even if you don't have (indiscernible). It was approved with the innovator with a weight of evidence conclusion of what carcinogenic risk already is. So now you're talking about a generic that comes along.

You should be able to take that weight of evidence -- you should know enough about the innovator to say, is one dose or two dose or three doses cancerous. The data should already be there. I certainly wouldn't advocate doing additional or new studies to try to determine, well, was that old weight of evidence wrong?

Is it actually a carcinogen? Because now, you're screwing around with the innovator, too. (indiscernible) something.

MAN 6: Can I clarify this?

MAN 7: Yeah, sure, because I'm a little confused by this.

MAN 6: The studies done in healthy volunteers with the innovator may not have been done in the United States, and so we may not have weighed in on the risk/benefit determination. Now, the

generic comes in and wants to do a study in the United States that changes the paradigm.

MAN 8: You do also have the opportunity to do some post-market surveillance and ask if there's any data on cancer in patients who have been taking the drug, which is something that you could never for (indiscernible) entity.

DR. AISAR ATRAKCHI: We can. We have to have a good reason to ask for it. [OVERLAPPING SPEAKERS].

MAN 9: We're also trying to protect your patients, because you have -- you now have a drug of higher risk that you know you cannot address in the normal drug development process, but you have data in the patients in the United States who've been taking the approved drug and you can ask, is --

DR. AISAR ATRAKCHI: (indiscernible).

MAN 9: You can ask. I mean, this is the hot topic de jour of Health Canada and a lot of the genetic toxicologists is whether mutagenicity is in and of itself a risk factor and so that's part of the current scientific debate, if you will, of our

time and so now, when you get a generic drug which is Ames mutagenic, you can answer that question because you have access to the data.

DR. BOB BRASH: Just to circle back on kind of (indiscernible) question about that weight of evidence and going back and reevaluating the -- I really wouldn't go back to reevaluate mutagenicity or carcinogenicity (indiscernible) for sure. That's left to the NDA. We would go on the given information in the RLD label. We would go further in characterizing the safety of the impurities in the generic formulation.

For example, if they were not present in the RLD, so it's those things outside of the RLD that we might characterize the safety of to make sure that they're in bounds, but we wouldn't further the API safety (indiscernible).

MAN 10: I just wanted to add --

DR. AISAR ATRAKCHI: Identify yourself. Name.

MAN 10: Oh, sorry. (indiscernible). I'm (indiscernible). I just wanted to add that the

bioequivalent studies done (indiscernible) are to advise the viability of generic drugs for the API and the reason is that (indiscernible) changed so the (indiscernible) of drugs my change (indiscernible) the toxicity of API. They're looking at the bioequivalent (indiscernible) difference in the (indiscernible) drugs.

DR. AISAR ATRAKCHI: So for Part A, which test results should received the greatest consideration and the weight of evidence? So would we follow up with another mutation test or would we follow up with (indiscernible).

DR. ALAN BOOBIS: I mean, I'm assuming you have the data.

DR. AISAR ATRAKCHI: That's true. We do.

DR. ALAN BOOBIS: Yes.

DR. AISAR ATRAKCHI: So --

DR. ALAN BOOBIS: So you're looking to see if there was an appropriate followup of any positive.

DR. AISAR ATRAKCHI: Correct.

DR. ALAN BOOBIS: Because I assume what's why the question was asked in the first place.

DR. AISAR ATRAKCHI: Right.

DR. ALAN BOOBIS: So there's a positive somewhere in vitro. You'd want to see what was the followup on that, which would contribute to your conclusion. I mean, as I've said, if you've got a bioassay, which you should have on the innovative drug, then that would also be substantial evidence it was a clean bioassay and a clean followup, then you see, well, I don't see there's any real concern of a Phase 1 study or bioequivalent study in healthy volunteers.

MAN 11: (indiscernible), CDER. Actually, I saw one consult to the (indiscernible) for generic drug, so the results are (indiscernible) toxicity is a little bit (indiscernible) and then a followup study, like, (indiscernible), that was clearly positive (indiscernible). And then the (indiscernible) toxicity study was in that case, however, the two-year (indiscernible) study is clearly (indiscernible) complications.

So the label is more or less misleading folks, (indiscernible), because the label claims that based on (indiscernible) evidence, it's not a mutagenic. So (indiscernible) little hard for the generic drug. It's hard, like API (indiscernible), do you consider safe (indiscernible).

DR. BOB DORSUM: So you recalled a very interesting case. Typically, with generics, in order to inform how generic applicants should develop their drug, OGD, the Office of Generic Drugs, will post a product-specific guidance out there that's available for all applicants to see and it will inform how to do the trial, and is that trial that I mentioned on the slide (indiscernible) include healthy subjects, include patients only.

So in order to come to that determination currently, what the reviewers will do is go back and take a look at what is the overall profile of safety from that reference listed drug (indiscernible). So that may be, in a sense, looking for whatever signals may exist, it is certainly not solely limited to gene toxic carcinogenicity

information.

I'm actually posing some of these questions here today so that we can increasingly use triggers from that drug label to initiate (indiscernible) so that we can use the weight of evidence more appropriately. So currently, we're kind of trying to mirror what was done before, and that may be sufficient in many cases.

But in cases where we have more information that we could use better, that's what we're trying to do and aiming to do, and so that's where the questions are coming from and so I don't know exactly how that product-specific guidance was for that product, but my guess is that it was evaluated according to the prior development program and looked at by the clinical discipline to make that assessment, according to the various present safety signals for the product.

DR. AISAR ATRAKCHI: So do -- since you're up there, Bob, do you think we have enough comments to answer this question?

DR. BOB DORSUM: Yes. I think that 3B,

the question of are there any other factors relating to generic toxicology, in intentionally left that open as a question because, of course, we have to answer these questions -- these sorts of questions, do some thinking on it frequently.

So I had thought maybe weight of evidence would be appropriate. I thought maybe the rodent bioassay might be reasonable to use. But I really wanted to also leave it open for our expert panel to say, you know what, yeah, there's rodent bioassay but what you should really be attempting to do is this other thing.

So I'd like to leave it open. Is there anything that we should consider as a trigger for, let's look at this further, that we haven't already talked about here today?

DR. TIMOTHY MCGOVERN: The only thing that comes to mind for me is if, say, you had an Ames positive that was also positive in carcinogenicity assay and the sponsor, as part of the overall package, went forward and conducting various studies to show that that positive finding and carcinogenicity study

is not related to the mutagenic positive.

So that could possibly build an argument to say, you have a threshold -- say, might be more based on pharmacology, so you may have a threshold effect in place that could potentially allow dosing in healthy volunteers just because that positive carci finding wasn't tied in with the positive gene tox.

DR. BOB DORSUM: Thank you. So that gets more towards, consider your margins, consider the mechanism, science-based decision making if it is a positive, is it relevant.

DR. TIMOTHY MCGOVERN: Right. And it could be a question of how -- the label may just say, positive gene tox, positive carcinogenicity. May not have any of that underlying information that actually supported approval.

DR. BOB DORSUM: That's right.

DR. TIMOTHY MCGOVERN: Yeah.

DR. BOB DORSUM: But fortunately, there are some cases and labels that clearly state where, perhaps, there is a signal of what are those margins

and sometimes the labels are (indiscernible) descriptive in ways that are very informative, so --

DR. TIMOTHY MCGOVERN: Yeah.

DR. AISAR ATRAKCHI: But if you do have -- if you do know from the label of the individual drug that it is -- caused mutagenicity and it was positive in carci study, would you still be comfortable using healthy volunteers or at that point you will not to the bioequivalent study in healthy patients -- healthy subjects but you will go to patients?

DR. BOB DORSUM: Positive carcinogenicity --

DR. AISAR ATRAKCHI: Yeah, positive carcinogenicity and positive -- right. Well, Ames or the (indiscernible) or if they did the two-year bioassay on the rat and they did the transgenics in mice, it’s positive.

DR. BOB DORSUM: Well, then data are mounting towards, we need to be very cautious with this and we would have to look at the mechanism (indiscernible).

DR. AISAR ATRAKCHI: So you (indiscernible) give it to healthy subjects (indiscernible)?

DR. BOB DORSUM: That's not what I said.

DR. AISAR ATRAKCHI: You said you would look at other things, right? But --

DR. BOB DORSUM: Well, safety is a very -- it's across all end points. It is not just (indiscernible).

DR. AISAR ATRAKCHI: Correct.

DR. BOB DORSUM: So that's what I mean by that. But that's mounting evidence towards, there's risk there for healthy subjects, that that would be --

DR. AISAR ATRAKCHI: So you'd still consider doing the healthy volunteers, all this mounting positive gene tox and carci?

MAN 12: Can you (indiscernible) as well?

DR. AISAR ATRAKCHI: So I was wondering, now you have all the data you wanted to

decide. Are you going to say no to healthy volunteer study at this point because the gene tox data and positive carci study, or you still look at other data, other mechanistic study or dose ranges or any other evidence to show it's not -- it's no harm to healthy volunteers? I didn't get your response yet.

DR. BOB DORSUM: Okay. I think I'm just being too indirect.

DR. AISAR ATRAKCHI: Because, I mean, I think we're (indiscernible) answer the other question, so you do have -- this is for generic, so the drug's already approved. It's in the label. That says this drug is mutagenic and carcinogen. Right? And now, it comes to generics and you want to do the bioequivalent study in healthy subjects.

You have that information. Would you -- because here it's asking, are there any other factors relating to genetic tox that should be considered when determining if the study can go in healthy subjects. So if you know those two end points, mutagenic and carcinogenic, would you still -- what else would you want to have --

DR. BOB DORSUM: I don't think that you can really ask for more information. I think (indiscernible) or you can try to (indiscernible), but it is data that are concerning at that point. I'd be interested if the panel would suggest that there are other information that we should be more thoughtful about, so those data in and of itself, do raise (indiscernible).

DR. ERROL ZEIGER: Well, presumably, if you're talking about later date, you hopefully -- you may have a lot more information about the clinical effects of that particular substance or that class of substances. You may also have, as was mentioned, more information about the mechanisms of carcinogenicity and how irrelevant that mechanism may be for humans as opposed to compared to rats or hamsters.

So, which means, you might have to go back and reassess the initial chemical, but it gives you some guidance as to what data gaps you may have for the equivalent and are there any areas of knowledge that are now relevant to your question that may not have been relevant 10 years ago when it was

first approved.

DR. AISAR ATRAKCHI: Yes, this was exactly what I was going to say. I mean, the drug is approved. It's been on the market for a while and now it's coming as a generic, so -- and it's positive with the Ames or any other genotoxic, it's positive with the carci, so this must have been for a serious indication. So probably it was given to healthy volunteers when they started with the actual drug, so I would go to the original NDA and see what happened, did they give it to healthy volunteers, how many doses, and just go from there.

WOMAN 4: But healthy volunteer study may have been conducted without the (indiscernible) point.

DR. AISAR ATRAKCHI: Yes.

WOMAN 4: So now, you have a different stage to decide.

DR. AISAR ATRAKCHI: Yeah, we do have the carci data but it's probably for a serious indication. If it wasn't for a very serious indication, I don't think we would see it, right?

DR. BOB DORSUM: My FDA label (indiscernible), but there's some pretty interesting profiles for gene tox and carci out there.

Do the search, and these are things that are sometimes either available over the counter, there are things that are commonly used mainstream, and there are these results that are either positive or negative in one study or another and I just want to make sure that we're asking the experts the right way to navigate those because sometimes a positive is popping a way that's seemingly problematic when we do have 10, 20 years of apparent mainstream usage (indiscernible).

What about the signals (indiscernible)?

DR. ALAN BOOBIS: Can I just ask for clarification? When you say there's some positives, do those positives always include an Ames test? Are you talking about somewhere in the in vitro gene tox battery there's a positive which could be carcinogenicity or aneugenicity but not gene mutation as well? I mean, alternatively.

DR. BOB DORSUM: Right. There are some

that are positive Ames and then end up being negative in carcinogenicity, for example.

DR. ALAN BOOBIS: But are there some that are negative in Ames but positive in other in vitro gene tox --

DR. BOB DORSUM: Yes.

DR. ALAN BOOBIS: Because the reason I'm asking that is that I don't think that all in vitro gene toxicity is equal. I think the interpretation of a positive Ames is a little bit different from the interpretation of a positive aneugenicity assay or even a (indiscernible) exchange assay.

DR. AISAR ATRAKCHI: Yeah, we're going to get -- that's question four.

DR. BOB DORSUM: I'm looking forward to that answer. Thank you.

DR. ROBERT HEFLICH: So presumably when the reference compound was approved, you didn't do the transgenic gene mutation assay and that would be a new piece of information that might inform the decision over and above the cancer (indiscernible).

DR. ERROL ZEIGER: Yeah, what I was going to say is the various in vitro tests are not complimentary. If something is positive in Ames and it's not a carcinogen, it's more likely to be positive in the other in vitro tests as well because we're not measuring carcinogenicity in these tests. We're measuring DNA reactivity or chromosome reactivity.

So the fact that it's positive in Ames and also positive in in vitro micronucleus or mouse lymphoma? doesn't add anything to the weight of evidence that, wow, it's positive in three tests as opposed to one; therefore, it's more likely. It doesn't work that way. And for a number of the in vivo tests, they're not -- and I don't know the data -- the current data for the transgenic or the PIG-A, but as a rule, they were not as sensitive as the in vivo -- as the in vitro.

There are quite a few mutagenic in vitro carcinogens that are negative in the bone marrow assay, for example, and bone marrow micronucleus assay.

So a positive in the in vivo assay may

add some feel-good assurance to the positive in vitro, but a negative in vitro -- in vivo assay doesn’t detract from the predictivity of that positive Ames test and there are a number of publications on this from the NTP database and from larger databases that show the in vivo bone marrow assay is not that sensitive and not -- it doesn’t correct the negative Ames assay all the time.

I’m using the word “correct” as providing the right concern.

DR. ROBERT HEFLICH: I think the lack of sensitivity, though, (indiscernible) based on target exposure. I mean, as far as any end point (indiscernible) into that, but you’re only measuring bone marrow.

DR. ERROL ZEIGER: Yeah, exactly.

DR. ROBERT HEFLICH: If it doesn’t get to the bone marrow, you’re not going to see anything.

DR. ERROL ZEIGER: Exactly. But another concern I have for in vitro assay is that (indiscernible) by the NCTR data on acrylamide, is where you’ve looked at a number of tissues for

mutation and looked at a number of tissues for cancer and the mutation tissues to not -- tissues that show mutation do not always show tumors and the tissues that show tumors do not always show mutation.

So there is a disconnect there, some sort of mechanistic disconnect that we don't understand.

DR. ROBERT HEFLICH: Yes. Well, the next step is to measure the mutations that are actually relevant to the end point, which means measuring cancer-driving mutations which I'm not suggesting that you do, but I mean, the reason why you get expanded call maybe a data or mutation or it may be promotion of a particular preexisting clone, and both would be relevant to carcinogenicity.

DR. ALAN BOOBIS: Could I just clarify? As I understand it, genetic toxicology community do not consider an in-needle bone marrow micronucleus assay as an adequate followup of a positive Ames in vitro. They are talking about different set of assays and we have come across this and we're writing the guidance on it now for WHO; we should make this very

clear.

DR. AISAR ATRAKCHI: Well, (indiscernible) micronucleus as a followup to the Ames.

DR. ALAN BOOBIS: Yes.

DR. AISAR ATRAKCHI: Yes. I mean, it's a different end point.

DR. ALAN BOOBIS: It's a different end point.

DR. AISAR ATRAKCHI: Right, it's a different end point and you need to follow up, which, we'll get to that question. You need to follow up at the end point. If it's a mutagenicity in Ames, you need to follow it up with a mutagenicity test.

MAN 13: I have a very simple question to all the expert panelists regarding mutagenicity and carcinogenicity. Do we have a list of the two-year study tumor list from rat, from mice that we know this to be positive, if they are relevant to human? Can you tell us what they are?

DR. ERROL ZEIGER: Well, I'll ignore that last half question, but through the NTP database,

going onto the NTP bioassay database, I think they can -- you can break it out by tumor type because we've done it -- we did it a few years ago for mesothelioma. You could identify all the chemicals that produce mesothelioma and then look in the database to see if they were Ames positive or Ames negative.

This doesn't go to the relevance, whether this particular tumor is relevant to humans, that's another issue and that's going to be decided by the people who know rodent tumorgenicity and know human cancer because there are certain -- for example, the Zymbal gland carcinoma, I think it's in rats, we don't have a corresponding Zymbal bland. If something's positive just in the Zymbal gland, it's called a carcinogen.

So I don't know how to bridge that gap at this point. You need to get the pathologists from both disciplines talking to each other.

MAN 13: And I believe there's one (indiscernible) cancer is not relevant to human.

DR. ERROL ZEIGER: Well, it depends on -- I think it's a mouse or rat thyroid cancer,

depending on the particular cell type, that's considered not relevant to humans. But then again, there are good mutagens that only produce thyroid cancers. Is that relevant for humans? Only produce thyroid in rats or mice. Is that relevant to humans, because they are genotoxic.

A lot of the thyroid carcinogens are not genotoxic, so --

MAN 13: I assume somebody should have complete list of those.

DR. ERROL ZEIGER: Those lists are available from the NTP studies and you may be able -- even able to get them through the IR, if there are IR compilations, that let you search across (indiscernible).

MAN 13: Thank you.

DR. ALAN BOOBIS: Can I --

DR. AISAR ATRAKCHI: Yes.

DR. ALAN BOOBIS: I just wanted to say, it's not as simple as a list of tumors, types. It's also, has to include mode of action. It's quite clear hepatocellular carcinoma can occur in humans from

certain chemicals that do it in rats. But it's also true there are many chemicals that cause hepatocellular carcinoma in rats by a mode of action that's totally irrelevant to humans.

The same with kidney. (indiscernible) rat's kidney is a mode of action that is irrelevant to humans, but we can get real tumors by other mechanisms or modes of action, so you have to look at tissue and mode of action.

DR. ROBERT HEFLICH: I think the issue of using rodent carcinogenicity data to make decisions is somewhat problematic. I mean, but it's the best we have.

Of course, we know there are differences between rodents and humans as far as their mechanisms of carcinogenesis and just to go back to cancer driving mutations, I mean, there are different sets of cancer driving mutations in rodent than human -- tumorgenicity, finding those that overlap and relate to one another is the trick for making the rodent carcinogenicity assay truly predictive of human cancer.

I think that's where there's (indiscernible).

MAN 13: I just wanted to follow up (indiscernible) for you, sir. I assume when we make a decision, whether that's relevant or not, depends on the mechanism of action, correct?

DR. AISAR ATRAKCHI: Yes.

MAN 13: But we might not know, are there mechanism of action that we don't know. Isn't that true?

DR. ALAN BOOBIS: Yes, and you're not going to assume, therefore, on the side of caution that if we don't know that it's potentially relevant. That's just the way risk assessment works.

DR. AISAR ATRAKCHI: So if -- we should move on to question four. Moving a little bit away from mutagenicity and the question is, certain drugs may be clastogenic but not mutagenic. Should consideration be given to the mechanism of action and genotoxicity, in designing studies is healthy subjects?

We have talked about this but I think

the questions is here, so I'd like to hear from the panel.

DR. ERROL ZEIGER: The nice thing about clastogenicity is that it's something you could easily monitor in human subjects as opposed to gene mutation which is not as easy to monitor.

DR. AISAR ATRAKCHI: What do you mean, you can do it in humans?

DR. ERROL ZEIGER: Well, you draw the blood from somebody and you essentially can look for chromosome damage in the white blood cells or --

DR. AISAR ATRAKCHI: We have tried to do this over the years, but it's not a very -- sponsors don't like to do that, for liability issues because, especially in healthy -- well, it's actually neither one, healthy or patients -- if their test becomes positive, the result is positive for chromosomal aberration, what's the obligation of the sponsor to inform or not to inform that patient or that subject?

So it is difficult to do. Otherwise, even lymph (indiscernible) test can be done and -- but

it’s not done. I think that’s a little bit -- ideally, yes.

DR. ERROL ZEIGER: Well, there is enough data to show that an increase in the peripheral blood micronucleus or chromosome aberration is associated with increased cancer risk. So to answer this question, I don’t see, if you have a scientific -- forget about the legal, the other thing -- if you have a scientific way of simply answering the question, is there an increase in chromosome damage in patients, then that essentially addresses your question here.

DR. AISAR ATRAKCHI: Sure.

DR. ROBERT HEFLICH: You can measure -- excuse me -- PIG-A and HBRT in humans --

DR. AISAR ATRAKCHI: Right.

DR. ROBERT HEFLICH: -- correctly, although the end point is not validated the way -- same way the micronucleus and the chromosome aberrations were, respect to the kind of study. I mean, that was an incredible undertaking to do that.

DR. KEVIN PROHASKA: I’d like to add to

that last point that was made. I'm looking to go about being able to monitor for something. It's not my area of expertise, but if there's a reasonable way, a reliable way of monitoring for an adverse event, it ought to be included in the safety mitigation plan for the study.

And with regards to the liability that sponsors may have for finding these problems, it's hard to have much sympathy on that, I'm afraid. You guys, there is not only just a legal liability to identify these problems but a moral liability. They really are (indiscernible) they've identified some serious adverse event they should be aware of.

DR. ROBERT HEFLICH: Well, that's always been the pushback, the liability problem.

DR. AISAR ATRAKCHI: I mean, I just --

DR. ROBERT HEFLICH: -- industry is (indiscernible).

DR. AISAR ATRAKCHI: I guess one way around that is probably to collect -- especially in healthy subjects -- you can collect the blood from any -- X number of subject, therefore, you're not going to

really know -- as long as they're healthy, you're not going to know the blood belongs to who and you can do the study that way.

DR. ROBERT HEFLICH: There should be a way of knowing whose blood is whose, you know, I would hope. Yeah, so (indiscernible) conversation.

DANIEL LEVY: A followup comment for -- question for -- (indiscernible) from Biogen. (indiscernible) going back to in vitro testing with (indiscernible) NIEHS.

So your comment about monitoring patients for, say, increasing their chromosomal breaks and lymphocytes or micronucleus in the blood, but isn't that a population-based analysis in that you can't be precise for each individual whether their increase in micronuclei will be a liability. Is that correct?

DR. KEVIN PROHASKA: Well, it's not my area of expertise, of course, but that's why I added the caveat, if it's reliable and actionable (indiscernible). So if there's some reason to believe that the information is not reliable, then there could

be an argument for not informing people, but if it's reliable, people ought to be informed.

DR. ERROL ZEIGER: Actually, everything we've been talking -- all the numbers we've been throwing around about genotoxicity and its predictive value, that's in a way, all population based. It's all retrospective study. The fact that 75 percent of the salmonella positives are rodent carcinogens doesn't tell you what that -- what this chemical today that's positive, whether or not it'll be a rodent carcinogen. Leave it at that.

DAN LEVY: You can -- this is Dan Levy again. You can design a study where you take samples before and after administration of the drug and see if there's an increase in micronuclei and I think most of us would think that's a pretty reliable way, if it's positive, of saying there's a pretty high risk of clastogenic damage in that individual.

I will remind you that the micronucleus assay, while it, in terms of correlation of rodent micronucleus results and rodent cancer results it's extremely specific but very insensitive. That is,

many compounds including Ames positive compounds that are carcinogens, are negative in the rodent micronucleus assay.

In human micronucleus assay, there are a lot of studies in the literature of biomonitoring of people who are exposed to a variety of known clastogens and known carcinogens and, for example, cigarette smoke is known to have a variety of both mutagenic and clastogenic compounds in it, but most micronucleus studies of cigarette smokers do not find an increase in peripheral blood micronuclei.

It’s simply not sensitive enough to detect it in people who are exposed to what we know is an environmental carcinogen -- an environmental genotoxic carcinogen. So getting a positive result in a patient or a healthy volunteer in the micronucleus assay, I think, would be a very strong indicator of risk and I think very actionable and I -- considering relative noninvasiveness and inexpensiveness of that test, I don’t understand why you wouldn’t be doing it.

But a negative is not as definitive as a positive result, which is fine. I mean, in some

ways, you want to get the most potent things. You want to remove the most potent risks, and if you had a relatively simple and reliable test to get rid of some of them, you can consider using it.

DR. ALAN BOOBIS: I think this is -- I have some thoughts on it, but I think this doesn't really get to the answer to question four. But before I do try and answer question four, I agree what Dan said, but the fact is, I suspect -- I know of no study of that type. Do you? Do you know of that --

DAN LEVY: I'll send you a list.

DR. ALAN BOOBIS: Sorry?

DAN LEVY: I'll send you --

DR. ALAN BOOBIS: In which they gave -- they did the study in a group of healthy volunteers before and after --

DAN LEVY: Oh, no, no, no.

DR. ALAN BOOBIS: No, I know (indiscernible) studies in populations as far as -- but I suspect the reason they'd be doing C-positives is because pretty well nobody is going to get a drug which is going to cause a positive micronucleus test

in a patient -- in a volunteer. That would have to be a pretty good genotoxic, and to do that.

DR. ROBERT HEFLICH: It was done with AZT.

DR. ALAN BOOBIS: Sorry?

DR. ROBERT HEFLICH: It was done with AZT.

DR. ALAN BOOBIS: Yeah, but that's a particular compound, particular class -- group of patients. I mean, the sorts of drugs we're talking about here today, which is the present forms of that. But that's -- I mean, this is (indiscernible). I think the answer to question four is, very much so.

I would want to know about the mechanism of action, so how does that compound cause a non-gene mutation, genotoxic effect because we know of many modes of action or mechanisms which are thresholded and would not translate into a significant risk at the sorts of exposures we're talking about in these clinical trials.

There are others that would, but there are many that would not. So that information would

really help moving forward.

DR. AISAR ATRAKCHI: Thank you. I just want to say, AZT pretty much does it always.

DR. ALAN BOOBIS: Yeah.

DR. AISAR ATRAKCHI: It's a transplacental carcinogen. It's also a clastogen. It's also a mutagen. So it doesn't... Okay.

DR. ROBERT HEFLICH: So just to follow up on Dan's, the problem with cigarette smoke is that it's an inhalation exposure which is very inefficient in exposing the bone marrow. That's the basic problem, why cigarette smoke is negative in most bone marrow type assays.

DR. AISAR ATRAKCHI: Yes. I do have a question from people online. I was going to go through the questions and then ask or, should I do it now? I could. You want me to do it now? Okay, so one of the -- the first question, I'm just going to read them. For Kevin, Dr. Prohaska's presentation, what is told by FDA currently to patients in the informed consent or exposure to an Ames positive drug? I don't know what that --

DR. KEVIN PROHASKA: I'm sorry, I don't know if I follow the question. They asking whether or not we have any policy as to what --

DR. AISAR ATRAKCHI: I think so, yes.

DR. KEVIN PROHASKA: Okay. None that I'm specifically aware of; however, if there are preclinical concerns, my thought is they should be discussed or described in the consent document.

DR. AISAR ATRAKCHI: Okay. Would this drug be suitable for a healthy volunteer study? The parent was negative in Ames without S9, but there are human-specific metabolites that are Ames positive in TA100. Is there an allowable threshold for the level of these metabolites, 1 percent, 10 percent versus 50 percent of total exposure?

DR. TIMOTHY MCGOVERN: Sounds like a case-by-case type evaluation.

But getting back to that initial question, I mean, what I typically would see for any kind of positive gene tox result, it would be in the informed consent stating what the response is, our concern being that there's an association with that

positive response to induction of cancer and some type of -- it's always difficult words, especially -- I haven't seen one for a positive Ames assay, but some statement regarding risk -- potential risk to the subject.

So there definitely would be something in the informed consent.

DR. AISAR ATRAKCHI: Okay. The next question is from -- this is for (indiscernible) presentation. From what was presented so far, it is still not clear to me what Ames positive IND does mean here. Ames positive is the only information we have as gene tox information. The drug is Ames positive but negative for clastogenicity in vitro and/or in vivo.

The drug is positive in Ames and both in vitro and in vivo for clastogenicity or the drug is Ames positive and considered clearly mutagenic in vivo in humans. The different scenarios imply different potential risk for the patients as potential of in vivo relevance may be different.

Does the single dose causing cancer

correlate with the 25,500-fold compound-specific TTF -- which is the TD50 over 50,000 -- for those compounds with lifetime carci studies available?

DR. BOB DORSUM: I think the first part --

DR. AISAR ATRAKCHI: Can you use the mic?

DR. BOB DORSUM: I think the first part there, inquiring what type of data are available and what's the relevance of that, so as we discussed earlier, the only data that are available at the point of Phase 1 clinical trials are the Ames data. For the second part of the question, I'm not sure I have the expertise to answer that.

DR. AISAR ATRAKCHI: What is TTF? Maybe it's TTC? It's a typo? So the question is, does the single dose causing cancer correlate with the TD50 divided by 50,000 for those -- I guess that's the acceptable intake -- for those compounds with lifetime carci studies available.

DR. BOB DORSUM: I don't know that those comparisons were ever made for any of the

studies we looked at.

DR. AISAR ATRAKCHI: Okay. So this next question --

DR. TIMOTHY MCGOVERN: I'll take a stab. So you have, yes, TTC is based on lifetime carcinogenicity studies. I don't think it incorporates any of the single dose-type studies (indiscernible). So it probably does not -- I mean, it may call into the point that TTC of 1.5 micrograms per day is still protective in considering the results of the single-dose studies, but it wasn't used to develop that curve, if you will.

DR. AISAR ATRAKCHI: The next question is, are there certain Ames assay strains that are particularly predictive or suggestive of positive carci studies.

DR. ERROL ZEIGER: No.

DR. AISAR ATRAKCHI: Some better than others?

DR. ERROL ZEIGER: No. They're measuring different target sites, so the fact that something hits a 5C sequence or 5G sequence, this one

hits a 6G sequence doesn't tell you anything about a relative potency or relevant predictability.

DR. AISAR ATRAKCHI: Is exposure to a metal really the best approach when considering duration? Maybe that was beryllium question. Is exposure to a metal really the best approach when considering duration? I'm not sure why not. Okay. Currently ICH S1 only requires carcinogenicity testing for drugs used for a total of six months or more. Do these data suggest that all drugs should be tested regardless of their duration of use?

DR. ALAN BOOBIS: I mean, I don't think it does. I think we're talking about the value of genotoxicity testing data and (indiscernible) what more information you would be asking for, I think that if you have good negatives in genotoxicity, good repeat dose toxicity, you can certainly get to a situation where you wouldn't be asking for a carcinogenicity study for all drugs, as we do now.

DR. AISAR ATRAKCHI: Tim?

DR. TIMOTHY ROBISON: I just want to come back the Ames positive metabolite that -- in a

lot of situations, we (indiscernible) the same as the API, sort of pursued them, and I think there was one case example where it was extremely low and we sort of went through an argument where maybe nothing was made of it. For the most part, we treated them equivalent to the API and it needed to be pursued in terms of testing.

DR. AISAR ATRAKCHI: But presumably that metabolite is, like present -- it's a major metabolite.

DR. TIMOTHY ROBISON: Well, I mean, yeah. I mean, generally, but we didn't use the 10 percent threshold for an Ames positive metabolite. We pursued them in terms of further testing.

DR. AISAR ATRAKCHI: Then moving on to question five relating to ICH S2R1 guidance provides recommendation for followup for a positive in vitro (indiscernible) clastogenicity assay. If a drug is a mutagenic, Ames positive, are there followup studies to assess risk that should be conducted prior to conducting studies in healthy volunteers?

If so, would a 28-day transgenic rodent

mutation assay, which includes a PIG-A end point, be appropriate or -- well, okay, and if it was positive, then you stop there. If it was negative, the tissue evaluation should proceed.

Alternatively, instead of the 28-day transgenic, would a 26-week (indiscernible) mouse carci study or (indiscernible) bioassay be requested?

So now, we need to know if -- what would be the followup test which we kind of talked about a little bit earlier, about the followup for positive Ames test.

PATRICIA ESCOBAR: Patricia Escobar from Merck. I just have a clarifying question in your (indiscernible) you're assuming that we do have (indiscernible) mutation assay and to that, we advocate the PIG-A end point. Actually, there are two different assays. You can do a transgenic 28-day study and you can do a PIG-A Assay, so they're different -- two different (indiscernible) so I just want to clarify (indiscernible).

DR. AISAR ATRAKCHI: Yeah, I think the point is the followup with in vivo mutation.

PATRICIA ESCOBAR: But it could be either.

DR. AISAR ATRAKCHI: It could be either.

DR. MIRIAM POIRIER: -- build the end point into the transgenic mutation assay.

DR. AISAR ATRAKCHI: Oh, yeah, yeah. That's right.

DR. MIRIAM POIRIER: (indiscernible) end point into the assay.

DR. ROBERT HEFLICH: the idea was to make it easier. If you got a positive PIG-A, the transgenic becomes not important.

PATRICIA ESCOBAR: Yeah, but, for example, they said we're going to use it. You can do a 28-day study as your first -- (indiscernible) 28-day study and you have an inform -- a PIG-A end point.

DR. ROBERT HEFLICH: Right, but by --

PATRICIA ESCOBAR: -- positive, you stop it, right?

DR. ROBERT HEFLICH: Yeah.

PATRICIA ESCOBAR: Like, you're not

going to do a 28 (indiscernible) study.

DR. ROBERT HEFLICH: The problem is if the PIG-A is negative, then can't really rule out a tissue-specific response you could pick up with the transgenic.

PATRICIA ESCOBAR: But I thought that (indiscernible) between the PIG-A assay was actually to be another -- a surrogate in vivo mutation assay comparable to the 28 transgenic.

DR. ROBERT HEFLICH: I'd like to think that, but I mean, that's not an accepted...

PATRICIA ESCOBAR: Because I (indiscernible). As far as I understood, the (indiscernible) that's how we've been kind of thinking about it. We have not used it a lot. There's a lot of information out there, just not as much, but that's the idea, to do one or the other, both of them in vivo mutation end points, which is the way --

DR. ROBERT HEFLICH: The way we recommend doing it is combined assay at this point because of the uncertainty about the PIG-A.

DR. AISAR ATRAKCHI: Thank you.

MAN 14: So I have a question about the 28-day transgenic. Even the most mutagenic carcinogens generally only cause tumors in three, maybe four tissues in a rodent study.

Bob mentioned earlier about the acrylamide study where the mutations and the tumors weren't in the same tissues or there was some overlap but not total overlap, and so the question is, how do you decide how -- in which and how many tissues you need to sample in the transgenic assay before you've got enough data to make a decision and how can you say that that's equivalent to a single PIG-A end point?

So we know -- we really know very little about the relative sensitivity of various tissues in the transgenic mutation assay. There's very, very little data on that. I spent two years, three years on a HESI committee looking over a lot of those data and it's very sparse. And so it's not clear to me how you come to a conclusion that you have enough data to understand which tissues and how many tissues to sample. And so I would be curious how you plan to develop that recommendation.

DR. ROBERT HEFLICH: I think in TG488, there's recommendations as to how we choose tissues, based on what you know about the distribution of the chemical and its metabolism. Just follow that -- those guidelines, as far as setting up a transgenic assay.

The data in the transgenic gene mutation database has been collected over 30 years and some of it's pretty bad. I mean, it was generated using antiquated methods, so I think if you look at it in total, we can say, this is junk, you know.

But if you -- I think, I'd like to believe, if you conduct a transgenic assay following the current guidelines, that you'll get a reasonable estimate of mutation in particular tissue. It's clear that there's a lot more data in some tissues than others, but anyway, in theory you can look at anything.

DR. AISAR ATRAKCHI: But I just wanted to clarify, I think the point here from A is you're doing the 28 transgenic rodent mutation test, but incorporate into it the PIG-A. But you're saying

that’s -- you’re saying they’re two different studies. I don’t think we will object to how it’s incorporated unless there’s technical issues that --

PATRICIA ESCOBAR: No, no, you can incorporate them if you want to, but we were seeing those assays as separate assays, so you could choose one or the other. So not necessarily that you needed to do the two at a time. Here, you’re suggesting to do them at the same time.

Doing the assay at the same time is feasible because at the end of the day for PIG-A, the only thing you take is blood and then you just run the PIG-A. So that’s the easy part.

That’s why we thought, if you do a 28-day study, the (indiscernible) study or a 28-day study, you could actually at the PIG-A end point and get that important -- not necessarily going into this 28 transgenic --

DR. AISAR ATRAKCHI: So 28 days, non-transgenic?

PATRICIA ESCOBAR: Yes, or 28 days non-transgenic. Yes.

DR. ROBERT HEFLICH: If I can predict how the guideline is shaping up, I would say that it's possible to do just PIG-A if you can -- like the in vivo micronucleus assay, if you can argue that you're getting adequate exposure of the bone marrow to the reactive metabolites, that may be by alteration in the reticulocyte frequency or something like that.

But you'd be taking a risk of having your data -- if you got a negative in PIG-A, the FDA might come back to you and say, well, you should do this (indiscernible) or something.

DR. ERROL ZEIGER: And as you know, if you do it all in the transgenic, you can still look at PIG-A, but freeze away other tissues and then you have -- you might throw them out or you might need to analyze them or want to analyze them.

DR. ROBERT HEFLICH: That was exactly the argument.

PATRICIA ESCOBAR: No, that's a good option. I'm just saying, the way we were interpreting it the last couple of years was completely separate. This is a different way of seeing it. I'm not saying

it’s right or wrong.

DR. ROBERT HEFLICH: Well, we’re forward thinkers here at the FDA.

DR. AISAR ATRAKCHI: So just to wrap up this question, is there a preference of using either the 28-day transgenic or the 26-week Tg.rasH2 as a followup?

DR. ERROL ZEIGER: I’m not sure what -- how much information is available on the effectiveness of the Tg.rasH2. I know it was tested with a lot of known carcinogens, a lot of alkylating agents, but as far as other chemical classes, I have no idea of that. I don’t know if the data exist or if somebody just hasn’t pulled them together yet.

DR. AISAR ATRAKCHI: So you’re saying the 28 -- you’d go the 28 day?

DR. ERROL ZEIGER: I don’t know enough about the Tg.rasH2 to say yes.

DR. AISAR ATRAKCHI: Okay.

DR. ROBERT HEFLICH: I think without knowing very much about it either, I think there is a history of positives in it that may not be

informative.

DR. ERROL ZEIGER: Looks like --

DR. ROBERT HEFLICH: To put it elegantly.

DR. ERROL ZEIGER: It's like the early TGR transgenic studies. People only tested chemicals they expected to be positive. I don't think anybody put a Ames negative -- more than one or two Ames negative chemicals into a TGR study in the early days, because they were looking for positives. They were trying to develop the system and see how well it works.

And from the little I know about the Tg.rasH2, we might be in the same situation. We know these are carcinogens. Let's see how this responds to it, which doesn't really tell you anything.

The example was, a number of years ago somebody came into a lab saying, I have this wonderful bacterial test that identifies all carcinogens. And he showed that the dozen or two dozen alkylating agents were positive and carcinogens. And one of us asked the question -- I don't remember who -- well,

how does this test do with non-carcinogens? And the answer was, well, we're not interested in finding non-carcinogens.

So yes, if you're going to only test chemicals you think will be positive, the test will look good.

DR. ALAN BOOBIS: The other point about the transgenic mutation assay is it's available in both rat and mouse; whereas, the Tg.rasH2 is only available in a mouse. That could be significant. It's a lot quicker to get turnaround on results and I think there's a lot more data now in positives and negatives. And there's fewer false positives in the transgenic mutation assay. The Tg.rasH2 assay responds to not only genotoxic carcinogens.

PATRICIA ESCOBAR: I wanted to clarify this and actually, and actually some other information out there, I don't know the literatures, but (indiscernible) part of (indiscernible). They're accepting the Tg.rasH2 to cover a full set of chemicals, genotoxic and non-genotoxic (indiscernible). So it is a known and well accepted

assay.

DR. TIMOTHY MCGOVERN: I was going to add that maybe more of a practical stepwise approach, if you have a positive Ames assay, you might want to start with a 28-day study just to get their clinical program up and rolling again eventually, assuming it is a long-term administration drug, you'll need your two rodent model carcinogenicity which could include the 26-week Tg.rasH2.

But you could, probably, if you wanted to bypass the 28-day study, you could go straight to the 26-week Tg.rasH2 as well.

DR. BOB BRASH: Well, one thing about -- Dan probably would like to comment on this is there's an interest in substituting the in vivo common assay for a gene mutation end point and there's data in the literature that just came out this year, arguing that this is inadequate substitute. I wonder how other -- whether you'd accept common assay data in lieu of gene mutation data for a decision like this.

DR. ALAN BOOBIS: It depends. I think common assay has a lot of merit, but I think it also

has to be interpreted very carefully because we know you can get high dose false positives from non-genotoxic modes of action. If you -- because it's not measuring a direct gene mutation (indiscernible).

It's something consequence to that and that's a valid end point, but I think as long as it's interpreted properly and the study was designed correctly, then yes, it can (indiscernible). I think in the absence of other in vivo follow-ups, it could be (indiscernible).

DR. DOUGLAS BRASH: One thing to keep in mind with the common is (indiscernible) assay is very sensitive to the first couple of hits and then it just plateaus and so if you're also looking at DNA (indiscernible) for example, you're going to see a dosage bounce. It goes up, it's flat (indiscernible) higher doses.

Now if you wait for repair, you're going to think, (indiscernible) there's nothing, nothing, nothing, nothing's happening, then boom, the last couple of things (indiscernible) you see it. So you do have to be very careful.

DR. AISAR ATRAKCHI: We have three or so questions from online. With respect to ICH M7 referred to earlier were 120 micrograms for 30 days would be acceptable for an impurity with clear Ames positive data and not belonging to a class of well-known, highly potent mutagenic carcinogens, how likely would you assume such an Ames positive compound to represent a new class or highly potential mutagenic carcinogen?

DR. TIMOTHY MCGOVERN: It's a tough -- how likely? Repeat that last part.

DR. AISAR ATRAKCHI: Okay, not belonging to a class of well-known, highly potent mutagenic carcinogens, how likely would you assume such an Ames positive compound to represent a new class or highly potential mutagenic carcinogen?

DR. TIMOTHY MCGOVERN: I say we generally would assume it not likely to represent a high potency.

DR. AISAR ATRAKCHI: Yes, I think so. If a drug is shown to have an equivocal Ames result, what followup studies would constitute an acceptable

rate of evidence argument as shown to be negative? For example, a panel member mentioned the in vivo PIG-A, or would more need to be done?

WOMAN 6: (Indiscernible).

DR. ERROL ZEIGER: If it's equivocal, you go back to basic scientific principles. You do a repeat assay and you do it with maybe a little bit more substance to it, more doses or additional strains. But if it's equivocal, it calls out for repeat tests because the difference between a negative and equivocal and a positive could just be a few mutants on a couple of plates.

MAN 15: Have you published a paper recently on that?

DR. ERROL ZEIGER: So did you.

DR. ALAN BOOBIS: Can I just say that this is a decision -- mission-critical decision. In other words, the answer to the question is, do you need a resolution of the answer or could you move forward, assuming a positive? And maybe you can. If you come up with a strategy that says there's a certain amount you could give as a single dose or two

doses to your volunteers, then making that assumption allows you to move forward, okay. If it's critical to resolve the question, then yes, you'd follow the strategy of repeat dose and then think of followups. So I think it's not necessarily essential that you resolve every question, if you can move forward (indiscernible).

DR. ERROL ZEIGER: Well, based on some studies that were done a number of years ago, these weak positives are much less likely to repeat than a clear -- something that's clearly negative. So I've always been an advocate of repeating your results with a positive or negative, doing a repeat test because these weak positives, like I said, two people with slightly different interpretations of the twofold rule.

They have one coming up called negative (indiscernible) equivocal or positive. It takes less time and less effort to repeat it than to discuss it.

MAN 15: One of the things I want to point out is there's a big difference between repeating a two-year cancer bioassay and repeating an

Ames test. It is the cheapest, quickest test in our armament and in a few weeks, you can get an answer and if that settles your question, that's a lot easier than a lot of other things you might want to work on.

WOMAN 7: Yeah, actually (indiscernible) panel of experts, especially from FDA so the (indiscernible) a question. For example, (indiscernible) clinical (indiscernible) for first the human side (indiscernible) for Phase 2 study, it can be (indiscernible) and of course there are some (indiscernible) for the formulation (indiscernible) test, but for the solution for (indiscernible) clinical test.

And also (indiscernible) to rely on a possible Phase 2 we will rely on Phase 2 (indiscernible) and the (indiscernible) move forward, the healthy volunteer study (indiscernible) or we need to provide all this (indiscernible) more relevant Phase 2 study. It's a (indiscernible).

DR. TIMOTHY MCGOVERN: I think you'd want to submit all the data. And I would be looking for rationale as to why you're getting different

results in your two assays because sometimes you could be purifying material, the original result could've been due to potent impurity that was present and no longer there, but I think it would probably raise some eyebrows initially.

They had one positive, one negative, and we'd be looking for some investigation as to why you saw a difference in that result and why we should rely on the negative one as opposed to the positive one.

WOMAN 7: Yeah, that's a very good comment. We all think about that, actually. (indiscernible) in different labs, but all (indiscernible) and (indiscernible) from one country to another, so the labs (indiscernible) different vision, too, and of course the Phase 2 one is more mature clinical (indiscernible) potential.

DR. AISAR ATRAKCHI: I mean, that's like Tim was saying. You need to -- once we see differences in results, clearly it's better -- we will ask the questions. So instead of us asking you the question, just provide the explanation why you think

there was a difference in (indiscernible) as opposed to the back and forth.

WOMAN 7: Okay, thank you. So basically the whole information need to provided and a real story line thing, you guys will judge on that.

DR. AISAR ATRAKCHI: Another question is, it was mentioned that the PKC for mutagenic impurities was 3.8 milligram for one day for ICH M7. If so, then what is the justification for giving one to four doses of an Ames positive compound to healthy volunteers that is likely a much higher dose than for the impurity?

DR. DOUGLAS BRASH: That's why we're here today.

DR. AISAR ATRAKCHI: Correct. Okay. Coming back to the main topic, how would the panel rate negative results from a combined comet micronucleus study? I assume it means, if you have results from comet micronucleus test and they are negative, how would --

DR. ERROL ZEIGER: If the Ames test was positive, I would --

DR. AISAR ATRAKCHI: And those are negative. I think that's what --

DR. ERROL ZEIGER: And those are negative, they -- going along with what was said, I would not denigrate or I would not reduce the impact of the Ames positive.

DR. AISAR ATRAKCHI: Right.

DR. ERROL ZEIGER: The fact that it's -- and we did a study of this for -- in was a couple of (indiscernible) committee that was formed five years ago to look at this. What does it take to cancel out the implications of the Ames positive? And it clearly came out at -- two papers were published. David Kirkland is the first author on it. If the Ames test is positive or negative in any of the in vivo studies, did not -- an eliminate the probability that it would be a carcinogen, but did not affect the probability it would be a carcinogen.

DR. AISAR ATRAKCHI: It's different end points. The assay (indiscernible).

DR. ERROL ZEIGER: Different end points.

DR. ALAN BOOBIS: That may be the conclusion in that paper, but there are other groups who's come to different conclusions, who's concluded that if you've got good negative data on a measure of the same end point, so comet assay and/or transgenesis in vivo, then it might -- and with no evidence of precursor effects in repeat dose study up to 90 days or six months, it's enough to discount the positive in vitro.

DR. ERROL ZEIGER: Sounds as if we need a new group to get together and pull together all these data.

DR. AISAR ATRAKCHI: But I think you're saying is the overall weight of evidence.

DR. ALAN BOOBIS: Absolutely.

DR. AISAR ATRAKCHI: Yes. I don't think you're saying the results of the Ames, ignore it.

DR. ALAN BOOBIS: No.

DR. AISAR ATRAKCHI: Right. It's a weight of evidence.

MAN 16: (indiscernible). All of us

know that the Ames positive mean something. We need to deal with that. But I don't know if we remember that as about -- over 20 percent of Ames test positive will be negative in carci studies, so do we over-emphasize the positive if there's other in vitro study or in vivo study (indiscernible) study is negative and we say decrease a little bit (indiscernible) Ames test?

DR. AISAR ATRAKCHI: The 80 percent prediction of the Ames test, it's -- when it's positive, that's giving you its predicted -- these compounds going to be likely they will be carcinogens. So you will be -- they are two positive (indiscernible).

You're missing the point percent and it's a decision that we have all made that we are okay with (indiscernible) missing 20 percent of the data as a false negative. Or rather, false positive, actually. False positive.

DR. ERROL ZEIGER: Part of that, there's a fallacy in looking at sensitivity and specificity, because a lot of these studies were done

with 50 percent to 90 percent carcinogens in the population. So when you've got -- and the population that you're looking at is -- Ames positives are much less or the -- much less than that, carcinogens (indiscernible) be much less than that.

If you look at -- there are some publications that Ames test is 90-some-odd percent of the publications where the Ames test is 10 to 20 percent. The more carcinogens you'll have in your population of chemicals, the better your sensitivity will be. Ideally, you have 100 chemicals and all of them are carcinogens, you check every one, you've got sensitivity of 100.

Ten percent of non-carcinogens, you check off every one, you've got a sensitivity of 90. It still looks good. But if only 10 percent of them are carcinogens, you check off every one, your sensitivity is only 10 percent.

This is something that was pointed out by Cooper in one of his originally two-by-two table presentations, that the proportion of true positives in the population that you're looking at will look at

how effective the test looks.

MAN 16: Yeah, another problem with these correlations is that we really do not have a very accurate list of genotoxic carcinogens and non-genotoxic carcinogens and genotoxic non-carcinogens, so for example, estrogen is definitely a carcinogen. There are actually some studies that show it's genotoxic.

But nobody thinks that it's a carcinogen because of its genotoxicity. The mechanism is well known that its qualities as a hormone binding to a receptor are what makes it carcinogenic.

DEHP, non-genotoxic carcinogen. We know a lot about how it causes liver cancer. It has nothing to do with genotoxicity. If you were to do a two-by-two table it would seem, oh, the Ames test misses DEHP because it doesn't detect it as a carcinogen. Well, of course it doesn't. We don't want it to detect it as a carcinogen.

And a lot of the lists that you see of genotoxic carcinogens and non-genotoxic carcinogens are merely based upon Ames results without knowing,

without mechanism of action when it's known, and there are a fair number -- unfortunately, many of the compounds for which the mode of action, mechanism of action is really, really well understood, the data comes from studies that are not in open literature but they're in the files of CDER, the Office of Pesticide Programs, and so forth where they -- not a lot of these kinds of studies and as far as I know, nobody's really assembled that very high-quality data into an overall study and so you have to understand the correlations in those two-by-two tables are just a rough estimate and you cannot live and die by them.

MAN 17: Another problem is, there was no clear, agreed-upon definition of what is a genotoxic. There are dozens of tests that measure mutation, recombination, strand breakage. Where do you stop? Where do you stop? Right now, the current is, if it's positive in one of the ICH assays it's considered genotoxic.

MAN 16: I mean, glyphosate is genotoxic because it causes (indiscernible) exchanges.

MAN 17: Yeah. (indiscernible) nobody

does anymore, because it was very (indiscernible) is very sensitive to the particular protocol you're using. That's why nobody does it, but yes. There's no definition. John Ashby used to use, if it's positive in salmonella, it's a genotoxin. But then, he would define genotoxin by positive in salmonella, positive in salmonella --

DR. TIMOTHY MCGOVERN: Circular.

MAN 17: But this is part of the problem and there are a number of chemicals that we know are genotoxic that cause rodent tumors but do not cause rodent tumors based on their genotoxicity.

One example that I was involved with that came to FDA is something that was weakly mutagenic in the Ames test, which doesn't mean that much, but it produced tumors only in the presence of chronic inflammation and chronic necrosis, and only in the animals where you had the chronic inflammation and chronic necrosis.

Now, is that -- is it a genotoxic chemical? Is it a genotoxic carcinogen? Or is it some other mechanism? We don't have the science at

this point and I don't think we have the discipline at this point to say, yes, it's genotoxic but no it is not a genotoxic carcinogen.

DR. ALAN BOOBIS: Well just to -- for information, the chemical (indiscernible) which is the European counterpart of (indiscernible), I think it is, long range initiative, has funded the construction of a database along exactly the lines you're suggesting where they're try -- I mean, it is a judgment call, but they have people like David Kirkland involved, to try to determine which of the carcinogens are carcinogenic by a genotoxic mode of action and by non-genotoxic mode of action and which chemicals have they got which are negative.

And they're going to release a curated database of several hundred chemicals later -- early next year, probably, that -- and the idea was, the start of this was to try to underpin the TDC for genotoxic carcinogens more substantially with a curated database, but grounded to try to provide the sort of information we're asking for now. Which of the carcinogens are carcinogenic by genotoxic mode of

action and which are not?

So hopefully we'll get a quantify of data (indiscernible).

MAN 17: I thought ECVAM had already done that.

DR. ALAN BOOBIS: Excuse me?

MAN 17: I thought ECVAM had already done that.

DR. ALAN BOOBIS: They did, putting together a list of genotoxicants but not -- they haven't curated the CPDB database.

MAN 17: (indiscernible).

DR. ALAN BOOBIS: Well, David Kirkland's involved in both.

MAN 17: Okay, yeah, I know it was the first offer from the ECVAM study. One thing I've learned from doing two-by-two tables for the PIG-A gene mutation validation exercise with David Kirkland is that the outlier, the non-concordant chemicals in explaining why they're non-concordant, is often the most valuable part of the study because there are always going to be things that are negative from what

your prediction you would like to have because of a reason.

And the reason is very informative as to the nature of the assay.

DR. AISAR ATRAKCHI: So I'd like to move on because the last question I'd like the panel to address -- and I think question six, perhaps, can move on quickly because we've discussed a lot of the points here.

Can you provide guidance for a path forward for development of a DNA reactive drug, for example, the need for a mechanism of action, structural considerations, functional groups at the molecular level, (indiscernible) cross comparisons (indiscernible) molecules with a known safety information, observed genotoxic response, mutagenic, clastogenic, aneugenic, or followup assays that described in the earlier question, which is the alternative (indiscernible) test or the two-year bioassay?

Is there anything else we can add to this information? I think there is an agreement that

we would like -- it's helpful to know the mechanism of action. It's helpful if we know there is a structural alert that can add to the weight of evidence. Whether -- is it more important to know if it's mutagenic or clastogenic or aneugenic?

But if we have this information, I think we can add that to the weight of evidence and I think we just discussed the followup assays. Is there anything else that we can add to this?

DR. ALAN BOOBIS: I think C, read across from (indiscernible) can be extremely powerful. If you know what -- you've got a very good idea what the chemical reactivity of the new entity that's driving the positive and you've got an existing compound with the same reactivity and a similar profile in vitro, but you've got a vast amount of human experience because it's been used for years as a human medicine. Then, you can use that information for read-across very effectively.

MAN 17: I think you're saying here if you have an Ames positive (indiscernible), we say stop, not going to allow this to be given in humans

because there is a risk, although we can't clarify it very well, you're going to have to give us more information.

DR. ALAN BOOBIS: This is sort of (indiscernible).

MAN 17: And that's what we're -- yeah.

DR. ALAN BOOBIS: Yeah.

DR. AISAR ATRAKCHI: Okay. And the -- oh, and I guess Part F is to allow microdosing of such drug without any followup assessment. I think we've addressed that as well earlier.

The last question, are there drug classes or specific drugs targeted to the (indiscernible) that should never be administered to healthy subject? Think we had some slides to show on this. Okay, there we go. So this is an introduction to the epigenome consist of specific (indiscernible) modifications of chromative components which include DNA, RNA, and proteins that (indiscernible) inheritance of differentiating states.

Structure and function of the epigenome are controlled by these covalent marks which are

applied by enzymes which are the riders to the (indiscernible) 47 base pair of DNA and the eight (indiscernible) components of a nuclear cells. These marks instruct and the proteins that recognize them, the readers, to identify and remodel particular genomic regions to modulate gene expression, plasticity of the epigenome (indiscernible) much to the existence of erasers that is the enzymes capable of (indiscernible) active and repressing marks.

Tumor cells not only are activated by genetic, epigenetic alterations, but also (indiscernible) epigenetic processes to ensure their escape from chemotherapy and host immune surveillance. And there has been a growing emphasis of recent drug discovery efforts on targeting the epigenome that includes (indiscernible) modification. Several new drugs are being tested and some are already approved by the FDA.

Neoplastic, for example, lymphoma and pre-neoplastic legions have been observed in toxicology studies with (indiscernible) as short as three months in duration which is highly unusual. Is

it appropriate to use healthy subjects for these types of (indiscernible)?

DR. ALAN BOOBIS: There are a number of drugs which we've been giving to healthy volunteers and patients for a long, long time which affect the epigenome, so valproic acid is one of them. It's a (indiscernible) modulator. And we have never been concerned about the risk of giving a few doses to volunteers of these drugs.

The question is, are we going to reappraise our entire approach to giving any drug to ensure that it doesn't affect epigenome before we give it to a patient, because it may -- or a volunteer, because it may not be designed to hit as an anti-cancer drug. It might just be an incidental effect. And we have the clinical experience of some of these compounds already.

DR. ROBERT HEFLICH: Isn't it somewhat outside the scope of this workshop.

DR. AISAR ATRAKCHI: It is.

DR. ROBERT HEFLICH: I mean, because these things are likely to be Ames positive.

DR. AISAR ATRAKCHI: Yeah. It was a question that --

DR. ROBERT HEFLICH: Unless you (indiscernible).

DR. AISAR ATRAKCHI: -- wanted an answer to.

WOMAN 8: There's been no correlation between results of gene tox and findings of carcinogenicity in animals or in humans, so that's why the question is out there. We don't know which ones are carcinogenic and after how many doses.

So while we're seeing an animal carcinogenicity, we hear carcinogenicity in animals with several of these drugs, we don't know if that -- a single dose will be priming the gene such that effects could be seen later on or this is after chronic administration or multiple dose administration that these effects would be seen in animals. So again, it's been seen in animals, medications.

DR. BOB BRASH: What's the indication? Are these anti-cancer drugs?

WOMAN 8: Yes, but animals are healthy.

Yes, so if we see carcinogenicity in the animals, but patients -- you see it also in patients (indiscernible) is not strong.

DR. AISAR ATRAKCHI: It is anti-cancer drugs, yes?

WOMAN 8: Yeah.

DR. ALAN BOOBIS: Are these both types or the methylase -- demethylase types? The methylation demethylation or with the histo modulations as well?

WOMAN 8: Well, I don’t know how -- how much information --

MAN 18: Methylating inhibitors, not with the (indiscernible).

DR. ALAN BOOBIS: Okay, that makes sense.

WOMAN 8: Yeah, not the -- yeah. Okay.

DR. ALAN BOOBIS: (indiscernible) that we know, that don’t --

DR. AISAR ATRAKCHI: Have you mentioned the target? No.

MAN 18: We had experience with the

Ames positives. We have some basis of making a decision, but we have -- with these drugs that particularly target the epigenome and single dose, we don't really know what a single dose might do multi generation. So without that data gap, question is, is that that under the current climate, should healthy volunteer studies be excluded with drugs designed to target the epigenome? (indiscernible).

DR. DOUGLAS BRASH: So I'm guessing that this class of drugs is going to get bigger and bigger over the next few years. And so the concern I have about it for the fact (indiscernible) although nobody talks about it, we (indiscernible) mutations. Years later, you become mottled with tumors (indiscernible) which by itself does nothing.

But if you have a mutation you now get the tumor. These go back to experiments. You know, the most famous (indiscernible), so forth. These go back to the (indiscernible). So then if you have these, this class of drugs, it becomes relevant -- maybe not whether this one is the Ames -- the positive drug, but was there something else ever, or your

hamburger last week or whatnot, that then does show up (indiscernible).

WOMAN 8: So some of these are negative in all three battery of assay. A mechanistic study --

DR. DOUGLAS BRASH: Thank for reminding me to say it. So in the ’70s, there was a push to have an assay system or (indiscernible) support promoters and for various reasons it never took off. But so the answer is, I don’t think we have any database to compare to as to when this would happen.

MAN 18: Yeah, I mean, remember, the battery is based on specific mechanisms, none of which apply to that particular class. I’ll point out that there are a lot of things that affect methylation -- folic acid, folate, vitamin D (indiscernible) -- so I think the problem we have is that we don’t have a screening assay that can tell you whether your drug is a potent enough inhibitor to create those tumors, and so that’s a huge hole and without any assay, the answer is, we can’t tell anybody what to do or not to do because we don’t have any rational way of approaching it.

I mean, if we know that these drugs are being developed specifically as anti-tumor agents, what's the chance that somebody will accidentally develop a similar compound for another indication against which it appears to have efficacy and it turns out it's doing the same thing that these drugs are doing on -- the same effects on the epigenome, do we have a mechanism that will find that out in our entire armament of pre-clinical screens?

And that's the question I think we really need to answer is, what do we need to start looking at to pick up the most potent or at least -- either this mechanism or at least the most potent versions of it? Now, if a 90-day study is all you need because you're seeing tumors in a 90-day study, then maybe that's your answer.

WOMAN 8: Well, we see pre-neoplastic lesions in one-month studies with some of these drugs, but -- so until such a day that we have the mechanistic studies or something else, should we exclude healthy volunteers?

MAN 18: I don't think anybody -- I

think that's an easy question to answer. I think, to me, the real conundrum is whether there are other drugs that are being developed like that that we should start worrying about. That, to me, is the bigger -- drugs and other products. That's the big concern that -- you've uncovered the tip of the iceberg of a new mechanism of toxicity for which we may not have an adequate screen, for not only healthy volunteers but also for patients.

DR. BOB BRASH: For sure, you should exclude healthy smokers.

WOMAN 9: So if carcinogenesis is -- carcinogenesis is complex. So we're still talking about DNA damage, but now we know there's inflammation, so to me the tipping point is metastasis and I remember reading one paper, there's a model and this was in a mouse and metastasis was dependent on epigenetic changes.

So I think we have to leave open the concept that different interactions are -- may affect different steps in carcinogenesis and maybe we need to develop some tests to look to these mechanisms.

MAN 19: Talking about oncology, I mean, I'm in the Division of Psychiatry. We have a lot of neurodevelopmental diseases and now they're supposed to start entertaining this kind of epigenetic (indiscernible). They're trying to do something like that.

Then, the other concern for epigenetics, it could be transgenerational so you might not pick up anything in the -- in your carci study, but you can pick up in their offspring, but we usually don't do that. I mean, we don't do carci analysis in their offspring.

For repro studies it go up to data or something like that or a little bit longer, but that's not long enough to pick up any tumors, so I'm just wondering what's the approach here or do the experts have any recommendation for that?

DR. TIMOTHY MCGOVERN: Sounds like you should reexamine the yellow mouse and model. Maybe Trosko's metabolic cooperation test -- I mean, going back a ways there, but, I mean, people have thought about this in the past.

DR. ERROL ZEIGER: Also, if you're thinking transgenerational, then you're thinking a lot more than cancer reduction. You're thinking unknown mental --

MAN 19: Right.

DR. ERROL ZEIGER: -- effects.

MAN 19: Right.

DR. ERROL ZEIGER: Which would be easier to detect because they'd be detectable at the young age of (indiscernible) and that could be (indiscernible).

MAN 19: So for, like, specific to the question, should the drug be administered to healthy subjects, I mean, especially for neurodevelopmental diseases and...

DR. ALAN BOOBIS: You could start by -- I don't know how ethically permissible it would be, but you could probably make an argument that you avoid women of childbearing age or potentially pregnant women. That would overcome a transgenerational impact.

MAN 19: That's true.

DR. ALAN BOOBIS: Most likely.

DR. AISAR ATRAKCHI: But so -- sorry, you're done?

MAN 19: Yeah, I'm done.

DR. AISAR ATRAKCHI: So are there other classes of drugs -- not necessarily just the epigenome but anything else that you can -- that the panel can think of that should not be administered to healthy subjects?

DR. BOB BRASH: Well, you're talking about a whole series of non-genotoxic carcinogens. I think a lot of mechanisms --

DR. AISAR ATRAKCHI: Yeah, I mean --

DR. BOB BRASH: Several mechanisms.

DR. ALAN BOOBIS: You don't give them to healthy volunteers (indiscernible).

DR. BOB BRASH: Well, then there might be a different answer for each class.

DR. ERROL ZEIGER: Well, at this point, where we are now we don't know they're non-genotoxic carcinogens. We just know they're non-genotoxic.

DR. AISAR ATRAKCHI: Right.

DR. ERROL ZEIGER: But where do you go from there? Well, one thing -- didn't mention that relates to some of the other questions, one thing we haven't discussed is look at the chemical class. There are some chemical classes that seem to be the predictivity of a positive (indiscernible) carcinogenicity is 90 percent or more. There are others, like single aromatic amines.

Half of the false positives in the NTP database are these aromatic amines; although they're very good mutagens and not coming up positive in the animal. So looking at the chemical structure and the relationship of that chemical structure to carcinogenesis can give you a lot of interesting information.

DR. AISAR ATRAKCHI: Okay, any other questions? I only have one more question from online, but I thought we had discussed this. If a compound has (indiscernible) specific metabolite S9 related which caused Ames positive but not in mice and human, what is the suggested followup study?

DR. ALAN BOOBIS: What do you mean by

not in mice and human?

DR. AISAR ATRAKCHI: I guess it was not positive.

MAN 20: (indiscernible) positive in the Ames (indiscernible) S9 and it (indiscernible) like other --

DR. AISAR ATRAKCHI: Oh, other S9.

MAN 20: (indiscernible) study and (indiscernible).

DR. AISAR ATRAKCHI: Oh, it’s a (indiscernible) specific metabolite. I think if it is, then I don’t think we care. Right, I mean -- what?

DR. ALAN BOOBIS: Well, your weighted evidence would argue --

DR. AISAR ATRAKCHI: Yes.

DR. ALAN BOOBIS: -- that there is not a cause for concern.

DR. AISAR ATRAKCHI: Right. Exactly. Okay, I think in absence of any more questions and we’re one minute away from 4:00, I really appreciate all of you coming in and listening to this workshop as

well as a great and big thank you to all our panelists and I think it was a very helpful workshop and discussion and hopefully we can -- as an agency, we can come up with some information that can advise us and give us recommendations to move forward for -- have some plan in place to address these types of compounds. Thank you so much.

(Whereupon, at 3:58 p.m., the proceeding was concluded.)

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