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CLINICAL TRIAL DESIGN CONSIDERATIONS FOR MALARIA DRUG DEVELOPMENT

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A P P E A R A N C E S

Clinical Trial Design Considerations for Malaria Drug Development—June 30, 2016

Speakers and Panelists

Paul Arguin, M.D.
Chief, Domestic Response Unit, Malaria
Centers for Disease Control and Prevention

Shukal Bala, Ph.D.
Microbiologist
Division of Anti-infective Products (DAIP), Office of Antimicrobial Products (OAP), Center for Drug Evaluation and Research (CDER)

Rana Chattopadhyay, Ph.D.
Biologist
Division of Vaccines and Related Product Applications, Office of Vaccines Research and Review Center for Biologics Evaluation and Research
Food and Drug Administration

Dakshina Chilukuri, Ph.D.
Pharmacologist
Office of Clinical Pharmacology, Office of Translational Sciences
Center for Drug Evaluation and Research (CDER)
Food and Drug Administration,

Ingrid Felger, Ph.D.
Head, Molecular Diagnostics Unit
Swiss Tropical and Public Health Institute (Swiss TPH),

Noel Gerald, Ph.D.
Biologist
Division of Microbiology Devices, Office of In Vitro Diagnostics and Radiological Health, Center for Devices and Radiological Health
Food and Drug Administration

Karen Higgins, Sc.D.
Mathematical Statistician
DAIP, OAP, CDER

James Kublin, M.D., M.P.H.
Executive Director of the HIV Vaccine Trials Network Medical Director of the Seattle Malaria Clinical Trials Center
Fred Hutchinson Cancer Research Center

Matthew Laurens, M.D., M.P.H.
Director, International Clinical Trials Unit, Division of Malaria Research Institute for Global Health University of Maryland School of Medicine

James McCarthy, M.D.
Senior Scientist
Queensland Institute of Medical Research (QIMR), Berghofer Medical Research Institute
Brisbane, Australia

Jörg Möhrle, Ph.D., M.B.A.
Vice President, Head of Translational Medicine
Medicines for Malaria Venture, Geneva, Switzerland

Sean Murphy, M.D., Ph.D.
Assistant Professor and Assistant Director of Clinical Microbiology
University of Washington, Seattle, Washington

Sumathi Nambiar, M.D., M.P.H.
Director
DAIP, OAP, CDER
Food and Drug Administration

Elizabeth O'Shaughnessy, M.D.
Medical Officer
DAIP, OAP, CDER
Food and Drug Administration,

Michael Proschan, Ph.D.
Mathematical Statistician
National Institute of Allergy and Infectious Diseases

David Saunders, M.D., M.P.H.
Clinical Pharmacologist and Internist
US Army Medical Material Development Activity

Bryan Smith, M.D.
Principal Medical Consultant
Clinical Network Services, Washington, DC

Kalavati Suvarna, Ph.D.
Microbiologist
DAIP, OAP, CDER
Food and Drug Administration

Peter Weina, M.D., Ph.D.
Chief, Department of Research Programs
Walter Reed National Military Medical Center

Tim Wells, Ph.D.
Chief Scientific Officer
Medicines for Malaria Venture, Geneva, Switzerland

P R O C E E D I N G S

MR. COX: Good morning. If we could have folks move towards their seats, we'll get going here in just a minute.

Good morning, everybody. I just wanted to start out the day by saying thank you to all of you that have come to join us. I'm Ed Cox. I'm the Director of the Office of Antimicrobial Products, here within the Center for Drugs at FDA.

We welcome everybody to today's workshop on Clinical Trial Design Considerations for any Malaria Drug Development. We're grateful to the many folks that have traveled from far and wide to come and join us. We recognize that there are tremendous rigors in travel and we thank all of those that have endured and managed to get here to do so in good shape and we thank you all for that.

Today we look forward to discussing several important issues in clinical trial design for treatment -- for antimalarial drugs for

treatment of malaria. We all know that malaria remains a major global health problem, with an estimated number of malaria cases globally at 214 million in 2015, with an estimated over 400,000 deaths. So it continues to be a major issue is global public health. And we do have agents to treat patients with malaria, but unfortunately, know that resistance erodes away at our therapies. It's currently a problem and we can expect that will continue to happen in the future.

So it's really important that we do have the development of new antimalarial drugs. That we have new treatments to be able to utilize for patients out there to be able to continue to treat patients with malaria and not lose the ground of success that we've achieved so far.

We also know that developing drugs, really in any therapeutic area, isn't easy. And that's particularly true in the setting of developing new therapies for treatment of malaria. We also recognize, too, that, you know,

we, here at the FDA, regulate drugs in the United States, but we're also very mindful of the fact that what we do here in the U.S. and our recommendations with regards to trial design have global implications. So it's something that we think is very important to take into consideration as we're talking about trial designs and new drug development for malaria.

So today's meeting we'll focus on really, two specific areas. We'll talk about clinical trial design issues first. And one of the issues that comes up fairly commonly is studying various different combinations of drugs. And there are a variety of ways to approach this. It depends a little bit on the drug, it depends a little bit on the disease. Whether you can use the drug alone, if you can use the drug alone. How long can you do that for?

Bottom line is that anything that's done really needs to be acceptable from an ethical standpoint and also provide adequate patient protection. So I look at the issue of

Page 10

addressing, you know, how to study combinations of drugs. It's essentially a solvable problem and the solution just needs to be appropriate for the circumstance in the particular drug that you're studying. So we look forward to the discussions today. I think it will help inform on that particular aspect of any malarial drug development.

In addition to talk some about trial design combination issues, we'll also talk some about methods of detection. As we work through this, I think we'll hear a lot of important information about the attributes of one test versus another test. I think that will be helpful in moving the discussion along about different types of tests that you might utilize to diagnose malaria to detect malaria in the setting of clinical studies.

And I would encourage people -- this is a workshop, so it really is meant to be an open discussion. So please do. Feel free. If we get the opinions out on the table, if we get the

Page 11

science out on the table, I think that's really the best way to move things forward, so don't be shy, okay. I'm sure we'll have a rich discussion. And maybe what we'll do too, just so that everyone's aware of who's at the table, why don't we start and we'll just work this way down the table here. We'll start with Professor McCarthy and then we'll move over here to Dr. Felger, and then we'll go down this side.

MR. MCCARTHY: So my name is James McCarthy. I'm a clinical investigator in Brisbane, Australia and I lead the blood stage human challenge system.

DR. NAMBIAR: Good morning. Sumathi Nambiar, Director, Division of Anti-Infective Products.

DR. KUBLIN: And I'm Jim Kublin, the Medical Director of the Seattle Malaria Clinical Trial Center.

DR. SAUNDERS: David Saunders. I'm a clinical pharmacologist and internist with the U.S. Army.

Page 12

DR. SMITH: Brian Smith, I'm a principal medical consultant for clinical network services and the chief medical officer for 60 Degrees Pharmaceutical.

DR. WEINA: Pete Weina. I'm the Director of Research Programs at the Walter Reed National Military Medical Center. I worked for almost 20 years with the Walter Reed Army Institute of Research and Drug Development.

DR. MURPHY: I'm Sean Murphy. I'm a clinical investigator at the Seattle Malaria Clinical Trial Center and I'm an Assistant Professor at the University of Washington.

DR. LAUREN: Matt Lauren. I'm a clinical investigator at the University of Maryland School of Medicine, the Institute for Global Health.

DR. FELGER: Ingrid Felger. I'm coming from the Swiss Public Health Institute in Basel.

DR. ARGUIN: Paul Arguin, Chief of the Domestic Malaria Unit at the Centers for Disease Control and Prevention.

DR. MOHRLE: I'm Jörg Möhrle from the

Page 13

Medicines for Malaria. I'm heading the translational medicine group there.

DR. WELLS: Tim Wells, Medicines for Malaria Venture in Geneva.

DR. O'SHAUGHNESSY: I'm Elizabeth O'Shaughnessy. I'm a medical officer in the Division of Anti-Infective Products at the FDA.

DR. PROSCHAN: I'm Michael Proschan, mathematical statistician at the National Institute of Allergy and Infectious Diseases.

MS. HIGGINS: Karen Higgins, I'm the statistical team leader supporting the Division of Anti-Infective Products.

MR. COX: Great. Thank you all. And just so folks know, the meeting is being transcribed, so there'll be a transcript. So I just want to let folks know that. And also, too, we provide information on a potential conflicts of interest. There is information available at the front desk. We think that's helpful for folks and available should folks need it.

So now let's move onto our first

Page 14

presentation. Professor James McCarthy will be speaking first, stepping for Dr. Dondorp. We appreciate that very much. I'll just say a little bit. Not too much but a little bit.

Professor McCarthy is a senior scientist at the Queensland Institute of Medical Research and Infectious Disease for the World Brisbane and Women's Hospital, which are both in Brisbane, Australia. And his clinical and research training were undertaken in Australia, the U.K., at the University of Maryland and also at the Laboratory of Parasitic Diseases at the National Institutes of Health.

And we were talking a little bit earlier. I was actually a fellow at the same time that he was a senior fellow within LPD at NIAID. So the world is a lot smaller than we think. The major focus of his research has been on the development application and clinical trial systems that entailed deliberate infection of human volunteers of malaria parasites, via intravenous injection of plasmodium effected red cells. So we're

Page 15

grateful that Dr. McCarthy has joined us here today and we look forward to his talk.

So James?

DR. MCCARTHY: So thanks very much, Ed. It's a great pleasure to be here amongst friends and colleagues. I know many of the people in the audience through the work that we've been doing or from previous lives, as Ed has just mentioned. I'm stepping in for Arjen Dondorp, who many of you would also know, who leads a lot of the work going on in the Mahidol Oxford Research Group in Bangkok, Thailand. He was unable to make it due to some problems with travel that I don't want to go into.

So he's kindly provided me these slides and I'm going to talk, really to set the scene about antimalarial therapy and why we need to be concerned and to develop new drugs and to deal with it as efficiently as possible. So here's a picture of Arjen, just so you recognize him. I'm really pointing out the center of the hot spot of mission resistance and he's led a lot of the work

Page 16

that has this to the fore.

And here is the slide of the four malaria parasites plus the zoonotic malaria parasite, plasmodium knowlesi. Just to make one point, we're going to be talking mostly today about plasmodium falciparum, which is the most lethal form of malaria. But many of the issues are equally apparent to the other three species, and in particular, plasmodium vivax, which I don't think we're going to get time to talk about today, but certainly there are some specific issues about relative activity of some drugs against P. vivax as opposed to P. falciparum.

So here's the last stock of the life cycle of the malaria parasite. I really don't need to go into that this with this audience, just to make the point that we're going to talking mostly today about the blood stage, which is the stage that causes clinical illness in humans. But there is going to be quite a bit of discussion about the gametes I expect as well because this is the life cycle stage that is

Page 17

transmitted to the mosquito and is the focus of increasing interest, both in terms of drug efficacy but also if it's to eliminate malaria.

So artemisinin drugs are the key drugs that really saved the problems we were having with chloroquine resistance that all the antimalarials that were viable 20-odd years ago were filing in principal amongst those with chloroquine, which had been effective for 50-odd years. But the other aspect of the artemisinin was its key attribute in terms of -- we do see mortality.

And these are the two pivotal clinical trials that were untaken, one in Asia in children which showed a 35 percent reduction in mortality when intravenous artesunate was used instead of quinine, which was then the drug of choice for severe malaria. And likewise, a larger study done in nine African countries where there was also a 23 percent reduction in mortality. So this is key issue that we need to think about when we're worrying about the need for new

Page 18

antimalarials. If we lose these drugs, and there are data that we'll be showing in a moment, we are going to be faced with worldwide increasing immortality for malaria.

And the reason why the artemisinin drugs are so effective is because they work across the whole parasite life cycle and not just at the letter. Part of the malaria parasite life cycle where most of the drugs that we have developed in the past only work from the trophozoite on through to sporozoite, whereas, the artemisinin also works in the early stages where the ring stages are present, and therefore, are more rapidly acting. This early stage of the malaria parasite life cycle, the artemisinin drugs seem to be losing their activity again, and therefore, are reducing their efficacy.

The other aspect of the artemisinin drugs is the rate of which they kill parasites. So this is a slide that Nick White published and reviewed several years ago, showing the relative decrease in the number of parasites in a human

Page 19

host over weeks of treatment. And you can see when you give the artemisinin drugs, you can effectively eliminate all the malaria parasites by a week of treatment, whereas, with a more slowly acting drug such as Mefloquine, Piperaquine, and Malarone, there's a much slower decrement in the parasite clearance. And then a drug such as Doxycycline, which is widely used for prophylaxis, really means that you've got to give treatment for upwards of three weeks if you're going to achieve cure. So that's the other key attribute of the artemisinin drugs as their rapid activity.

Now, as well as their different activity, these drugs have got very different pharmacokinetic profiles. So shown on this graph is plasma concentration, the drug vs. treatment time in weeks. And what you first see is that you can't see the artemisinin drugs because there, a plasma half-life is measuring now, so you don’t really find the drug in the blood after 24 hours. Quinine is a little bit longer in

Page 20

terms of being around for up to 24 hours. And then many of the other drugs that we use for both prophylaxis and treatment such as Piperaquine, Chloroquine and Mefloquine have a half-life measured in many weeks.

So this means you can give a loading dose at the start of treatment and continue to have drive efficacy out for quite a long time when the drugs are effective.

So the story of artemisinin resistance is really, first, properly documented in 2009 by a paper that Arjen and I were first author on in the New England Journal of Medicine. And it showed that we were seeing decreased rights of clearance of a malaria parasite in different sites in Asia. So this is site in Cambodia, where you can see the parasites are still disappearing from the blood, but there's a significant decrease in the right of clearance of the parasites. And this was the first clue that we were actually seeing a failure of the malaria parasite to kill those early life cycle stages of

Page 21

the parasite.

Since then, things have become worse. So in 2012 and 2013, here you've got a Kaplan Myer probability of cure estimate. So now you're seeing out at 70-odd percent cure rights in one particular location in Pursat compared to the other locations. So clearly, a very significant decline in the efficacy of antimalarials. And indeed, it's become even worse now. So you can see here, you won't be able to read the scale here, but you're seeing it in these locations in Cambodia. Basically, you're having the inability to cure the malaria with DHA Piperaquine, which is dihydroartemisinin, a derivative of artesunate. So you're getting a 42-day failure rates in the order of 80 or 90 percent.

And we now know very well the mechanisms of this. This is the Kelch K13 propeller protein, whose function is not completely well understood, but there's a mutation at residue 580, which confers significant resistance. This is now a molecular markup of artemisinin

Page 22

resistance that is now well-described and can be used for epidemiologic purposes to map the spread of artemisinin resistance.

And this is what it was in 2013, where we saw the focus of resistance really being along the border between Thailand and Cambodia and in certain parts of Cambodia. But more recently, it's moved into Myanmar as well, which will be shown in the next slide. But you can see in other parts of Asia, there was no evidence of resistance at all.

Now, this is a genetic slide put up to map the origin of the artemisinin resistant mutation. And the point of this slide is really to show that mutation arose independently in Myanmar to Cambodia. So what this tells us is that the parasite is actually developed resistance at the same location in its genome, independently in two different places. So that really raises an issue, as the drug has become more widely used in Africa, that that mutation is likely to occur as more use of the drug takes

Page 23

place. And the genetics of this is quite independent. And this was published some two years ago. So we now have clear data to suggest that we are going to lose artemisinin some time. And the question is when. And that will depend largely upon the pressure that is applied to the parasite by increasing use of the drug.

Now, the situation in the greater Mekong subregions have become even more worrisome. This is a study published in the Lancet Infectious Diseases a year ago. And this is a map of Myanmar. And up in this far corner here is India. And you can see that the red spot is the mapping of the prevalence of this K13 propeller mutation. And you can see it's come right up to the border of India. So it's very likely that we have already got spread of this mutant parasite into India, which suggests that we are going to see the problem spread across Southern Asia in the near future.

And this is just more data showing the increasing in prevalence of the drug resistant

Page 24

parasite. But in this case, in conjunction with Mefloquine, which is the partner drug used, where you have seen a major jump in the parasite clearance half-life, which is a major of how quickly the parasites are killed in 2010. And that's also associated with a decrease in the efficacy of artesunate-mefloquine also, occurring about that time.

So not only is the artemisinin resistance developing, but it's also occurring with the partner drug which is, in this case, Mefloquine. And this is a slide prepared by Arjen, showing where we are with artemisinin resistance now. In Cambodia, the drug of choice in Cambodia, which is dihydroartemisinin Piperaquine, is by simply becoming ineffective. And new treatments are going to be required as well as with artesunate-mefloquine, which is the other widely-used combination in the greater Mekong subregion is now no longer effective in the Thai-Myanmar border.

So this is the situation right now where

Page 25

our two most potent combination therapies have become effectively resistant to treatment. And this is also been mapped for mefloquine to be shown to be associated with the MDR drug transporting pump. So as you get increasing copies of the MBR, copy number from one down to more than two copies of the MDR, you can see your cure rate drops from 100 percent down to 60 percent. So clearly, we've got good molecular data as to what's going on here. And the question is how long are we going to last?

And the issue is not only will we see resistance, but we'll also probably see increased transmission because people who have decreased cure from the antimalarial drugs carry gametocytes. The sexual stage of the parasite ran for longer, so that means there's going to be a larger reservoir, more clinical cases, more drug used, more resistance. So this vicious cycle will continue. And this was certainly case when chloroquine resistance arose.

So I don't need to again remind the

Page 26

audience that was spread of chloroquine resistance that arose independently, both in South America and also in the same region of Asia and it's spread over a period of 10 years across Africa and caused probably an excess of millions of deaths in children in Africa when chloroquine was no longer effective. Likewise, we've seen the spread of resistant sulfadoxine-pyrithiamine, again, from Asia or across to other parts of the world.

So this clear historic precedents when you see these resistance events occur that they are going to spread. And the question really is how quickly that's going to happen. So when Arjen and I were doing infectious diseases training, one of the paradigms we were taught by our mentors was you never add one drug to a filing regiment. So whether it be tuberculosis, HIV, or any of the other combination of infectious diseases that require multiple antibiotics, adding one drug to a filing regiment, really only buys you a little bit of

Page 27

time and is not going to be a definitive solution. So the solution is being contemplated now in the greater Mekong region, basically doing exactly that. So we're adding mefloquine to dihydroartemisinin Piperaquine or amodiaquine to artemether-lumefantrine. In the following slides, I'll show why this might have both pharmacokinetic and pharmacodynamic rationale, but the point being what we're doing is really only buying ourselves time.

There are some new drugs around, arterolane, formerly known as OZ439 is a synthetic antimalarial which is being tried with the piperaquine, but the data with this aren't particularly encouraging in the dose regiments that have been tried to date. But whether this will be an effective regiment when used for longer, we'll wait and see. And there are two clinical trial programs going on called Track 2, which you're looking at that. And then there's obviously the options of increasing the duration of treatment, which is certainly a possibility,

Page 28

but then the adherence of your patient certainly diminishes. And there's the possibility, I suppose, of crop rotation, where you rotate different drugs around to try and decrease pressure on a particular drug, hoping that the survival advantage of the parasite with the wild type completes the mutant parasite.

And then there are sequential uses as well or potentially, artesunate-pyrithiamine, which is otherwise known as Pyramax, which has recently been more widely licensed as the liver signal has diminished. So these are some of the strategies being contemplated in the greater Mekong region to try and reduce -- to try to really buy some time. And I think that's what my take on this is, what we're doing is buying time.

So these are just some data on the combination therapies. The first is that there seems to be some mutual antagonism between piperaquine and mefloquine when you use them together. That there is no enhanced cardiac signal as far as we can tell because both of

Page 29

these drugs, you'll know, have some effect on the cardiac conduction but there doesn't appear to be any problem with that and that there is both some pharmacodynamic and pharmacokinetic reasons why these drugs can be used together. So that's the rationale for why the AK-piperaquine can be used in combination with mefloquine. And potentially, it will rescue the situation in parts of Cambodia where the DHA-piperaquine regiment is no longer effective.

And likewise with artemether-lumefantrine, the proposal is that amodiaquine which is a log off of chloroquine, has both a pharmacodynamic and a pharmacokinetic reason for combination here and using these two drugs together. So this is otherwise known as Coartem or (inaudible). It is a very widely-used, in fact, the drug of choice in Australia and the U.S. for the treatment of malaria now is adding amodiaquine to this regiment actually probably will improve drug efficacy and reverse the situation where you're losing activity of the

Page 30

artemisinin combination.

So in conclusion, we're going a little more quickly than I had planned, the fast-acting drug, the artemisinin has a major survival advantage. And if we lose this artemisinin, we're going to lose that survival advantage. And in subsequent talks, they'll be a discussion about selecting a drug that does have a fast-killing property, which is obviously going to be critically important for saving the lives of people who severely have malaria. Certainly, this also means that you can select a partner drive with a long half-life, which really means you can identify a regiment of antimalarials that can be given in a very short course, which is clearly ideal when you're dealing in a situation in many countries that have malaria where having people come back to complete course of therapy can be a difficulty. And obviously, a drug combination, just in other areas of antimicrobial therapy, you increase the genetic barrier to resistance as you coformulate drugs with

Page 31

complementary mechanisms of action. This is a concept that I don't think I need to explain to this audience, but it means that we really need to be thinking about this when we construct coformulations. A major theme of today's talk is that we have a significant increase in the complexity of drug development once we start to think about developing and licensing a combination antimalarial, given some of the difficulties that we'll discuss later in the day in terms of clinical trial design.

So the other conclusions to make is artemisinin resistance is now with us. It's expanding across Southeast Asia. To date, there is no evidence that it's arrived in Africa, but because, as I said, the mutations have arisen independently, there's no reason to believe they weren't arised in Africa either. It contributes the treatment failure and we're seeing clear cases of treatment failure in part of the greater Mekong region. It selects for the partner drug resistance. So once you lose your fast-killing

Page 32

effective artemisinin, you're going to lose your partner drugs. So you really need to anticipate that happening. And also, you're going to probably increase the transmission of malaria because there's going to be increase in gametocyte carriage.

In terms of partner drug resistance, this is an increasing problem across Southeast Asia, and particularly in the greater Mekong subregion, where the partner drugs are basically running out of juice. And finally, this leaves us with the situation where there are now very few options left in this part of the world. And as I discussed, these triple combinations are now becoming necessary. In my view, at least, this is just buying us time. So therefore, we urgently need antimalarials and we need to think very carefully about the partner drug and the developmental pathways for licensure of the drugs.

And just as a last slide, this is the global list of drugs that are available at the

Page 33

moment. So you've got drugs under research, which you're doing lead optimization, translational work, which will be discussed later in the morning. And then we only have one drug, which is a combination that is currently in clinical trial that doesn't include an artemisinin drug.

So despite the fact that we got a large number of drugs all across this point, the only drug we currently have at the moment, having Phase II is the combination of OZ439 and ferroquine.

So we really are in a very urgent situation because we need to somehow develop new combinations so that we have drugs in Phase II and Phase III so that we don't start having thousands of people having adverse outcomes because we don't have a drug to treat them with. So I think I might stop there. I'm not sure how we're doing for time. We got time for questions or we keep going?

DR. NAMBIAR: Yeah. I think we have time

Page 34

for a couple of clarifying questions. Any questions from the panel members? Any questions from the audience?

We have one question.

DR. BERMAN: Hello. An excellent talk, Professor McCarthy. I'm Dr. Berman from Fast-Track Drugs. Since you do have a little extra time, let me sort of think in broad terms for the last 20 or 30 years, we've had a prohibition about anything more than three-day dosing. And as you've well said, presented the origination of resistance in a certain part of the world and then spread to the rest of the world. So there are two questions that occurred to me as a listened.

The first is this prohibition against anything more than three days really a strong barrier, just something that grew up with traditional chloroquine treatment and may be something that's not so much of a driver. It doesn't have to be so much of a driver these days.

Page 35

And the second question and this is just one of knowledge, is using the example of mefloquine. As mefloquine resistance jumped from the Mekong River locale to the rest of the world, the reason I'm saying that is very few of us travelers and people in the Western world really go to the Thai-Cambodian border or the Myanmar Cambodian border. And so the broad question of drug development is it to make products against what's there because it will spread or to make products against issues faced by 99.9 percent of the rest of the world which can use present drugs in spite of some of their difficulties, for example, mefloquine?

DR. MCCARTHY: Maybe I'll tackle the second part first. To my knowledge, there may be people in the audience knowing who can answer this question better than I, but I don't believe that there's been widespread use of mefloquine in, for example, sub-Sahara in Africa. Certainly it's used in South America, but we have not seen the occurrence of mefloquine resistance like

Page 36

we've seen in this part of the world and there has been speculation about particular epidemiologic factors located there, as well as drug use, counterfeit drug use. There's been a range of proposed explanations as to why the occurrence of these resistance mutations has taken place in that part of that world.

It's also true to say that the mefloquine resistance mutations did revert once artemisinin in combinations came into use there. So there clearly is a fitness cost to the parasite carrying these extra MDR gene copy numbers and that is readily able to revert. So very quickly after artemisinin resistance arose, we saw reversion to these multiple copy number MDR copy number parasites in that part of the world.

So I think it's an open question as to whether mefloquine will be widely resistance would be more widely seen if we used it, for example, in heart transmission settings in Africa. But also, as you're well aware, there are some significant toxicity issues that have

Page 37

really, I admit, although it may not be a problem with severe malaria there, obviously a significant opposition in the general public about the use of mefloquine, and certainly in Australia, we've had very recent examples with our military activists -- or ex-military activist blaming mefloquine for psychosomatic illnesses.

The second part, I suppose, is a more philosophic one about duration of treatment. That the CIRCaP concept that was really promulgated with the malaria and control initiative that came about five years ago has come under question that we may need to consider longer courses of treatment, but we all know that in clinical practice that longer courses of treatment are not necessarily well adhered to. And if you have to have those long courses, it certainly increases the probability of failure.

So ideally, one has a drug where you can have a prolonged period of time above the MIC with a drug that can be given in loading dose. So I think it is a philosophic question and I

Page 38

don't pretend myself to have an answer to and I think this is something that could be debated, perhaps, later on.

DR. NAMBIAR: Great. Thank you, Dr. McCarthy. So again, thank you for the excellent overview. And we'll looking for your clear message on the need for new antimalarial therapies and the need for combination therapies as well. So with, I think we'll move into the first session that Dr. McCarthy and I will co-chair. And the focus of this session is on clinical trial design considerations and use of multiple drugs in combination.

So we have four speakers. And in the interest of time, what we'll do is we will get through the four talks and have time for questions and answers after the last speaker. The first speaker of this session is Dr. Elizabeth O'Shaughnessy. Dr. O'Shaughnessy is the medical officer in the division of anti-infective products in the Office of Antimicrobial Products at the FDA. We've been very fortunate

Page 39

to have Dr. O'Shaughnessy as one of our reviewers. She has had experience in reviewing antimalarial products. She is trained in internal medicine and infectious diseases and has started her medical training in Ireland before moving to the United States.

With that, welcome, Dr. O'Shaughnessy. Thank you.

DR. O'SHAUGHNESSY: So my presentation today will be a high level description of the regulatory and scientific issues related to the development of antimalarial drug combinations. So I want to start with providing a regulatory framework or backdrop that pertains to the development of drugs in combinations for the discussions later this morning and then I'll comment on the challenge we encounter here with the development of antimalarial drugs in combination, and then go onto to talk about the FDA guidance document and the co-development of drugs, and then comment a little bit about study design options to assess the contribution of

Page 40

individual drugs to a combination regiment.

With regard to regulations, for all new drug applications, we need substantial evidence of effectiveness that needs to be demonstrated through the inadequate and well-controlled clinical trials. If we look at the definition of substantial evidence, it means evidence consisting of adequate and well-controlled investigations, including clinical investigations performed by experts which demonstrate the drug or the combination of drugs that will have the effect it purports to have under the conditions of use prescribed in the label.

And if anyone wants to look up the adequate and well-controlled trials, they're under CFR 314.126. So we also need data to demonstrate that each component of a fix-dose combination contributes a measurable advantage over the individual components. And this we refer to commonly as the combination rule. And this can be either increased efficacy, reduced emergence of resistance, better safety or, for

Page 41

example, a simplified regiment. And even for drugs that are not developed in a fixed combination are either not physically combined, we also require data to show that the individual components of the combination contribute something to the combination and that there's a measurable advantage.

So the challenge here is how to demonstrate the contribution of individual antimalarial drugs to a combination regiment. And we can do this through preclinical studies and clinical studies and it's usually in a combination of both.

For preclinical evaluations and antimalarial drug combinations may include in vitro activity of the combination versus the individual drugs against laboratory strains and clinical islets. And we can also look at the activity of the combination versus individual drugs in animal models. And we really look to the panel today to give us more information or help us to look at what in vitro studies and

Page 42

animal models would be suitable to study the contribution of individual drugs to an antimalarial drug combination.

With regard to clinical studies, well, one approach to obtaining a rapid proof of concept for the activity of a malarial vaccine or an antimalarial drug in humans is the controlled human malarial infection study, which would be covered later this morning. And we would like to ask if a CHMI study in any way could help to assess the contribution of an individual component in an antimalarial drug combination as a whole. And also, we would like to ask your opinion on the feasibility of a factorial design study in adults in a semi-immune population, for example, with uncomplicated malaria. Obviously, there are ethical considerations. There is potential for some optimum efficacy, the safety of the patients and the development of resistance, if one includes a monotherapy arms. And obviously the patients require close monitoring and prompt rescue therapy and we would

Page 43

like your opinion on the feasibility of such a study.

I'm now going to switch to the FDA Guidance document. So the FDA has a guidance document for the co-development of two or more unapproved drugs. So it applies to multiple therapeutic areas. It's not specifically related to infectious diseases and it's intended to provide guiding principles for the concurrent clinical development of two or more investigational drugs to be used in combination.

The focus is on approved drugs, but we also refer to it for co-development of an approved or with an unapproved drug, for example. We had a malaria guidance that was published in 2007. I say "had" because it's withdrawn from the FDA website right now and the plans are to update it and hopefully the discussion this morning will help us with this.

In the co-development guidance, they provide different study scenarios for the evaluation of the components of individual

Page 44

combinations and their contribution of the combination as a whole. One is where each drug alone has activity can be administered individually and they describe a factorial design situation where one compares the combination and in this, the combination is A and B and the SOC is the standard of care.

So one can compare the two together versus A versus B versus the SOC, or one can consider adding the drugs to the standard of care. And we heard from Dr. McCarthy the issues with that. Before you compare the combination with the standard of care versus each of the components and then compare it to the standard of care plus placebo. And we just posed a question, could we consider administering drugs for a short duration of time, but long enough to establish proof of concept, where we look at the effect on malaria parasite reduction as an early time point after the start of treatment. And of course, all the ethical considerations that I described would apply to this kind of study.

Page 45

So the goal, from our perspective, is to try and get a handle on the how the two drugs or the three drugs in a combination contribute to the combination as a whole before we get to Phase II trials because if the findings from in vitro and in vivo studies adequately demonstrate that, then the Phase III trials can compare the combination regiment up against the standard of care and that should be generally sufficient to establish effectiveness of the regiment.

Before I finish, the combination rule can be very complicated. These are just some examples of how it's been applied in other areas. I'll do an example for malaria first then -- actually, one from -- just look at Hepatitis C and tuberculosis. So as we heard, actually, Coartem was the most recently approved artemisinin combination. It was approved by the FDA in 2009 for the treatment of uncomplicated falciparum malaria. And the NDA for Coartem, among other studies contained two factorial design studies which evaluated artemether-

Page 46

lumefantrine alone and in combination.
These studies are older studies. They were done in the early '90s in China, when issues related to monotherapy and antimalarial drug resistance were not as well established as they are now. And one of them was a double-blind comparative trial of Coartem versus artemether and versus lumefantrine alone. There were monotherapy arms in that study. And then a partially blinded comparative trial of Coartem versus lumefantrine tablets and capsules.
So as I mentioned, these are older clinical data that the sponsor happened to have access to. And of course, it raises lots of ethical considerations now regarding the use of monotherapy; however, they did have access to this old data. And if there is old data out there that one can access, it certainly should be submitted to us.
So with regard to Hepatitis C, that guidance talks about an alternative to a factorial design study where sponsors can show

Page 47

the contribution toward efficacy of a multiple direct-acting antiviral combination using in vitro and clinical data. And the guidance goes on to describe that subcultural data showing that the antiviral combination slower prevent the emergence of resistance compared to single drugs. Our early Phase II data, where the addition of a drug to a combination improve sustained viral response reduces emergence of resistance. So the point being here that one can use a combination of in vitro and clinical data to make the case.
My last example is tuberculosis. And of course, we have the early bactericidal activity studies which are used to evaluate individual drugs and combinations of drugs with -- using a microbiological outcome in patients at early time points from 7 to 14 days. And the MDR-TB, if a superiority study can be done, one could look at adding the investigational drug with an optimized background regiment versus a placebo with an optimized background regiment. Of course, a lot depends on the efficacy of the optimized

Page 48

background regiment.
I think that's my final example. Yes, it is. So the assessment of the contribution of individual drugs to an antimalarial drug combination is challenging. And we look forward today to hearing from the panel what in vitro studies, animal studies and clinical studies could help look at this issue. And before I finish, I would certainly encourage sponsors to communicate early with division when they're considering co-development of antimalarial drugs so that we can address the kinds of questions that I've discussed earlier, early in development.
Thank you for your attention.
(Applause.)
DR. MCCARTHY: Thank you for that presentation. Our next speaker is Jim Kublin, who is director the HIV Vaccine Network based Fred Hutchinson in Seattle. He's also the medical director of the Seattle Malaria Clinical Trial Center, faculty member of the Department of

Page 49

Global Health at the University of Washington. Jim trained extensively in clinical research in HIV and malaria across South America, Asia, and Africa, including clinical trials of therapies and vaccine.
Jim completed his B.S. and M.D. at Georgetown University and then his MPH residency of preventative medicine at Johns Hopkins.
DR. KUBLIN: Thank you, Jim. And thank you for the organizers. As disclosure across the HIV TB and malaria fields, we're funded by GSK Novartis and Santa Fe.
I'll hopefully help set the stage for the application of CHMI to the therapeutic potential of antimalarial compounds. And we're focusing today primarily on the target product profiles for therapeutic purposes, but of course, we have extensive experience in applying the CHMI model for preventive drugs and vaccines.
We'll discuss briefly the methods of infection in the CHMI model of diagnosis, of product administration. Of course, highly

Page 50

dependent on the potential for the antimalarial drug and opportunities here and for discovery. So of course, the target product profile is first and foremost in the thoughts of individuals who are trying to develop antimalarial therapies for the purposes of preventive and therapeutic purposes.

For the purpose of today's discussion and focus on therapeutic outcomes, this is highly dependent, of course, on the plasmodium species; the focus and the control of severe disease. Control of further transmission, as was highlighted by James in his introductory talk and of course, in light of today's discussion, combination with other drugs. And personal interest is how diverse and complex oftentimes the endemic subjects biome is with the occurrence of concurrent infections.

As James highlighted, the malaria cycle is one that it first transfixed me in the early '80s with regard to my interest in the basic biology of the parasite, and particularly, the

Page 51

gametocyte oogenesis and fertilization in the mid-gut of the mosquito.

As a matter of interest, it's interesting to look back historically, and what breakthroughs occurred to make progress in moving forward into the development of the controlled human malaria infection models; the first being Ronald Ross's segment of work over 100 years ago. And then followed up by the second Nobel awarded for malaria in the application of the therapeutic value of malaria inoculation and the treatment of dementia paralytica at the time, eventually attributed, of course, to treponema and syphilis infection, making significant headway in the application of malaria infection for the treatment of neurosyphilis.

And this was most recently applied as late as the 1960s in which you see here an individual was intentionally infected with plasmodium in the great city of Boston in the 1960s. But of course, since then, we've applied the CHMI model extensively and in a very

Page 52

controlled fashion, and very much adhering to GMP. And now we're testing it, applying it for the evaluation of drugs and vaccines and have essentially three methods in which we can expose individuals to malaria, resulting in 100 percent infection rates among those in control arms. That's the sporozoite-induced malaria infection via direct venous inoculation, currently Sanaria is providing the crowd preserved sporozoites and of course, via the Gold Standard natural root of the infected anopheles bites. But also, thanks to James and his team, the induced blood stage malaria infection gaining great progress for the evaluation of acute therapeutic antimalarial drugs.

The methods of sporozoite-induced malaria infection, as I mentioned, include both the infected mosquito bite and via direct venous inoculation. We have an ongoing study in Seattle in which we have the opportunity to compare these two methods of exposure and infection to malaria in a clinical trial. And to my knowledge, is the

Page 53

only clinical trial that has the opportunity to compare these in identical cohorts. There are a variety of pros and cons to each methodology. The infected mosquito bite model is particularly, you know, I think the best for understanding any immunomodulatory effects, starting directly from the inoculation from the proboscis of the mosquito. And we know there are tremendous amount of dermal interactions between the parasite and people right up front.

There are some cons to this, as is highlighted by some of the subsequent slides in describing what is necessary for the rearing of these infected mosquitos. And similarly, there are pros and cons to the direct venous inoculation method. There are certainly some advantages by easier implementation, a lower cost by not having to maintain the insectary at the clinical research site and location. It appears to have a more consistent infectious dose and I think Sanaria is working on optimizing the crowd preservation process to improve the viability of

Page 54

those parasites. But it does, of course, bypass the skin immune system by directly inoculating through the vein and the sporozoites transfer directly to the liver without the intradermal exposure.

So the mosquito infection, of course, in Seattle we have the facility at the Center for Infectious Disease Research, in which we can rear the infected mosquitos. We pass these infected mosquitos eventually through a pass-through to the where the exposure does occur. They're returned to the facility for assessment of the bloodmeal dissection and assessment for sporozoites and grading of those sporozoites. And all of this is documented and we repeat this process until five infected bites with a greater than equal to two plus rating is achieved. And these are just images highlighting the process and the approximately seven weeks that it requires from the (inaudible 53:33) sites in culture to the ready and infected anopheles.

The mosquito challenged kinetics is

Page 55

something that we've been focusing on well because this is something that we want to compare the crowd preserved sporozoite application. And so far, without a direct comparison, they appear to be quite similar. This is data from an infection treatment vaccination study that we conducted and presented at Trial Med a couple of years ago, demonstrating in the red and green, some very consistent kinetics with regard to the emergence of the asexual erythrocytic stage. And in the black, highlighting individuals who did demonstrate partial immunity and protection to the asexual stage.

And then more recently we have experienced in Seattle with the malaria challenge, via the direct venous inoculation. This requires transfer of the prior preserved sporozoites to the clinical site and liquid nitrogen and dilution in PBS with the direct venous inoculation via tuberculin syringe, which is very quick and quite easy.

So in investigating and looking further

Page 56

at the crowd-preserved sporozoite challenge kinetics, on the lower left is data that we've just collected last month, reflecting a very similar kinetics to what we've seen in the mosquito. And Sean has compiled a comparison of the various crowd preserved methods of application, whether intradermal or intramuscular or the direct through the vein, and appear to be consistent with the thick blood smear and nucleic acid test. They're compared on the right.

And then there's been, fortunately, very good success by James and colleagues in the inoculation of blood stage malaria, evaluating the parasitemia of the falciparum red blood cell banks that they've established and just recently published on, looking at 78 percent parasitemia in those cell banks. There's confirmation of identity, evaluation of the viability. Of course, adventitious agent testing, identity testing and an extensive quality review highlighting that now with these red blood cell banks of infected RBCs, there's a tremendous

Page 57

opportunity to apply the inoculation of blood stage malaria model in future work.

Similarly, the growth kinetics has been published, appears to reflect that of the merozoites as they exit the liver and is very typical of the asexual replication in the periphery.

Moving on, methods of malaria diagnosis is also something that we'll be discussing and has various pros and cons with regards to its application. Of course, the standard in the field is the thick blood smear, rapid diagnostic test are also more frequently used now. In Seattle, we're using the quantitative RTPCR, and we're hear more about that from Sean.

So in our hands, the diagnosis versus clinical symptoms is something that we've had experience since our first demonstration project that we conducted in close collaboration with our good colleagues at Walter Reed to help establish the Seattle Malaria Clinical Trial Center.

We looked at the days of incubation

Page 58

period and the onset of symptoms. And here in the lower right, you can see that the blood smears were frequently positive after the initial presentation of symptoms with the application of nucleic acid testing, and in particular, that of the QRT PCR in our hands, were able to identify and diagnose most people prior to the presentation of symptoms.

So product administration and the methods vis-à-vis CHMI is also something that is under much consideration when looking forward to designing a clinical trial in the CHMI model. For the preventative and prophylaxis studies, I presented previously extensively on how we establish those different models. We've called them a time shift of single administration. That's being at fixed dose prior to CHMI and provides a tremendous amount of precision with regard to the PK and PD.

There's a dose de-escalation at a fixed time point prior to CHMI, and the, of course, we're looking at designing multiple dose,

Page 59

multiple CHMI exposures which may be more representative of the field.

For therapeutic studies, and particularly those in drug combinations, there's quite a bit more potential for these factorial designs. Questions around dose de-escalation, or escalation in the context of multiple combination is also something that can be integrated in such a factorial design. What the diagnostic threshold and the endpoint will be. The timing of the rescue therapy may be contingent upon the diagnostic test, intermittent presumptive therapy and how to translate IPT that may be the end target product profile reversed back to the CHMI model is something that we've also discussed. And again, this issue of co-infections and how that may impact anti-microbial chemotherapy and even drug resistance is an issue that's come up repeatedly and even more frequently.

So the method of product administration, of course, and the dose and the timing of that is highly dependent on the preclinical work and the

Page 60

first in man studies and which one looks at that early PK data, what the metabolites are and what the combinations may be, of course, will heavily influence how one takes this initial PK data and translates that into the clinical trial design.

And then what model of challenge, whether we use the sporozoite inoculation method or the inoculation of infected red blood cells is again, highly dependent on the factors I've discussed. This is an example of CHMI via the sporozoite inoculation method diagnosed with nucleic acid testing, which one does potentially provide a multiple therapeutic purposes, whether it's three days or longer is something that I think we have to consider.

In the case of thick blood smear, that will be shifted to the right and the application of drugs and the PK resulting from that, of course, will be a primary focus and target for the endpoints of the clinical trial. So we do a lot of work in HIV vaccines, and of course, the Holy Grail are the major focus of much of our

Page 61

research is to identify correlate of protection for further vaccine development. Pierre Gilbert is our statistician in that, and I know many of you have worked with us in this effort in trying identify correlate of protection in that area is a tremendous focus of our efforts.

We do have opportunities for discovery in the controlled human malaria infection model that I think is quite unique in the conduct of HIV preventative vaccine studies, we must go into the field and enroll thousands of individuals. And I think the CHMI model within our field here in malaria is a really tremendous opportunity to try to stay ahead of this wave of drug resistance that we've seen over the past 30, 40 years.

So with that, I'd acknowledge, of course, all of our study participants, as usual, but a tremendous team in Seattle based at Fred Hutch Center for Infectious Disease Research at the University of Washington and our funders and colleagues. Thank you.

(Applause.)

Page 62

DR. MCCARTHY: Thanks very much, Jim. I think we're running well with time, so we might see if there are any questions for Jim before we move on.

(No response.)

DR. KUBLIN: Most interesting talks to come, I think.

DR. MCCARTHY: Thank you. Thank you very much.

DR. NAMBIAR: Thank you, Dr. Kublin. The next speaker is Professor McCarthy, who needs no introduction to this group. So with that, we look forward to your talk on Induced Blood Stage Malaria: A Tool to Facilitate Development of Anti-Malarials.

DR. MCCARTHY: Thanks very much again. And thanks to Jim for introducing the topic. I first wanted to make a comment about nomenclature. We tend to use the CHMI acronym to describe what we do. We believe that it certainly can cause confusion locally in Australia because our IRB wants to know where the

Page 63

control group is. And we've got good data that we could share if there was more time to show that our system is very reproducible and therefore, we don't need control groups. And in fact, we are think it is ethically inappropriate to use a control group, not giving an antimalarial or given a different antimalarial. It increases complexity in the clinical trial design; so therefore, we've adopted to remove the word -- the letter "C" from our studies. And therefore, referring it to induced blood stage malaria. But I think I'm fighting a losing battle in terms of the literature and the nomenclature.

So with those comments, I'll move onto, just quickly, my disclosures. We've worked with both Novartis and Sanofi in some of the clinical trials that we've undertaken. So what I wanted to do in this talk is really to outline how our clinical trial system works. Really, not taking a historic approach but actually describing what we actually do today. Because as you'll see from

Page 64

the talk, we've been doing this now for several years and have had major developments in terms of how we do it.

I wanted to also discuss in some detail the study endpoints because they are obviously why we're doing the study, how we describe those. I want to also talk a little bit about generalizability. It's certainly a question in the field that we're using this time laboratory strain of plasmodium falciparum that would derive from an airport worker in the Netherlands in the 1970s and how is that in any way relevant to describing what will happen to patients with clinical malaria in endemic regions.

I wanted to also talk about safety issues. Safety is obviously extremely important in conducting any sort of clinical trial, but when you're giving a potentially lethal parasite infection to healthy human volunteers, there's obviously very major issues in terms of study safety. We would also like to talk about ethics, but we really don't have time for that today.

Page 65

And then if time permits, we can discuss a little bit about the future options of where we think this field may go.

So this is the outline of what we do. So what we've got here is our intravenous injection of effectively, 2,000 infected red cells on Day 0. So these are prepared by thawing out a prior preserved vile of malaria parasites that we have held since the 1990s. And then we have volunteers in outpatients. So they come in every day from Day 4. The phone calls take place in the first few days, and from Day 4, they would come in twice daily for a PCR test.

We have now accumulated data of over 170 volunteers, and I'll show you some of that data in a moment, to show that we reach a situation where we can get really very use PK/PD data by administering an antimalarial drug on Day 7. We admit the volunteers to our clinical trials unit at Q-Pharm for a period of three days, where we do PK sampling, as well as intensive PCR for getting the pharmacodynamic endpoint.

Page 66

We give a single dose of drug. We don't give more than one dose because we believe, and I'll show data in a moment, that we get adequate data from a single dose, and to date, have not been required to undertake the trial. We've given repeated doses. We obviously follow the volunteers after they leave the unit. And I'll show you data on rescue treatment that we give volunteers when and if they have a recrudescence.

We continue out to 28 days, and in fact, in some of our studies, we've gone out to up to 35 days. So we clearly have the opportunity to follow through recrudescence which is a really important endpoint. And then Sean will discuss later in the session some issues that have come up with regards to gametocytes, and I'll show you a little bit of data about this. And them more recently, we've become interested in looking at transmission as an endpoint when we're looking at transmission blocking activity of the antimalarial drug.

And a number of subjects that have been

Page 67

through this particular system now amounts to 178 people. So we've got data on quite a large population of subjects that really allows us now to get some really quite useful statistical analysis that we have yet to publish, but we are very confident that this information will be extremely useful when it comes to having regulatory interactions about what we're doing.

So here's a hypothetical -- it's not actually a hypothetical, but a redrawn clinical study in one single patient. So this is parasites per mL on a log scale and days on the X axis. And this is the typical growth in parasitemia that we see. This is incredibly reproducible. In a log scale, we first see parasites detected by PCR on Day 4. We see the typical sign of exponential growth phase of our malaria parasites. And we typically treat volunteers when they reach the threshold of parasitemia that you will detect with a blood smear of the order between 10 to 50 parasites per hour, which is 10,000 to 50,000 per mL. We give

Page 68

the patient the investigational drug and we typically see quite a rapid fall in the parasite levels in the blood by PCR and able to intensively sample by PCR the level of parasites in the blood over this time period.

You'll also notice, interestingly, and you'll see this in further data later on that there's this typical lag phase that we see with many of the antimalarial drugs which goes back to the early talk where we discuss the fact that many of the drugs only work against certain life cycle stages of the parasite.

We also see typically what is called the tail phase, when the parasite killing tends to tail off. And that's often due to the fact that that we're seeing clearance for the drug and therefore, decreased rate of parasite killing. So what we then do is undertake statistical analysis of the log linear phase of parasite clearance. So this is basically where we use a statistical technique to actually eliminate the lag phase and the tail phase and then using a

Page 69

modeling approach to actually measure the slope of this curve. And this is one of the key pharmacodynamic endpoints that we identified in our clinical trials. And the more recently accepted version of this is the parasite clearance half-life. So this is a measure of how quick your drug kills the parasite.

As well, we very frequently see recrudescence. And this is actually data from a single patient which has been redrawn. So we can see the parasites have come back and we've been able to watch them come back. What we see if we get rid of those particular things and then superimpose upon this the drug concentration, this time graphed on a log scale as well, so you see a rapid increase in drug concentration when the volunteers administered the drug. And then with log transformed there, you see a linear decline in drug concentration if you're dealing with a drug with first order kinetics.

Now, if you focus on this time point here with the volunteers, you've reach the asymptote

Page 70

of the parasite clearance curve. Here, you see the situation where parasite replication is equivalent to parasite killing. You're in an equilibrium situation. So if you draw a vertical line from this period of equilibrium up to where you reach your parasite or your drug concentration at that particular time point and then drop that line across to your drug concentration, this is actually a very good approximation of what the MIC of your drug is in your volunteer.

So what you've done in a small group of, and typically, I didn't say before, we typically do this is cohorts of eight volunteers. We effectively identified the MIC of the drug as well, as I've previously showed, the parasite clearance half-life.

So, you know, a very small study of eight volunteers we've collected two very key parameters and able to inform further development of the drug. This is data from a study that we published last year. Again, the parasite

Page 71

clearance is drawn out over a different timeframe with the lag phase and the tail phase. And you can see here some lovely reproducible log linear parasite clearance kinetics so that we can statistically model and then to perform an optimal regression line with a 95 percent interval. And you can see this data is very tight and we're able to really get, I think, very accurate estimates of this key pharmacodynamic property of the antimalarial drug.

So you can then go on and do modeling. And I'm not a modeler at all, but even someone like me can understand that the rate of parasite clearance, over time, it's dependent upon growth; parasite growth versus clearance. And the drug effect can then be modeled in here. And using differential equations with the data that we accrue in these situations, instead of modeling packages, we can really get quite useful data on this particular precise mathematical modeling. Jörg, who will follow me, will discuss the utility of getting these key data from the human

Page 72

challenge system so we can then develop a really good understanding of what the pharmacodynamic PK/PD relationship is between your drug and the parasite growth and clearance.

So many people ask me and I raised the question before, well how does the parasite clearance I see in my very subclinical malaria relate to what is seen in patients with clinical malaria?

So going back to the old literature, in fact, much of the old literature describes parasite clearance of blood smear and there is very little kinetic data available in the old literature about how quickly parasites had cleared by serial blood smears. But there are, as was mentioned in Elizabeth's talks, some very useful historic data -- and this is data from the study of mefloquine that was done in the 1980's where two studies were undertaken, one in Africa in children and adults with falciparum malaria, and one in Thailand in adult toy soldiers with chloroquine-resistant falciparum malaria. Both

Page 73

studies were published. So we extracted the data from these two studies and compared data from one of our clinical studies where one of our early clinical studies where we tested mefloquine as a single dose at 5 milligrams per kilogram, 10 milligrams per kilogram or 15 milligrams per kilogram. And just quickly, what we saw with five was that the drug failed and we had to rescue all the volunteers. With 10 and 15, we saw complete clearance of the parasitemia and you can see here quite nicely demonstrated the lag phase that you see with mefloquine in our human challenge system.

You also see that there is a five log difference here between the level of parasitemia in our human volunteers and the level of parasitemia in these clinical trial undertaking in Africa and in Thailand. But what you can also see is if you draw a linear regression of the parasite clearance in these human patients with quite severe malaria, if you draw the line of the parasite clearance as determined by a blood smear

Page 74

and compare that to the parasite clearance curve as determined by QPCR in our system, those two lines, the slope of those two lines are indistinguishable.

So this is one of our pieces of argument that makes us believe that what we really are seeing in the data that we're getting from our human challenge studies in this quite artificial system are actually clinically relevant and predictive of what's going to happen in the field with our experimental antimalarial drug. And that's not to say there may be exceptions with drugs that have specific effects. For example, one of the earlier ozonide compounds was clearly -- it had different properties, it's pharmacokinetic properties in patients with malaria. So this is something that needs to be closely observed. But certainly, at least this data is encouraging to say what's the data that we get in these very low level infections do have translational value in terms of what one would see in a real clinical trial in human subjects

Page 75

with clinical malaria.

So safety issues. So when it comes up, everybody asks me about this. So it's good to be able to speak about this briefly in this audience. There are obviously safety issues in terms of what's in this inoculant of malaria parasites. Are there any advantageous contaminants? For example, bacteria, viruses and prions. And I'm happy to report that the donor or the red cells are 20 years on from donating the unit of blood that is used to inoculate all my human volunteers still works in the pharmaceutical industry. So I think that speaks to his sanity that 20 years later he can still work in pharmacy. So I'm fairly certain that he doesn't have a prion disease at the moment.

There's also the issue of red cell alloimmunization we're giving these human volunteers, potentially, they had all this blood transfusion of the order of the couple marked liters of blood. And the question comes up do we actually institute an alloreactivity to donor red

Page 76

cells. We've actually done and continue to do a red cell antibody assays in volunteers, both at the start of the study and at the end of the study. And to date, in the 178 volunteers we've studied, we've seen nobody develop a red cell antibody.

So this also is consistent with the experience, in terms of generation of RHD antiserum for use in pregnant women. That's actually quite difficult to generate antibodies against minor red cell antigens, even when you give people 20 mls of mismatched blood for minor red cell antigens.

So then the other obvious safety question comes up, in terms of the malaria. What is the -- do we have malaria induced adverse events and severe adverse events? And I can happily say to this audience that we've seen no malaria-induced severe adverse events prior to drug administration in any of our volunteers. So people will get a small amount of fever. And I'll show you some data on that in a moment. But

Page 77

before treatment, we have seen no evidence of any safety issue arise. After treatment, we've seen some interesting side effects including a kidney stone that arose -- a left-sided kidney stone which made me worry that the volunteer had ruptured their spleen, but luckily, it was a renal colic and not a ruptured spleen.

We've seen a volunteer who went out to celebrate the end of the clinical trial in the usual way that college students celebrate the end of their exam and they had a fall from a height and broke arms, and legs, and ribs and ended up in their intensive care unit. So that had to be reported to their regulator, but we believe that it was not in any way related to the malaria.

(Laughter.)

DR. MCCARTHY: And then there's the issue of one with transmission that we have clear observation of our volunteers becoming PCR positive for gametocytes. And I'll show you this is a moment from one of our studies. We need to think about this as an issue, in terms of we're

Page 78

doing this in a setting where we may discharge someone from our clinic. And if there they are in a malaria infected environment, we need to worry about this. We are fortunate in Brisbane, although it's a subtropical area, we don't have malaria vectors in Brisbane.

So the safety of the inoculum. This blood has been given to 205 subjects at our site, 27 subject before I become involved. And as I said, 178 since then and 30 cohorts in 15 studies. So we've really accumulated quite a large safety database locally with this, as well as inoculum has been given to 55 subjects elsewhere in the world for vaccine studies conducted in Nijmegen in the Netherlands and Oxford in the UK.

As well as mentioned by Jim, we've actually improved the situation to develop resources in an ongoing -- in ways of developing other resources for doing a blood stage challenge. So we've successfully did a "wild type" P. falciparum. This was a patient who came

Page 79

back to our hospital with falciparum malaria, was shown to be infected with a single genotype. We've harvested his blood. We've tested it, validated it and released it. And I did a pilot study in two volunteers with a wild type strain. This is more-so to look at the vaccine efficacy, but it demonstrates the feasibility of looking at parasites with different genotypes and potentially different drug resistant patterns.

We've also remanufactured, under GMP, blood stage P. falciparum banks. I've got a 37 bank that we've tested in two individuals. The Goal Coast has manufactured an NA54 strain from the same strain that Steve Hoffman uses at Sanaria. As well, he also has a 7G8 bank that was recently produced. And some of these papers are in press or published.

As well, we've recently had great success in doing plasmodium vivax challenge studies using two banks, again, collected from patients back from endemic areas of plasmodium vivax. And this is greatly extended out to capacity. You'll look

Page 80

at plasmodium vivax, blood stage infection, both for our drug and vaccine development. And we now have data on 26 volunteers, using two different banks with plasmodium vivax.

So the other question that obviously comes up is can we identify recrudescent and safely rescue these volunteers? So on this slide that I've previously shown you before are drawn across the line of where one would find people being blood smear positive. And you can see here that we have got one, two, three, four, five, six, seven serial observations of PCR before the blood smears become positive. And we've been able to prospectively observe the recrudescence of infection way before we become blood smear positive and way before volunteers become symptomatic.

So we believe we've got several days of safety margin here present. And these are real data from a single volunteer. And in fact, in preparation for this talk, I went back and counted how many people we've had to rescue and

Page 81

the numbers are four. So I think that we can say with strong confidence in our system that we are able to protect our volunteers from having our recrudescent infection by ensuring, obviously, they come back to be tested, but also that we can identify people having a recrudescent infection way before they're going to become symptomatic and still being able to identify this key idea point, which really enables us to do pharmacodynamic modeling.

So we still are struggling with the issues, what is a safe treatment threshold. My modelers love to see lots of data points down here. So that means if we can get our parasitemia up to here, we get more data points, but at some stage or another, we are going to make people symptomatic. And we are not entirely sure this safety threshold is and we're obviously being very cautious about that. It depends as well on what your known drug potency is. So if you're working with a drug such as an ozonide antimalarial, well, you know you're going to get

Page 82

rapid clearance. You can probably with a little more safety margin. But if you're working with one of those more slowly acting antimalarial drugs where you want to be able to observe treatment effects over a longer period of time, you probably don't have the luxury of allowing your parasitemia get to a point where you may be seeing a clinical safety endpoint. So we still haven't answered this question clearly.

We've also developed a clinical score system. So we are really trying to standardize our way of recording what the symptoms our volunteers experience because this will be a way of comparing clinical outcomes and getting a good safety database that we can then accumulate that will ensure that what we're doing is really easy to record and therefore, gives everybody confidence, both our ethical committee, ourselves as investigators, and the regulator that what we do has got a reproducible system of collecting outpatient safety, in this case, volunteer safety data.

Page 83

So getting towards the end of this, I just wanted to highlight data from a just recently published paper where we're using the drug piperaquine. So piperaquine was developed by the Chinese back more than 20 years ago and there was very little pharmacodynamic safety data available for how effective this drug is.

So we were asked by medicines for malaria really to go back to piperaquine and do single-dose piperaquine to assess its activity. And as you can see here, these are two single volunteers in our study. And you can see in black shown here is the parasitemia growth in the volunteers. And in fact, in very dramatic and rapid clearance of parasites after a single dose of 960 milligrams of piperaquine.

What we then were able to do is follow these volunteers out, and this was because we had accumulated data on this. And in black, you see a reappearance of parasite genomes in the blood of the volunteer. And what you'll see here, and Sean will go into this is more detail is that

Page 84

there's reappearance of parasite genomes in the blood of these people, but at much lower levels than what one would see if there was an exponential increase in parasitemia. And consistent with that, you see this appearance of a molecular marker of a gene that's produced by female P. falciparum gametocytes called Pfs25.

So we see the appearance of this gene in the blood some seven to ten days after we treat them. And this particular transcript because you're using RT-PCR appears in the blood and persists for the duration of the treatment and it only disappears when you give the volunteers a dose of primaquine, which is known to kill female gametocytes.

So you see basically clearance of gametocytes and disappearance of the genomes from the blood, using your standard Q-PCR assay. What as well is present is that there's a molecular marker of asexual parasites. So this is an MRNA produced by asexual parasites, but not by gametocytes. And what you see on this X axis

Page 85

here are these red dots here. So you're seeing no replication of asexual parasites.

So if you then drop to this person down at the bottom, what you see in this person here is having a recrudescence because you can see there's a period of constancy in there, actual number of parasites in the blood, as well as a constant number of Pfs25 genomes in their blood. But what you're seeing here is this red line going up and that is an early appearance of asexual parasites in the blood that and it dates by three or four days, the appearance of the increase of genomes here. So this really gets to the point that we believe that we can confidently predict recrudescent infection by using a messenger RNA marker that is produced by asexual parasites.

So getting towards the end, we've also been working very closely with meds and some malaria. So this is a typical drug development pathway, where one would be doing a Phase I study to look at safety in pharmacokinetics of your

Page 86

drug. You would then move into a Phase II study; for example, doing -- study sizes of increasing complexity endemic areas with patients -- with all patients initially but then escalating to get efficacy for antimalarial drug.

Working with MMV, we've been able to develop an integrated program now where we can nest within the Phase I study, a human challenge study. So within one year, we're able to get safety and pharmacokinetic data doing a dose escalation study, but once we hit our target point for doing human challenge, we move straight into human challenge. So within 12 months, we can do a package of data that really is very informative for drug development. And Jörg will be following me, talking a little bit about this.

So we believe using this system, we can get really good early safety in PK data; obviously from the standard Phase I assay. But really, we can identify the dose for efficacy at a very early stage. So within the 12-month period, we've got data to guide a design of a

Page 87

later phase clinical trials. This has been working very closely with our local ethics committee. We've been able to generate quite a flexible adaptive design so that we build into our clinical trial protocol a range of options that we can go down, depending on the outcome of the first cohorts. We can also kill drugs early. The drug is not working in the system, we don't waste time taking in a later development stage. And I think you'll see an example of that.

And then we can obviously go back where we see a problem in terms of pharmacokinetic properties that suggest that we're not going to reach our input. We go back and reformulate before going into the human challenge.

So I just wanted to finish. Again, return you to the meds of malaria development pathway and look at the -- I think we have a good fortune, there is quite a large number of drugs in research in terms of lead optimization. There are drugs that are listed here that are without the permission of MMV that list some of the drugs

Page 88

that are going nowhere but are still listed on the website. Some studies that are in human volunteers and then studies that are actually now in more advanced development.

So what we do, we count the numbers here. So we've got eight drugs in preclinical development. We've got two drugs which we know are in the Phase I study already and we've got six drugs that are in patient exploratory. So then if you do the numbers, you've got 16 Phase I studies where you get Phase I and safety in PK. Now I think we've got ample global capacity to do 16 Phase I studies with these drugs. The problem comes is that you then need to do 16 proof of concept antimalarial drug activity. So you've got to go somewhere, do a clinical trial with people with malaria and figure out which of these drugs is worth moving on with.

In the global situation with malaria, where we need to do these studies doing these 16 proof of concept studies will become a logistic challenge. And then if you're thinking about, as

Page 89

the topic was started today, we need to then do a combination study. So if you go back to your high school mathematics and do the factorial analysis, this requires 120 possible combination studies to evaluated with these 16 drugs. This is clearly not an option that we've got available to us. And with the early discussion that we had about drug resistance, obviously we're not going to do 120 factorial designs, but we need to figure out if we need to do 20 of them. We really don't have global capacity to do this, probably within the bounds of my professional career, given this pace which some of these drug studies are done.

So I really think we've got to think creatively about how we're going to actually move the promising candidates from this particular pile here into this particular pile here. And given the conversations we had early on about how we need to combine these drugs together, how we need new targets and we need drugs with different targets to work together, we really do face some

Page 90

challenges that require, I think some creativity, both from a clinical trial design perspective, but also working very closely with our regulators so that we can actually reach a point where we can actually do something about the fact that we're really in (inaudible) therapy, and certainly in the Mekong region now because of artemisinin resistance becoming more and more of a problem.

So just in conclusion, in blood stage malaria provides a rapid, safe, and efficient means of having pivotal early efficacy data. It can be integrated and combined Phase I pharmacokinetic safety study, a standard Phase I study. And then it provides actionable data for modeling activities to predict clinical dosing for light stage studies.

So I just really would like at the end of this to thank all my collaborators, particularly my colleagues at Medicines at Malaria who has supported me along the journey that we've been over in these last several years, as well as

Page 91

funders from the Australian government and the Bill and Melinda Gates Foundation. And the wonderful team of people whom I collaborate with because these studies really are a very large team activity and in order to be able to carry this off successfully and ensure volunteer safety and good data integrity requires a really large team effort.

So with that, I think I might stop.

(Applause.)

DR. NAMBIAR: So are there any clarifying questions for Professor McCarthy. Yes? You might want to introduce yourself for the transcription. Thank you.

DR. PROSCHAN: Pardon? I didn't hear.

DR. NAMBIAR: I said it would help if the speaker introduce themselves so that the transcriber can capture your name. Thank you.

DR. PROSCHAN: Okay. Mike Proschan. You gave a differential equation for parasitemia over time. In that equation, obviously P is a function of time, but all those other parts of

Page 92

that equation are just constants, right? They do not or they do?

DR. MCCARTHY: I'm not sure. We can go back to it. I'm not a modeler, so you probably going to have to help me along the way here. There we go. So P is the parasite concentration. We know that. The parasite growth rate is at constant. We know that because we've done this in 178 people. Drug-specific parasite reduction ratio, we should be able to calculate that from the parasite clearance half-life, but you can solve this equation, obviously, different ways. And then there's a drug concentration effect, obliviously, and then the IC-50. And then there's also a fudge factor, which is the optional non-linearity parameter defining the steepness of the concentration effect.

This is not my work. This has been a standard equation that's been used in the past. And obviously, it could be optimized and there are people who are very skilled at doing this sort of work. I couldn't possibly understand the

Page 93

language that they talk, but certainly, it's my job, I think, to generate data that enable modeling activities so that we can really arrive at a more precise understanding of the concentration effect relationship between the drug of choice and the parasite. And particularly when you start to look at model drugs. I think you all will be talking about how we can use this with a combination therapy. Obviously, the complexity increases, but we believe that you can use these sorts of approaches to actually model combination approaches and that's the standard approach that is in other clinical pharmacology applications.

PUBLIC COMMENTER: Thank you. It was really very well done. I had one specific technical question. When you're looking at the rate at which parasite counts fall and you're looking at the slope, did you see any consistent differences in the slope when the drug like artemisinin was being used as opposed to say drugs like piperaquine or mefloquine or Coartem?

Page 94

DR. MCCARTHY: So in the interest of time, I didn't put up a slide, but obviously, one of the luxuries I have is being able to put up a slide with every single drug I've tried and you see very dramatic differences between them all and you get a very quick read-out. About a week after I begin the study, I can graph out, in a preliminary way, how the drugs are doing, feed that back to the sponsor and say well, look, this is how your drug is doing. And obviously, it takes a little longer to get that data all formalized, but certainly there are very significant differences.

With the artemisinin, it's an interesting story. It was the first drug we ever used and we weren't as good at doing it as what we did then. So next year, one of our plans is to go back and look at artesunate with a Kelch mutant parasite to see what the effect is there. So we do have lots of opportunities now because we've got a lot more expertise, in terms of design of the studies sampling frames, getting our Q-PCR working as

Page 95

best we can so we can really improve data quality as we get better at doing this.

DR. NAMBIAR: Dakshina.

DR. CHILUKURI: Dakshina Chilukuri, FDA. You've shown one slide which showed the recrudescence source of the safety profile for the one patient and you said that there were 70 0or 80 other patients that you rescued.

DR. MCCARTHY: Yes.

DR. CHILUKURI: Did you see the profile for any other patients, a similar profile itself?

DR. MCCARTHY: Sometimes we see PCR being completely negative. So we interpret that as being the parasite count as below the limit of quantitation of PCR. So there may be only 10 viable parasites in the body. They may be sequestered somewhere and therefore, potentially protected from a drug. In fact, in some of our studies, we see recrudescence upwards of two weeks after the parasites have disappeared from the blood. So we certainly don't give up on them. We increase our intervals between PCR

Page 96

because they get sick of coming back every day for blood tests, but we certainly see quite frequently light recrudescences. I mean, in the malaria community, they go out to six weeks. We don't have the luxury of being able to do that, but our volunteers are not immune, so light recrudescences in an immune population, you've got to deal with the fact that there's probably an effect of the immune system in parasite counts.

You make a good point that maybe three weeks after treatment may not be sufficiently long to absolutely identify everybody who's going to recrudesce. We do, however, at the end of treatment, send everybody home, having been given a therapeutic course of Coartem. So nobody leaves our study without being cured of potential (inaudible) malaria.

DR. NAMBIAR: Maybe we can go to the next.

DR. MCCARTHY: Yes. Maybe we'll move on now. Our next speaker is Jörg Möhrle from

Page 97

Medicines of Malaria. He's the head of translational medicine, MMV. A career in development and pharmaceutical and biotech, followed by joining MMV in 2005. Since 2010, he's head of the translation medicine team and brings the new drugs from the laboratory to proof of concept in patients.

Jörg obtained his PhD from Basel University for work on protein kinases Plasmodia, and in 2006 he attained his MBA from Lorange Institute in Zürich and SUNY New York.

DR. MÖHRLE: Thank you for the introduction and especially for outlining the blood stage challenge studies so then I don't have to explain so much.

So I'd like to talk you through what our challenges are in moving from the challenge, that is from the early phase human studies into combination studies. I would like to illustrate and take you along a story of MMV's project OZ439 and DSM265. These are two projects -- OZ went in demand the first time in 2009, DSM in 2011/2012.

Page 98

So it's really -- I want to show you how we learned ongoing and I want to also show how the ongoing learnings from the different studies helps us to really get to better study design, better dose selection for the latest trials. I'd like to show through that journey how we can also show in early phase trials the contribution of each compound in the effect on malaria and briefly some words on the impact this could have using this early stage controlled human malaria infection trials, combined with Phase II A trials, what is the impact on developing new drugs and bringing new drugs to the market.

What our challenges are, I think we heard this morning, we need combination treatment to ensure that patients are cured and no resistance is developing. One of the questions is how do we get to the right dose of each individual component later in the fixed dose?

We don't have historic data. We're talking about NCEs. We don't have historic data like with lumefantrine or with piperaquine where

Page 99

they were used in monotherapy. There are operational and ethical obstacles to conduct full factorial design studies in the relevant patient populations. Remember the maturity of malaria patients are children. If you want to run full factorial design studies in the pediatric populations, the maturity of these children in the trials will be either on doses that are too low or too high. Not optimized. So we have to find a way to go into the target population for malaria with limited small number of doses that are likely to succeed and likely not to overdose.

Operationally, I think James made a very, very good point. The number of new trials in the pipeline and the number cohorts in the full factorial study make this study huge. If we have several full factorial Phase II B studies with different drug combinations, I'm not sure whether they are enough clinical trial sites in the world that could handle that burden.

Again, large monotherapy studies in clinically infected patients are not advisable

Page 100

because we will develop -- or we might already develop resistance at that stage in clinical development. I will also say change alluded to what I call the MIC study; so studies where you give a single dose, not a curative dose. You observe parasitemia and PK over a period to see when do we reach the nadir of parasites and when do we see regrowth of parasites? These are doable in the field. MMV has done a study of part of this, but they are very, very difficult to conduct in the field. So we need to find other ways to do this MIC studies in a better controlled -- where we have better access and where the volunteers or patients have better access to the healthcare facilities.

The question is yes, a lot of people ask me, "Why are you doing this, Jörg?" This is interesting experimental medicine but how can you use that later to really transfer the information into the clinical studies. So that is one of our challenges. And then yes, one question is we have here studies, the challenge studies where we

Page 101

have parasites per milliliter and in the field, we have parasites per microliter. So it's about 1,000-fold higher parasitemia. Can we extrapolate the information on MIC, on kill rates or parasite reduction half-life from the challenge studies into the real live situation?

So these are some of the challenges we are facing and I hope I can at least give answers to a few of them. Again, change is already shown. Most of that explains the graph of how the challenge studies are done. We are collaborating now with QMIR and (inaudible) in six years. These are four publications that have been published recently in the last two months. So now, finally, we're getting to publishing the work we are doing. These are available now and they are open access, so everyone can access the papers.

So the K study, or the story I want to take you along on two molecules in MMV's pipeline, one is OZ439, a fully synthetic ozonide, where we conducted the PoC study in the

Page 102

field. And the challenge study in this sequence, so it's the other way around. We went first into the field before we had the challenge study available. And then DSM265, a DHODH -- DHODH inhibitor, specific to falciparum malaria where we did the Phase I and the challenge study within one protocol. And we actually used both the information we generated in the previous study, but also the availability of these molecules to a combination challenge study last year at QMIR.

So OZ439 Proof of Concept Study, this was -- it's a new chemical entity. We did not know how it works against parasites. We went into patients because at the time when we did that study in October of 2010, the challenge model was not that developed yet.

Based on the discussion we had with the investigators, with the ethical committee, the study design was that the patients received, when they presented to the hospital with clinical malaria after confirmation that their criteria were met, they received a drug. They were

Page 103

observed for 36 hours. And after 36 hours, the patients received standard of care quarantine.

So the observation period, what does the drug OZ439 do to parasites lasted 36 hours. Afterwards it's mixture of quarantine and OZ439. So the output of this design of the study was that we had information is yes, the drug kills falciparum malaria. We could estimate the parasite reduction rate, the parasite clearance half-life, parasite clearance time and fever clearance time.

The study was conducted between October 2010 and May 2012. We had four, since we did not have any prior information of how much drug we need, what is the potency in humans. We actually had an open sequential cohort design and we recruited four cohorts of 10 volunteers or subjects each.

This slide, you have already seen from James. So what we are interested in is the parasite reduction clearance half-life -- sorry, I'm away from the microphone. Where is the curve

Page 104

turn? Where is the midlevel concentration where parasite regrowth starts again?

With the 36-hour design, we only could measure to the black line. So the next study we conducted was a challenge study where we investigated single doses of 100, 200 and 500 milligrams of OZ439. And at that time, we only could observe until study dates 16. So again, that was the early phase of the challenge. We could really see here are the individual graphs and here is the 100, 200, and 500 milligrams. At 200 milligrams, you can really see parasite PK line and regrowth. And if you look at the individual graph here, you can also see that we can estimate the nadir of the parasite growth and overlay that in green with the PK information and ozonide exposure.

It's also interesting that this study was conducted between September 12 and February 2013. So within half a year, we had three doses with the full information of parasite reduction rate and MIC and parasite clearance half-life. We

Page 105

have done in the meantime also done an MIC study in the field where patients presented, got a single dose of OZ439 and were observed over 28 days because the patients had to come back every day to the clinic in the field base. That study took one and a half years to recruit. Here we had half a year.

So the data, I have here the PRR, the parasite half-life of the MIC data between the field study and the challenge study is comparable. So there is no difference between the PRR and the MIC of no significant difference between whether it's in patients or in volunteers. Taking the learnings of the OZ program, we then went with a new compound, DSM265. And as James has already explained, it was one protocol single-dose in healthy volunteers and nested within that protocol when we reached a dose where we thought it has an antimalarial effect. We had a cohort of challenge volunteers.

The volunteers received 150 milligrams of

Page 106

DSM265. Four out of the seven volunteers experienced a recrudescence. The estimated PRR was two and the MPC estimated to be between 900 and 1,400 nanograms.

Based on that information, a new estimation for the human efficacious dose was made to be around 320 milligrams. We tested and used that information to set up a proof of concept study in patients. First of all, because we had already these prior information, we were allowed by the ethical committee to extend the observation period from 36 hours in the previous protocol with OZ439 to now a full 28 days. So the patients received the drug that were in the hospital until they cleared parasites and could then go home and return on a regular basis for a follow-up.

So we have now data of over 28 days for the patients. What is also interesting is that we had selected a stocking dose of 400 milligrams. And in the first cohort, 12 out of 13 patients were a treatment success. We dropped

Page 107

the dose to 250 milligrams, and there we had seven out of 10 volunteers or patients that were a treatment success.

If we compare the OZ proof of concept protocol, having the challenge information with better data because we can follow up for 28 days, we have PRR parasite clearance half-life estimation of MIC, but we also, instead of having to treat four cohorts to get some information, in this case, we got with two cohorts, a very good estimation on the dose and efficacy.

And now we use the information of both OZ439 and DSM265 to do a combination during this trial. We wanted to see what is the effect of the individual doses and we selected, deliberately low doses of both compounds. Remember DSM265, we had 150 milligrams for treatment for recrudescence out of seven. With OZ439, we had eight recrudescence out of the eight cohort.

We did some modeling work. And the prediction was 1,000 milligram DSM265, we will

Page 108

have a 40 percent treatment success or six failures. And with 200 milligrams OZ, 50 milligrams of DSM265, the 28-day success rate was predicted to be less than 5 percent. We didn't really trust ourselves yet, therefore, we said let's go with the higher dose before we don't see anything of addition.

So we started with 200 milligrams and 100 milligrams. And four out of eight volunteers had the recrudescence before the end of the follow up period. So close to the 40 percent. And on the bottom, you can see the estimation MIC. The apparent MIC of OZ in the presence of DSM and apparent MIC of DSM in the presence of OZ439. And I have a summary table later.

With the 250 milligrams of DSM, I think we only had unfortunately, five volunteers because of a recruitment issue, but we really can see the patient -- the parasite reduction and then the regrowth in the majority of these volunteers which had a very, very good handle on estimating and calculating the MIC -- that parent

Page 109

MIC for both drugs OZ439 and DSM265. And this is the summary table. So if you look at the single dose OZ439, single-dose DSM265, OZ in combination with a 100 milligrams DSM, OZ 200 with the combination of 50 milligram DSM, we can see there is an additive effective which is significant on the PRR for OZ439 from 2.2 to 2.8; from 2.2 to 2.7. And we also see that the MIC of OZ439, the apparent MIC in the presence of DSM, 100 dose goes down to .3 and in the presence of 50 milligrams goes down to 1.2 nanograms per milliliter. Similar apparent MIC of DSM265 is reduced in the presence of DSM265. So I think it's very clear that we can, in that model, by using two non-curative doses demonstrate that both drugs have an effect on the parasite and that effect is additive.

So the contribution, I feel we can demonstrate it very nicely. You've seen our model for the parasite growth and kill rate. We can also calculate a factor for the contribution of both drugs in that model now. So it's

Page 110

possible to quantify the effect and have a model for both drugs together.

So now, this is not the end of the story. We are using the updated model and the updated information based on the combination challenge study to prepare a study in the field. This planning is ongoing. One change, obviously from the challenge study into the field study is nontherapeutic doses are not acceptable. We cannot, in that field -- in that area, we want to do the study, we cannot have patients coming back every day and induce treatment failures.

So we're looking to select two cohorts, both cohorts with an aim to have an efficacious, curative dose. And a two-dose combination that predict treatment success based on the PK/PD modeling of the three controlled malaria infection studies that I just described.

And this is what we propose for the MMV drug development of combination drugs. We were looking at animal data and I haven't talked about that. Animal data of scid mice infected with

Page 111

monotherapy and combination, used that data, analyze, model it to prepare the human challenge study. Used the human challenge data to prepare a field study monotherapy but also field studies in combination. But at the end, using all these data from monotherapy human challenge doses, combination human challenge studies, monotherapy and combination Phase II A studies to be able to move into Phase II B already with a combination and with a limited dose so that we can avoid full factorial design studies at this stage.

So I hope I could explain that controlled human malarial infection studies, plus modeling and simulation were successful in generating already in Phase I, pharmacodynamic information by including challenge studies into the classical Phase I programs. That we can reduce the size of the first in-patient studies, OZ439, four cohorts, DSM265. Two cohorts, we can generate more and better data because we will have more studies, better studies, lower follow-up in the field. We can reduce the overall timelines

Page 112

because a) the studies in the field will be smaller. And we don't have to do additional studies in the field like what I described as the OZ439 MIC study that took one and-a-half hours to recruit patients. And I think these exams also shows that we can demonstrate the contribution of each compound on parasite reduction rate, apparent MIC and the probability of success through the challenge studies. So that early, we can already show the contribution of the individual compounds, even in combination.

And with that, I would like to thank you for inviting me and to thank our patients, the volunteers, their caregivers, our departments, especially the clinical side and especially the sides in Brisbane and in Seattle for the volunteer studies. Our mentors, our advisors and our colleagues and our funding partners, without them wouldn't be possible.

(Applause.)

DR. NAMBIAR: Thank you, Dr. Möhrle. Thank you to all the speakers in the first

Page 113

session this morning. So we'll take a 20-minute break and we'll be back at 10:50. We'll have a few minutes to ask clarifying questions of our four presenters this morning before we go into the panel discussion.

Thank you.

(Brief recess.)

DR. NAMBIAR: All right. So in the interest of time, we're going to get started. We see that you are all having a very interesting and robust discussion, but it would be great if people could take their seats so we can get the first panel discussion going because we have only about an hour to discuss many important topics.

Before we start the panel discussion, we wanted to check if there might be any clarifying questions for any of the speakers this morning. Does anyone on the panel have a question for the speakers?

DR. MURPHY: I have a question for Dr. McCarthy. I noticed in the recrudescent curve that you showed, I realize it was just from one

Page 114

patient, but in the initial phase pretreatment, there is the very characteristic secondary saw tooth rise of parasitemia. When people recrudesce, there was not that rise, there was a slower slope. So I'm wondering whether in addition to the point at which you declare that they are recrudescing, whether there's information to be gained in a secondary rise in parasitemia.

So for instance, if you saw an immediate secondary saw tooth slope, you'd say there's no adequate drug on board. The parasite is exactly back to its wild type state. And if there is a gradual slope with no saw tooth, you'll seeing persistent drug effect. Does that tell you anything about the drug?

DR. MCCARTHY: I think there are two questions there really. The first is that we don't always sample it at the same frequency. So when we are doing the early stage of assessment, we're sampling twice daily. So we've got a really good chance to actually identify that saw

Page 115

tooth rise and fall of parasitemia as sequestration takes place. But when we're doing assessment for recrudescence, we're not doing nearly as rich sampling. So I think that might be a sampling out effect. You do also raise the question of whether we can identify in vivo induction resistance. In the DSM265 study, we had clear evidence from preclinical data that it was possible to induce the resistance. And one of the potential mechanisms of resistance was a mutation in the target enzyme. So we were able to actually retrieve sufficient parasite DNA to sequence across the target at which resistance had been induced in vitro and demonstrate that the parasite genotype hadn't changed from the early stage of the parasitemia until later on.

DR. NAMBIAR: Any other questions from the panel for the speakers this morning?

DR. KUBLIN: James, I also had a question. Have you considered nucleic acid testing for either the discovery drug or the rescue -- the study drug or the rescue drug in

Page 116

the course of the blood stage inoculations?

DR. MCCARTHY: Deep sequencing is certainly something that's becoming increasingly sensitive in terms of being able to do single cell sequencing. And I think that's certainly the way things are going. At the moment, we haven't sought to do that, but one of the things that we are very careful in doing is preserving all nucleic acid material for purposes, for example, working with Sean and other, we're looking at market discovery to try and understand parasite biology, particularly focused on gametocytogenesis. So we'll looking at transcriptional activity, for example, of different singling pathways that may be important in terms of gametocytogenesis.

So I think all those things are going to be possible, it's just a matter of how many hours there are in the day and can I interest a molecular biologist to do that sort of work.

DR. NAMBIAR: Are there any questions from the audience for the speakers this morning?

Page 117

PUBLIC COMMENTER: I just wanted to ask a question of how predictable is the MIC that you are determining in the scid mice for the data that you have seen in your challenge model or for the data you see in the clinic. I don't know whether --

DR. WELLS: So just to rephrase the question. In preclinical, obviously we do cell biology studies and then was the routine testing vehicle. These days we use a scid mouse, so it has human red blood cells and P. falciparum, and we do it in two test centers. One is actually done GSK is a service to everybody in the community. So we see from the mouse model then we get parasite reduction rates and we also get an MIC. So the first thing is that the absolute parasite growth rate, so we're talking about growth and death before. The absolute parasite growth and death rates are different in the mouse from human, even though it's the same parasite and the same host cell.

But the correlation between the parasite

Page 118

reduction rates in generally quite good but not perfect. In terms of the MICs, the MICs actually transfer really well. I mean, James showed a paper that he's just published which has mefloquine in patients and volunteers, but we actually forced the mefloquine data in the scid mouse so you can actually see the correlation across.

I guess the question then becomes later on we do see some nuances. So for example, you'd expect that all of the formula quinolones will be equally active in patients and in volunteers and they're not. So I think it's fair to say it's good to use the scid mouse model as a way of triaging, but finally to get the data in real people is much, much more accurate for producing the clinical outcome.

PUBLIC COMMENTER: So I would like to perhaps plant the seed and ask a question to the colleagues from the FDA. You know, the flip side of antimalarial treatment is antimalarials that prevent infection. And I was wondering whether

Page 119

in fact the CHMI model, which has been used extensively, first in the vaccine and now being adopted very nicely in drug, whether there couldn't be a regulatory strategy of approval of antimalarials that prevent infection. It may not be the purview of this meeting today, but it ought to be at least considered. And also for antimalarials that interrupt transmission. I think those are two large efforts in the worldwide malaria community. And one could envision using CHMI as a regulatory strategy as the FDA has recently adopted approval of vaccines for (inaudible) based on CHMI alone.

So I'd like to hear maybe any perspectives, if that would be appropriate.

DR. COX: Yeah, so I think the question may go beyond what I can answer right now. But I think part of the panel here today is to have a discussion about these models and their potential utility. I do think that the models provide a lot of important information about how a drug is working. And the question is really how far you

Page 120

can take it. So I'm glad you're here. I'm glad you're asking the question, but let's talk some more about the models and see where we get today.

DR. MCCARTHY: I'd just like to make a comment as well. I think if you're able to define the MIC in vivo, then hypothetically, that should be the concentration you're going to need to maintain your blood stage prophylactic agent at in order to prevent blood stage infection. That's not to say if you're looking at causal prophylaxis in the liver, there is blood stage activity if we're able to define an MIC in vivo then that will be very informative.

And certainly, the data we have shown with primaquine, in terms of clearance of Psf25 as an endpoint of clearance of gametocytes, I think there is an ongoing interest in using the CHMI system that we have developed where we can actually deliberately make people gametocytemic. As a potential exploratory approach to validate preclinical data on the activity of antimalarial drugs against my own female gametocyte, which is

Page 121

certainly an important piece of the puzzle in terms of informing priorities for drug development.

MR. CLAY: Thank you. My name is Bob Clay. I'm a consultant to MMV. I also have worked in the pocket with critical path NTB. And I wanted to raise an issue and hope that this is discussed. What we see in malaria with the challenge that you've seen today and Dr. O'Shaughnessy's presentation highlighting viral diseases and TB, my observation is that we have an opportunity here to do something we really can't do with an EBA model.

So I think it would be useful for you, at least from my point of view, to compare and contrast across some of the different diseases and how reliable you think this information may be. I just wanted to highlight that. Thank you.

DR. NAMBIAR: So thank you for that comment. I think that takes us right into the panel discussion. So we do have five questions and we have about an hour to discuss them. Some

Page 122

of these questions, at least one of them has five sub-bullets. So we need to keep time in mind.

I think we have seen some promising data on how CHMI studies can be used for drug development. There are certainly some unanswered questions that we need to work our way through, but certainly encouraging information at hand so far.

So with that, I would be interested in hearing the panel's thoughts on the first questions which pertains to the CHMI studies and how one can use that to assess the effect of individual drugs. I think that the specific areas that we really look forward to getting your input on how one can use CHMI studies to predict the efficacy of a new drug to assess the effect of the drug on later endpoints, because typically these endpoints in CHMI studies are sooner than what we would use in clinical trials. Generalizability of the findings, which did come up in the presentation by Professor McCarthy given that certain specific strains are used in

Page 123

CHMI studies and certainly differences between that and what you would see in a field trial and how one might use the result of the CHMI study, again, it did come up in Jörg's presentation to design a future clinical study.

So I think these are the topics we would like to cover under the umbrella of the first question and welcome thoughts from members of the panel. And certainly, we'll take comments from the audience as well. Yes, Karen.

MS. HIGGINS: Regarding the assessment of drug effect on later endpoints, I noticed from the talks earlier today that in fact you do follow people out to 28 days, and in fact, maybe a CHMI study could be used to assess the later endpoint. Is that true?

DR. MCCARTHY: Yeah. It's certainly possible to go out. We find that our volunteers start to lose enthisiam to come back and have a blood test every day after about three weeks. We have been successful in increasing the interval between assessments, but you need to realize, the

Page 124

light public, particularly focused on the -- and I know there has been publication both over this side of the Pacific Ocean as well as in Australia about how much we're bribing our volunteers. So there's some really clear ethical and practical issues about extending study durations beyond a month that limit our ability.

I think the other thing in our favor is that we're dealing with non-immune. So light recrudescences in immune populations probably occur partly because we've got an immune effect on retarding parasite growth. In a non-immune population, I would propose that you're going to see recrudescences earlier.

DR. WEINA: Well, since nobody else will say anything, I'll jump in. I usually say really dumb things, so we'll get the ball rolling. The idea of CHMI studies and moving toward regulatory approval, the questions you have are actually quite interesting and I'd like to kind of turn it around and say why are we sure that the traditional trial methodology that we're using is

Page 125

any better or actually even gives us good information. If you look at infectious disease clinical trials versus, say, something for a new cardiac drug or a new lifestyle drug, the size is huge, as far as the difference. When we go out and do a Phase II in an endemic population or a Phase III in an endemic population, the amount of information we gather is very hit or miss. We gather so little amount of data that is out there. We really, you know, just addressing some of the issues like, you know, how many strains are going to be necessary? I mean, where did we say well, two Phase II's are good? Or two non-human animals populations are good enough? Where was the analysis that was done that actually came up with that?

But when we look at what information we're able to gather from such a small group of individuals, very carefully studies, looking at the PK and the effect on parasite clearance and everything else all put together in one tight little package, the information we gather from

Page 126

even eight people is better than we get out of 300 in a typical Phase II.

So the idea is, at least in my mind is that even ethical with this information to continue to use Phase II trials and Phase III trials as the basis of approval when we're getting so much better information and so much more controlled data out of the CHMI. And it just comes, as I said, I think it kind of comes down to the ethics of the issue of the ethical argument of the other. But the issue of cost and time associated with the development, the idea that we can do better dosing optimization and everything else, and early kill design for getting rid of drugs that are going to be a problem for us with very small populations rather than exposing huge endemic populations to a clinical trial that's probably flawed.

So my argument and the question that I think people should take on and think about is that even ethical with this background information for us to continue do in our

Page 127

traditional trials and shouldn't we be using the technology that we have and the massive amount of information that we're getting to modify how we approach regulatory approval.

DR. COX: Thanks, Pete. I figured that was the place to start. In your comment, you raised a lot of issues. Let me see if I can sort of sort through at least a few of them. So the Phase III trial really is designed to try and study the drug in the way in which it would be used in the population it would be used. So there's going to be heterogeneity in the patient population. There's going to be different strains and you're going to gather information that really should help you to understand how the drug would be used in the real world.

So there may be something specific that you're talking about when you say the trial is flawed. And I'm not exactly sure what you're referring to. I mean, I understand that you may be saying that it may not be the most efficient way to gather information; there's heterogeneity,

Page 128

there's noise. There is reinfection and other things that make it a difficult thing to sort of sort through. And maybe when you say "flawed," that's what you're referring to.

DR. WEINA: The whole argument that we get into as far as the difference between effectiveness and efficacy of a drug. You're absolutely right; how it's going to be used in the real world. But it's just like kids are remarkably resistant. They are ruined by their patients. Our patients are remarkably resistant to not using a drug the way that we've asked them to use it, no matter what you put on the label because half the time the label is not read.

DR. COX: So I do think there is a certain degree of messiness and noise and otherwise that make the trial less efficient. I don't know that I would say it was flawed, per se. It's got some traditional Phase III trial will have some issues that can make it difficult to interpret in some circumstances. It can make the trial less efficient. And I think, what

Page 129

you're getting at is really -- I mean, the science and what you all have brought the science to really is fairly impressive. You know, the tools that you all have developed to be able to look at drugs, you know, their effect on parasite count is really quite remarkable, quite helpful.

And to be honest with you, there are two things; I mean, that information that you are able to get from the various different tools and methods that have been developed in these experimental infection models can really even make the Phase III trial more ethical because you're less likely to venture into a Phase III trial with a drug or a drug combination that's not going to pan out or a dose that's going to be less likely to be effective. So I actually think that the tools can help to make the Phase III trials better.

And I think one more aspect of what it is that you're bringing up here is how far can we take these models? How much can we get out of the models? I think that really is sort of what

Page 130

we're here talking about today to try and figure that out. I mean, is it that we can use the models to get the combinations correct, get the dosing correct so that we go into a Phase III trial and that we're in the best circumstance to be able to come out with a successful outcome? Or I think the point you're raising is does the science allow us to even utilize that data for even more and is it so good that we can understand more? We're actually hoping to see what folks think about that? What do folks think? It's a laboratory strain. It's a controlled setting. It sounds like maybe in James's model it's mostly non-immune patients. Perhaps, in some of the data that Jörg was presenting, it was immune patients. So just sort of sorting through the science, I'll stop there. Good point.

DR. O'SHAUGHNESSY: I just wanted to add, though, from the Phase III trial perspective, we do need the safety of the drug in the population in which we're going to study. So we definitely

Page 131

should limit the CHMI as far as we can, but I think in regards to safety, we need the numbers in the patients who have the disease for safety.

DR. WEINA: I mean, you bring up a great point on safety and that's always paramount in our mind and yet, we still, you know, when we talk about an infectious disease agent, we're willing to accept numbers of 300, or 400, or 500 versus thousands and thousands or tens of thousands in an anti-hypertensive.

So where do you actually draw that line? And where you draw that line for safety is never going to be enough until you've tested every single person, right.

DR. COX: So just in general, I mean, safety databases, usually we're looking at the benefit that a particular compound brings the seriousness of the disease, the degrees of unmet need. And, you know, if you look across a variety of different drug development programs, or I should say at the point that the drug is approved, you'll find that that number does vary,

Page 132

you know, based upon whether you're treating, whether you're prophylaxing, and all these factors do figure in. So usually, safety databases, you know, probably on the lowest end is something in the order of a probably like 300 patients or thereabout. That is sort of on the lowest end. You're going to see safety databases more in the several hundreds and getting them to 1,000 or a couple of thousands for antimicrobial drugs, depending upon the seriousness of the condition, the availability of alternatives and such.

I mean, it does seem that as we're approaching drug development, we ought to be thinking about, you know, we do need some safety data and trying to strike that balance point how much we need to understand the risk, how much we need to bound the risk of the drug, balancing that against the seriousness of the condition that it's being used for.

DR. PROSCHAN: Can I go ahead? It's always a scary prospect to try and use short-term

Page 133

endpoints to predict the longer term endpoints. I do think the earlier comment, though, if you could extend these CHMI studies to get the longer term outcome, you would feel a lot better, I think about using information from the CHMI and saying maybe there's not as much of a need for Phase II or III. But I don't know, I always worry about anytime you try and make a conclusion based on shorter term endpoints and think that that that's going to have an effect on the later endpoint.

DR. MCCARTHY: I think the other issue is the PK profile of your drug. So if all your drugs are gone after five days and you have seen no recrudescence two weeks later, I mean, I think there's no logic in continuing to follow the volunteer further beyond that. If you're dealing, however, with drugs -- and some of the drugs we work with MMV on have -- well, depending on how you look at it, really encouraging with long half-lives or long half-lives that may select for resistance in other people's mind,

Page 134

then you've got, obviously, to address that issue in your clinical trial. You can obviously address that as well by giving sub-therapeutic doses or being clever in terms of how you design your study. So I think there are ways of getting at it. But I do agree, if recrudesce is your endpoint, then you're going to need to carefully study the design to be sure that your study design will be efficient, in terms of detection of recrudescence. And I think a non-immune population is a perfect population to study for recrudescence.

DR. MÖHRLE: I think there's not a big difference between the long-term endpoints in Phase III malarial trials and the studies we are conducting. As I said there was 16 days because it was at the beginning when we were doing these studies, but now we routinely go out to 21, 28 days in the challenge trials. We are at the 28-day time point, which was the primary endpoint, at least until now with FDA at the malarial trials. So I don't see that there is a big

Page 135

discrepancy between this trial design for challenge trials and the trial design for patient trials.

DR. COX: Would anyone like to comment on parasite burden or count? I mean, it seems like we're catching folks fairly early in the experimental models of infection. Any thoughts on that?

DR. SAUNDERS: Yeah. I looked at a couple of things. I mean, I think one of the things that you give up, if you were to rely only on challenge data would be the variation in parasites and geographic variation in parasites. In a couple of example, artemether-lumefantrine and artesunate do not work all that well in Cambodia and some other places in Southeast Asia. And we don't understand exactly why that's the case completely. There may be some evidence of cross-resistance, but had you relied only on challenge data and non-immunes, that would not have been revealed.

And I think your point about initial

Page 136

parasite burden and so forth, which is very carefully controlled in challenge models is never controlled in the field. So we've seen that, for example, artesunate efficacy may be influenced by baseline parasitemia. So these are things that you would sort of miss if you were just to rely on a challenge model.

DR. WELLS: One of the things that came out from the talks is the fact that the challenge models actually reduce the complexity of the problem. So if you look at the Phase II trial designs, you know, we normally talk about the factorial designs of a nice sort of 5x5 or 4x4, but in fact, if you throw on top, as you said, the geographic distribution, the difference between Africans and Asians, and then the fact that we're aiming to get drugs out for pediatrics simultaneously, or ahead of when we get the adult drugs out. So we've got the dose de-escalation. When you look at those charts of what you're trying to do in the Phase II B combo study, it's actually a full dimensional problem.

Page 137

So just being able to look at some of the problems and say we have a fair degree of confidence that the midpoint here is going to be this dose of Drug A and this dose of Drug B is really, really important for reducing the complexity. Historically, if you didn't do that, I mean, the historical thing to do is to do the whole Phase II program and then do it in children and then do it in the other population.

So in knowing what the starting points are and having some idea, you know, within a factor of three or whatever, then it really does make the information you get out of the Phase II B studies much more useful. And I was interested when Pete was pushing forward, initially, it sounded like you were trying to get rid of Phase III. Obviously, it would be nice if we could get rid of the Phase II B combos as well. At the moment, then the question you pointed out is can you use this to get rid of these Phase II A monotherapy studies where you're in a single population in a single country, is it really that

Page 138

much use? And I think there we've managed to show that we can predict the historical data and you've got one case now, maybe two cases where we're forward-predicting what would happen. And the question is how much more data do you need to get confident?

DR. MURHPY: So I have a comment about the number of strains. It's not that the CHMI model has just one strain. There are at least three strains that are being used in vaccine studies, including one that's chloroquine resistant. And James is working on some others. We typically infect with strains that are either pan resistant chloroquine resistant. And one of the things we tell subjects is we have a whole range of drugs to treat you, should you recrudesce or not tolerate the therapy.

But should we be developing CHMI strains that are selectively resistant for some of the drugs that we're encountering resistance to and that we're trying to work around with these combination therapies?

Page 139

DR. MÖHRLE: I think we should. I think the capacities we now have to do high quality GMP production of parasite banks. And the capacities we have now to do targeted gene disruption really provide us opportunities if we do the manufacturing and validation and release of parasites correctly. We have the opportunity to make designer parasites for use in CHMI studies. And while it may cause some people to become very concerned, I think it really will provide us with an opportunity to greatly accelerate studies and also to be -- given our slides, I think we need to be mindful of what the context here is that if we can sit around and think about what the world's most perfect malaria drug development strategy would be versus the possibility that we will see very large numbers of deaths in children because we have a parasite strain that's resistant to viable drugs. I think we need to put this into perspective and think about how we can be creative with the modern molecular tools we now have.

Page 140

DR. COX: And to the issue of do you want to construct various resistance strains and study them in a CHMI model, I mean, I think you have to sort of back up a little bit and think about the question that you're trying to ask. I mean, if in fact the mechanism of action of the drug is completely unrelated to the existing mechanism resistance, it's knocking out other drugs, then it may not be the most informative experiment to do. In all settings, the experiment would need to be one that didn't pose excessive or unacceptable levels of risk to the patient.

So I think the question is, at least as I think about it, if there's a resistance mechanism you're concerned about, you've got a new drug that operates via different mechanism, you know, to the extent that you can study that outside of humans, whether that be in another preclinical model, animal models, that may be helpful. But if the real question is does the drug have an effect on parasite count and it's mechanism of action is different or unaffected, then you may

Page 141

be able to essentially use other strains that aren't necessarily resistant to particular drugs to be able to address that question.

If you are in the setting where the particular resistance mechanism is one that may knock out various different drugs and have broader effects, then there may be real questions to be answered there. And we certainly want to proceed in doing that in a safe manner, whatever that was, you know, particularly starting in preclinical models and then deciding whether it's something that needs to be addressed in humans. So that's just my thoughts on that.

DR. WEINA: When I see that question, the thing that pops into my head is thinking about the indication and the labeling and how it's actually going to be used. So when you talk about what strain is used in there, the things that run through my head is okay, so most of the time we're targeting either falciparum or vivax, but we don't actually put on the label well, we don't know crap about obali (ph), so don't use it

Page 142

obali. That's just not what we put on the label. And the reality is that even if we come across one of the zonotic ones like, you know, the Brazilian crawled into our population, you know, you have that. You're going to go ahead and you're going to use whatever drug you have on hand. And if it works, great. And you're going to continue to use it. And if it doesn't work, then that's a data point that you can put out there and you can say okay, well, we've got to try a different one. This is how we're going to learn, but we're certainly not going to do clinical trials and say okay, well, now we have to test against malaria to say that this is an antimalarial drug.

So the question kind of becomes, as you brought up the issue of what is that strain going to be able to tell us about how that parasite is responding to what we're doing to its environment. So the number of strains that are there, whether it's one strain, the perfectly designed strain or if it's five strains that all

Page 143

have different characteristics. It kind of becomes, in some ways, more of a regulatory burden question than a true scientific one.

DR. COX: When I think about the regulatory approach, I mean, to the extent that the science is there, that allows us to do things that are scientifically valid. The regulations really shouldn't be pushing us to do things that we don't think are scientifically valid or important.

So what we may be in is a situation where the science is evolving and there may be sort of differences of opinion on the gray areas and all that, but I think we really are trying to figure out exactly what can we get out of these various different models. What can they tell us? It certainly, I mean, there's no question that it will help inform Phase III and prevent situations where you embark upon a program that probably was not a good choice or something like that.

There may be more to be learned too about the number of strains you're looking at and

Page 144

generalizability and how much, if it is from a CHMI study. If you're looking at one or two strains, is that generalizable to P. falciparum across the board? Are there exceptions and what do we know about that? I mean, it seems like that's really the heart of your question and the heart of the scientific issue at play.

DR. LAURENS: Thanks. Just to borrow from the malaria vaccine development community, we can see that RTSS is a case in point where the CHMI model did predict field efficacy of the RTSS vaccine and the CHMI model is still the basis of dose optimization choice. So I think that we can see the success of this vaccine product and borrow from it and be assured that there is high likelihood that CHMI would predict field efficacy for drugs as well.

So just to comment also on the use of field-adapted strains for a CHMI model, it would be great to get strains that are culture adapted that we could use in CHMI studies. Certainly, taking safety into consideration, we wouldn't,

Page 145

for example, want to develop an artesunate-resistant strain and use that in CHMI without having drugs that would work against it. But the use of field-adapted strains should be priority as well.

DR. WELLS: I think that's a really important point. If you look at the discussion about how we face artemisinin resistance and could you develop drugs that were working against artemisinin resistance, not just by killing all parasites, then one of the thing you come up against is you don't actually have very much to go on, in terms of developing because it's not a classic IC-50 shift, it's a shift in the speed of kill. So any in vitro assay, you know, we have in vitro assays, but you were worrying about how they fit.

And then going back to the animal model question, you said well, people put strains into mice and you have no idea that they link back to the clinical reality because in the clinical studies and the Phase II studies we do, yes, we

Page 146

have Kelch mutations in very, very small numbers. So in this Phase II study, you have 19 Kelch to the 10 genotypes in there. So it's not brilliant to be relying on the Phase II B study. So I think given the seriousness of the artemisinin resistance phenotype and given the fact that it's actually this weird kinetic things, it's not an IC-50 shift, then having something -- if James comes back with a model where he's got artemisinin change in slope in the CHMI, then you can go back into the mice and you say we see this in the scid mice and work backwards, is much more healthy than what we do at the moment, which is building up from cell biology to animals to people. But I think that could be really powerful because we can come up with new generations of drugs which solve the artemisinin resistance problem by completely different mechanisms. But if you had something that would just add to ACTs and bring them back to life then that would be worth having.

DR. NAMBIAR: Are there any questions

Page 147

from the audience regarding this particular question before we move onto the next? In the interest of time, we'll keep it short. We've got four more question to tackle. Thank you.

PUBLIC COMMENTER: I just wanted to ask about a slightly different perspective here. If we consider that perhaps, it's difficult to cover all the potential parasite strains, et cetera. Would we consider, would we think about taking the highest well-tolerated combination dose as our Phase III dose. So I'm wondering if there's a difference between finding the Phase III dose and showing the contribution. So is the Phase III dose the highest well-tolerated dose combination? That dose will always cure more patients, have longer prohphalis, have greater, longer protection against resistance. And then it's a question of how best can we show the contribution? And that, effectively, could be any of these approaches, including also Phase II, where we can show a concentration effect relationship. So we know that if we fix the

Page 148

concentration of one dose and increase the dose or increase the concentration or decrease the concentration that we can show that we've got a greater effect or less effect. That shows that each of those two drugs is contributing to the overall effect.

So what I'm trying to get at is, is there a difference between what we're going to use to show the contribution and what we're going to use to select the dose for Phase III?

MS. HIGGINS: I can comment briefly. Certainly. If you have a combination of two drugs and you hold one of them constant and show the dose response of the other one, I would say that is certainly a valid way to show the added contribution of the drug.

PUBLIC COMMENTER: (Off mic).

THE REPORTER: You cannot make a comment unless you're at the microphone.

MS. HIGGINS: So you said hold the concentration. It would depend on the design, how we could interpret it.

Page 149

PUBLIC COMMENTER: So if you characterize your exposure response relationship, then you can look at the effect of changing the concentration of each of your drugs and you show the contribution. So not necessarily dose because the thing is, when we're in Phase II and Phase III, we always variability. We don't necessarily need to vary the dose.

But anyway, it was a fundamental question about what is the dose for Phase III and how do you feel the contribution -- and they may not be the same sets of information.

DR. COX: So just one quick comment. You're raising a good point. If I understand your question, you're saying that the dose at which you might be able to show and effect when adding A plus B in combination may be different than the dose, the sort of maximal effect for A alone.

Yeah, it does seem important to understand if the response profile for each of the drugs and take that into consideration as

Page 150

you're trying to decide what doses to go forward. I get your argument. Your argument is that B may only show additional benefit beyond A alone at a dose where A and B and not on the flat point of the curve. So you're arguing that if you had both of these drugs on the flat part of the curve above, maybe you'd be better off and you'd get more benefit. But if you were to try and study that in a model at that flat part of the curve, B may not add much to A. I get your point. It a good question.

MR. MCCARTHY: I mean, the other side of that is the resistance selection issue that I think we see in just about any other area of any infected HIV, Hep C, TB, we want to have drugs that have different mechanisms of action at therapeutic levels in order to counter a selection for resistance. There has been some elegant modeling done for malaria about frequency of mutation that will be driven at a specific concentration. And also, I think it raises the issue of the duration of dwell time and how below

Page 151

the MIC you are with some of the more longer-acting drugs and whether that will be a selection mechanism for resistance. And that's not the topic or today, but it's an existential question that we face thinking about combination therapy for antimalarials.

DR. COX: Implicit in your question, too, is that the drugs were both well tolerated. So you're at a point where you're able to get to the flat part of the curve and not have adverse effects that would limit your dosing otherwise. Very good point.

DR. MCCARTHY: Moving on. I think the second question was about the factorial design issue. I think that both Jörg and I have made a strong case that factorial design, at least at the Phase II level is going to be particularly problematic. That we don't have capacity to do such factorial designs to the sky that we would like and also that we could obviate doing that by carefully designing our early phase human challenge studies and then as well, potentially

Page 152

doing a controlled human infection study with two drugs, varying the doses. If we move on to the Question 2 on the monitor, we might just ask if there are any points to be made from the floor or from the panel as to whether there are any issues that we need to revisit in terms of that. And then in terms of the in vitro studies that Tim spoke about, the relevance, the scid mouse model in forming drug development. There may be people who want to revisit either of these two questions and we should give them the opportunity to do so. So I'll open the floor up to that right now.

DR. WEINA: So I don't think it would be impossible to do large feasibility studies in a semi-immune population. It would be very difficult. You have, first of all, a very large trial. So you would have to enroll a large number of subjects. You have approval from IRBs to use monotherapy. That may or may not be doable, depending on which country you're in. And then you're going to have the issue of follow-up, which I think is maybe less of an

Page 153

issue in a controlled setting.

So clearly, it would be advantageous, I think, to all concerned, to consider evidence from a controlled study in assessing the combination rule first, compared to trying to do that in a field study. I think that would be very challenging.

I just have one quick comment on the idea of the factorial design and just again, stepping back from what we're looking at and that is one of the big paradigm shifts that we did was that we sat there and we looked at HIV and we looked at TB and we said, you know, we're not dumb enough to use a single drug against these organism, and yet we're arrogant enough to think that a single drug is going to take care of malaria and the big paradigm shift was oh, gee, let's not do that. Let's use two drugs. Let's combine the drugs.

Maybe one of the other things we can do is kind of step back and learn again from HIV and from TB, and rather than trying to push

Page 154

everything in a marketed fix dose combination or marketed, you know, these things are always put together, maybe we ought to just have a suite of drugs that a clinician can choose from, just like we do for TB, just like we do for HIV. And using different combinations in different places because where we get into trouble, I think, is the fact that we went ahead and mefloquine failed in Southeast Asia, so we added artemisinin to it. And sure, hey, it reversed mefloquine for resistance for a while but now it's failing.

Now both of the drugs are going to fail instead of single one, where if we had a suite of drugs to choose from, the clinician could choose from a bunch of different combinations. And yeah, maybe some of the combinations you have to stay away from, just like you do for HIV, but could be part of the packaging. It simplifies the development of it, first of all, in a lot of ways. Second of all, it helps kind of prevent these little pockets of basically monotherapy of two different drugs. So it's just a thought of

Page 155

getting around this idea of having factorial design studies and having all these ways of just marketing a single combination of drugs.

DR. MCCARTHY: I think if Nick Watt was here, he would point out that the loose combinations of artemisinin and the partner drug are really problematic in a clinic setting where you don't have good patient adherence. That someone feels a lot better after taking a couple of tablets of artesunate and doesn't want to follow-through with mefloquine. There are some real problems, I think with formulation and adherence if you have a situation where you give people the option of taking the drug that makes them feel better but doesn't cure them. So I think that's a real issue.

DR. WEINA: Same problem we've got with TB worldwide. And we fixed that by directly observed therapy or at least made a dent in it with directly observed therapy. So again, maybe another lesson of rather than being arrogant and thinking that we can just hand somebody malaria

Page 156

so easily taken care of, we can just hand somebody a drug and say take this for the next three days and forget about it. Maybe bring them back and make sure that they're taking the drug right in front of us, like we do with TB.

DR. MURPHY: So to me, this is my major reflection on really, the whole summation of the morning's questions is that it presupposes that the only way to move forward is to fulfill the combination rule. And certainly, one can imagine scenarios where that would make a lot of sense, but I'm wondering, are we ad-mixing the scientific issues and/or the practice of medicine and public health issues and the regulatory. And if we put it, really within the context of what do we have here within the United States to use, everything we talked about in the introductory slide, we have only agents and Coartem.

So within the confines of what would a sponsor do? Who would bring it to be able to fulfill those fixed combination rules become, even from the business side, very problematic.

Page 157

And to take Dr. Aukinshouse's point, if one then imagines what in the armamentarium of antimalarial use -- what other types of ways would clinicians want to use these drugs? Then suddenly, just the combination rule may not be exactly what you want to do if there was a drug that was available for intermittent presumptive treatment.

So these tools, to me, seem like they open up the ability to quarry triple drug regiments, maybe quadruple drug regiments. You were talking about it's inadvisable to add just one more drug to a failing regiment. So it may be two. But to think about doing that in a regulatory context where those have to be defined and then tested in that is overwhelming. Honestly, I don't think you can get there from here box, certainly not from a practical sponsor standpoint.

So I would just put out there that let's make sure we haven't set the criteria up front that have artificially backed us into a corner

Page 158

that we needn't necessarily have to be. That there may be some single agents and in ways that could get a regulatory approval and then be useful of multi-drug regiments. If a sponsor says I have no intention of putting it out and marketing it in these other kind of other ways.

I guess one thing to just follow on is let's face it, by forcing it into co-formulations, if one of those is an already approved drug, which can imagine all kinds of scenarios. Artesunate still works. It's just isn't working as well as it did, the shift in the curve, right. But it'd not like we lost our drug for severe and complicated malaria.

So one can imagine all kinds of partner drugs we want, but the second that comes, you've lost any of the incentive for the PRVs. You've complicated that, but that may be the only way that this makes any business sense for a sponsor to pick that up and agree to take it forward. So again, let's be careful to not back ourselves into a corner that we can't get out of.

Page 159

DR. WELLS: I just -- sorry. Go ahead.

DR. COX: Maybe I should just make a couple of comments. With regards to fixed dose combinations or singles, James brought up the issue of resistance. There are reasons to do fixed dose combination. Sometimes they're more convenient for patients. You don't end up with, you know, the idea there is to avoid therapy and gender resistance. I think Pete's bringing up the point of well, if the patient is already resistant to one of the drugs anyways, then you're essentially going back in with functional monotherapy.

So there is a setting when it's nice to be able to have the singles and have enough information to be able to determine what an appropriate treatment regiment is for the patient so that you're not giving them drugs to which they are already resistant.

So there's pros and cons to both sides of whether you're going to do fixed dose combinations or singles. If you look -- in many

Page 160

fields, what oftentimes happens is you see proliferation of a new entities. That happens for a variety of reasons. Sometimes a company has just one drug, so in the developmental phase it may be difficult to actually start to combine but at other times it's not such of a problem.

So it can evolve either way. You can either have singles or you can move to fixed dose combinations. And you can see there's pros and cons to both ways of doing this. Then the other thing is the combination rule. So when we were talking about this, you know, we said don't feel like you're a victim of the combination. The combination rule is really to try and figure out that the components that you have and the drug regiment are active.

And I think the importance of treating malaria with effective drugs, you know, you want to go in with Drug A being effective, Drug B being effective and adding something. That's really the heart of this. That's what we're trying to understand. We want to make sure that

Page 161

the components of the regiment are actually doing something, and there are a variety of ways to do it. And you can see that, if you look at HIV, we've been successful there in trying to figure out various different combinations drugs. The same, I think, more with Hepatitis C more lately with different combination of drugs being studied. So there are ways to do it. There are particular challenges in doing it in the field of malaria drug development. I think the real question is, is sort of gathering the scientific information and what can we learn from these various different experimental models of infection that will help us to understand how each of the components are contributing to the overall effect and is the science good enough it essentially establishes that and that's what I think we're sorting through.

DR. WELLS: I think there is a difference between HIV, TB, and malaria, the principal one being that you don't actually have many malaria patients in the country. So you get into this

Page 162

position where -- I mean, we talk about this all the time. It would be much easier if we could just register things as a single agent with the FDA and then put them in combination afterwards. The next step after an FDA, and Ed talked about that, approval, is it goes to WHO for going on the treatment guidelines where it has to be a combination. If you then say well, let's do the clinical trials and we do them in say, Uganda, the Ugandans want to know that WHO has approved it before they will register it.

So the idea of actually being able to do the clinical trial much easier just because the drug is approved by the FDA as a single, it just doesn't work that way. But I think it is important we make the drugs available as singles for testing in the right environment. And I'm not sure it has to be registered by the FDA for that, it's still part of the clinical trial. But we mustn't lock the combinations too early. Somebody was asking me that earlier, saying one of the things about malaria, which is important

Page 163

is that if the right combination is bringing two drug companies together, we can do that. And that's very different from some of the therapeutic areas.

DR. COX: So if I understood correctly, you're making a fairly strong push for combinations being the route to go here rather than singles?

DR. WELLS: I think it would be -- yeah, exactly. For doing the development, ultimately, our goal is not to register the drug with the FDA. Ultimately, the goal is to treat the first million or 10 million children. And once you start to map out that clinical path, then if the combination can't be defined, then the WHO isn't going to approve it. So far we haven't seen that much advantage of having -- apart from things like the priority review voucher, it's not that much advantage to being able to register as a single drug.

The other issue that comes up if you make a list of all the clinical data on monotherapies,

Page 164

then for example lumefantrine will work fine. It will give you a 90 percent cure if it give it for three days. Artemisinin will work fine if you give it as a single dose for seven days. But each time, what you're having to do it is you're having to extend the duration of therapy. So it's very difficult to set criteria of what a single dose, of what a single drug would have to do.

In a sense, the 95 percent ACPR, the WHO sets is an arbitrary one anyway. It's just sort of saying well, we can get there with ACT, so that's our new threshold. So I think if we went the single dose route, excuse me, a single drug route, we'd have to do quite a lot of work in thinking about what we were trying to achieve with a single drug anyway that has not been thought about.

DR. NAMBIAR: I'm sure there are some comments in the audience as well.

PUBLIC COMMENTER: The first comment is, mefloquine has saved a lot of lives in Southeast

Page 165

Asia, even though it was not an ideal combination. And for a small company active in this space, the PRV is the only financial incentive. So if monotherapy development through to the initial registration is taken off the table, then it eliminates a lot of private sector resources that could be brought to the bear on the problem as well.

Just reflecting on an earlier comment, ultimately, the genetic barrier to resistance is to combine the maximum tolerated dose of multiple agents. And so shouldn't that be the focus is getting regulatory approval for the safety of a drug that can them be used in practice of medicine? Thanks.

DR. COX: Before you leave, could you just clarify, you said monotherapy? Did you mean development of the drug not as a fixed dose combination or do you mean --

PUBLIC COMMENTER: Correct.

DR. COX: Okay. So you don't mean necessarily monotherapy. You might actually

Page 166

develop it in combination with another drug, but you would have it as a single agent in a separate table or something like that. Just to clarify.

PUBLIC COMMENTER: So even MMV, which is an organization that is well resourced don't have enough money to take every drug in their portfolio through using a standardized fixed dose combination approach.

So there are other organizations in the community that want to move forward with new approaches as well and they operate in a different environment where you have to be able to justify the financial return on investment. So the only thing that is attractive to investors is the PRV, which dictates a regulatory strategy, additionally around monotherapy.

So if that's your goal, then the challenge is to identify the maximum safe dose and take that forward to initial regulatory approval and then leave the issue of combinations to clinicians as a practice of medicine issue, combining it with other things that have also

Page 167

come through. And that just highlights what Colonel Weina was going with that point.

DR. NAMBIAR: Thank you. Dr. Möhrle you had a comment?

DR. MÖHRLE: I just wanted to follow up with what Tim just said. Currently, the monotherapies I don't think will change with the next generation very much. You have been given three to seven days to achieve adequate cure rates.

So if we now follow the experiments and let's register the drugs as monotherapy and leave it to the clinicians or the malaria guideline committee to tell us what would be the adequate combination, we have artesunate for seven days. We have mefloquine for three days. What is the evidence that a three-day artesunate combined with mefloquine or three days artesunate combined with piperaquine is working?

There would be a lot of work necessary to actually get the data on your different monotherapy option and the right dosing and the

Page 168

right dosing regimen. Even if you say let's keep the maximum tolerated dose, but you still have to combine the seven-day artesunate with the three days piperaquine or the seven days artesunate with the three days mefloquine.

So I don't see how, for the ultimate goal, which is new antimalarial drugs for people who have limited access to resources or clinicians. We can accelerate it by developing single compounds until registration because then the cost to get a combination treatment and the evidence for a combination and dose and regiment has then to start again after the single compounds have been registered.

DR. WEINA: I think I'm kind of missing something here because we're arguing about three days versus days. I mean, for TB, we're talking about four drugs for two months and two drugs for four months. So we're talking about six months of therapy. I mean, it's not working great. It's working, at least in some areas. That was just more of a random thought that came up.

Page 169

Jeff brought up an interesting thing about the priority review voucher and that is a big incentive. It's one of the great things that has happened for malaria. It's one of the worse things that's happened for malaria because whoever gets first through the gate is the one who gets a priority review voucher and can sell it for $200 million. I think that's the average latest price. But then nobody else is going to be able to get it after that.

So all the incentive that came from the priority review voucher goes away and instead, they're going to find something else to work on like Q fever or Zika or Ebola or something like that and try to get the priority review voucher so they can get their latest lifestyle drug through.

So great point, Jeff, but one of the problems is that whoever is first through the gate gets and it nobody else gets it afterwards.

DR. KUBLIN: It's a very interesting discussion because I think, you know, working in

Page 170

HIV, TB, and malaria, I baulk at the notion of on the one hand the development of a single therapy over time for malaria because of the three malaria treatment is, of course, most prolific in the community as ad hoc therapy. And there is plenty of evidence that fevers of unknown origin are just routinely treated at the stalls with monotherapy if it is available, and that's certainly contributing to the evolution of resistance.

But if here is medical care and if that can be directed in such a way, as it clearly is for TB and HIV, that's a different story. But given the ubiquitous nature of these antimalarials in the communities, you know, I think that's still a major concern.

DR. MURPHY: Just within the context of keeping possibilities open, we're sitting here at White Oak and discussing this within the context of the FDA. I can imagine scenarios by which medications are used in the United States on Americans in ways that would be wildly

Page 171

inappropriate in all these other places. But that doesn't mean that's not possible, given who does the FDA approve drugs for. And that may not be at all the same thing as what do we as a greater malaria community internationally need to do. And I can imagine a responsible sponsor who says this is my pathway. It is going to be a little more difficult but the same model than with an approved drug. I can take it back in there and say now I need to figure out where it fits in the combinations most appropriately.

Where are we going to put that in and do some work with that that then becomes the tool that can be used for the rest of the world. So at least for me, I can envision that as possible. And so I'm hesitant to just take off the table a discussion with the agency that may say this is our plan. This is our pathway forward.

PUBLIC COMMENTER: And also thinking back to James's first talk when he was channeling that as we need to think about adding new drugs to the ones that the combinations that are already

Page 172

failing in Southeast Asia that a new single drug might be what gets added to those.

DR. PROSCHAN: So as I understood the earlier presentation by the regulatory issues of combination drugs, that superiority of the combination to each constituent need not be on the primary outcome, right. It made it look like you could show that putting them together improve parasitemia more than either one alone. And that might be sufficient; is that right?

DR. O'SHAUGHNESSY: That's right. It could be a number of endpoints. It could be on fever clearance. It could be on any endpoint you choose.

PUBLIC COMMENTER: Does it even need to be on endpoints? It seems to me there are different levels of evidence for different things. And clearly you need a high level of evidence for establishing that a product is safe or that it is effective in its final form in the people you're going to use it in. But for just showing that the different components each have a

Page 173

contribution, that could rely on a much lower level of evidence and need not be clinical at all. It could rely entirely on MV Pro or preclinical evidence. And I'm not aware of anything in legislation that says you need clinical trials to establish that each component is having an effect.

DR. O'SHAUGHNESSY: And that's why we asked the question, you know, what in vitro studies, what animal studies -- what studies could help us to show the contribution of both components. So you're right, it doesn't --

PUBLIC COMMENTER: It's not necessarily both "and" because I think those can entirely replace. If we're relying on the argument that we can't possibly do these factorial studies as why we need all these other models, I'm not sure that we have any need for doing the factorial studies in the first place.

DR. O'SHAUGHNESSY: That's why we're having the workshop to discuss these issues.

DR. PROSCHAN: Well, I mean, I think it's

Page 174

a good thing that you can have lower levels of evidence because in a sense, if you find a combination that just works perfectly, who cares whether each component is needed or not, you know, you have something that works.

DR. NAMBIAR: So I think the point here is how one assesses the contribution of the components and there are many different ways one can do it. So it could be clinical if it's feasible. It could be microbiologic. So I think there are various ways. And as Dr. O'Shaughnessy said, that's the purpose of this workshop is to understand is the science with CHMI studies there, can we use that information because truly factorial designs in a clinical setting are maybe doable but very, very difficult is what we've heard.

So I think we're trying to see what other pieces of evidence could we use and CHMI could be one piece of that. There are limitations, but there are also limitations with other data which might be -- so I think that's the purpose of

Page 175

having this discussion.

DR. WELLS: But if you look at, as you said, looking at Question 3, the translation between the scid mouse, because it has the right parasite and it has human red blood cells through to the CHMI at least is very good. I mean, we can point out where there are problems and the factors of three here and there, but I think a combination of CHMI data supported by combination studies in animals would be actually quite solid.

DR. NAMBIAR: Right. So I think the science is certainly encouraging. It certainly does appear that there might be some more work that we need to do and we were hoping that at the end of today's discussion we would get some ideas and see how to move forward. I think that's the intent. I think maybe that could be the comment before we wrap up. Maybe there is one more comment from the audience.

PUBLIC COMMENTER: Like many of the people here I came fixed on the idea of a fixed dose combination. But as I listened to the

Page 176

discussion, I'm beginning to think that factors we cannot control may be removing that from us as a true viable, long-term option. The parasite is changing. We understand much little than -- less than we should about how drug combinations interact, including the fact that active drugs put together can occasionally produce a result which is less than either of them alone. I wonder if the discussion that's going around about shifting the paradigm and licensing or approving the single drugs as is often done in others parts of medicine is not an idea that deserves some serious consideration.

DR. COX: So we can work really with other circumstance, with either circumstance. I mean, whether it be singles or whether it be fixed-dose combinations, I think, you know, we can work through that. You've heard arguments for. You know we don’t want people to be taking monotherapy. We want to protect the drug. I think everybody gets that. You've heard arguments about well, what if somebody is already

Page 177

resistant to one of the drugs and the combination and wouldn’t it be nice to have singles to be able to tailor the regiment appropriately. You can see there's pros and cons on all sides.

One other point of clarification too, the question about 15 or 20 minutes ago was talking about the priority review voucher. So I'll preface this by saying I'm not a lawyer.

I'm not one of the folks that makes these interpretations. But I think it was about a year and-a-half ago we issued a guidance document that described our interpretation of a new chemical entity.

And there was a recognition that there were in many fields, infectious disease in particular, innovation happening where a new chemical entity, you know, in the old sense, a new drug, a molecule that had not been previously approved had been paired with a drug that had been previously approved. And that guidance document talks about -- it's more along the lines of talking about exclusivity, but I think that

Page 178

the issue of pairing a new drug with an old drug in a fixed dose combination, it certainly has been addressed from the setting of exclusivity determinations. Again, I'm not a lawyer, but I would think that would also have implications, too, for the priority review voucher for drugs that are being combined with previously approached drugs. So that's sort of an evolving areas, if you will, recognizing the value of fixed dose combinations in certain settings.

And of course, for a final rule on that, we'd need to go back to our lawyers to make sure what I'm saying makes sense and is correct. But that's at least my understanding.

DR. HAZELTON: John Hazelton, head of Malaria for GSK, based in Canada. It's actually my group that actually works with Tim and James very carefully, in terms of doing a lot of the animal models specifically around the scid mouse model.

But just to add some context to Question 3 because that's what we're here for at this

Page 179

workshop, actually discussing those at relevant animal models. As Tim has just suggested, it would be great to have that combination model in the scid mouse to be able to test these combinations. And that's what we do. That's what we're developing now. We're developing those assays, both ex vivo PRR, in vivo combination models, not to look at synergy. And this answers the question around looking for contributory. Does A work? Does B work? Is A plus B better than A or B alone?

Actually, that's not what a combination scid mouse model will answer. It will tell you if A plus B works and it works just as well as A or B that's fine, but what you don't want it negative interaction. So we are actually looking at using the in vivo and the ex vivo PRR model to actually assess which combinations work and then you will have armamentarium of drugs whereas the industry can work with the people at MMV, the Gates Foundation, Well Trust, et cetera, to put together those right combinations based on

Page 180

informed data from in vivo animal studies, ex vivo, and also, James, as we've discussed in the past, retrospective validation of existing clinical combinations to build that data set and I think that's what you're asking in terms of what animal models are relevant out there that we could use in the future. So that's really just to add some context to Question 3 there.

DR. MCCARTHY: I just wanted to make one other comment about combinations. I think if you go back to my slide on the pipeline for antimalarial drugs, where I think quite fortunately that we've got a number of novel targets that are already in the clinic that we provided the adverse problems don't occur. We're likely to have completely new target with potentially more than one drug available to use.

So the concept of hypothetically only having one drug to add to already licensed drugs I think is a little naïve. Jörg spoke about OZ439 and DSM265. There are other examples there where I think we really have hopes that in five

Page 181

years' time we will have these drugs in the Phase III trial and we need to think about how we're going to license them and how we're going to put them together and what appropriate regulatory environment that we're going to be working to get those drugs licensed, both here or in the developed world where malaria is a rare disease, but in the developing world where there are millions of cases and hundreds of thousands of deaths every year. And I think that's where, at least those of us who work in this community are highly motivated to try and get those things to move forward.

DR. COX: So maybe we'll bring the morning session to a close and break for lunch here in just a minute.

I do want to say that you've heard a lot of the complex issues that are dealt with here and I think the science and the advances and the science really are impressive and I think that's really a credit to all the folks in the field that have really moved things along here. You

Page 182

know, that scientific information, I think, is very important, as we start to look at the roles of combination that would be developed for treatment of patients with malaria.

There are ways to work through the combination issue. It shouldn't impose an impediment to development. It really should be trying to get at the information that you would need to use the drug appropriately. So as people are developing drugs, don't hesitate to engage us. Don't hesitate to engage us early. I think that we can work through this issues in a way that I would hope would be acceptable and scientifically based to help really address the question of what's the role of the different components of the combination.

You know, clearly, we need more drugs for malaria. And this is an opportunity to try and work through some of these situations so that drugs can be developed efficiently and we can have new options out there for patients, both here in the U.S. and recognizing the tremendous

Page 183

global burden and have drugs for patients where the larger burden of diseases are so that new therapies are out there.

So with that, why don't close the morning session and we'll be back after lunch at 1:00 p.m. So we'll have everyone back at 1:00. Thanks.

(Whereupon, at 12:00 p.m., a luncheon recess was taken.)

Page 184

AFTERNOON SESSION

(1:00 p.m.)

DR. BALA: I'm Shukal Bala with the Division of Anti-Infective Products, CDER, FDA. I'll be co-chairing this session with Dr. Ingrid Felger.

Dr. Ingrid Felger is -- okay -- is a full-time employee at the Swiss Tropical and Public Health Institute in Basel, Switzerland, where she heads the Molecular Diagnostic Unit, or Swiss TPH. Her research focus is molecular technology of plasmodium falciparum and plasmodium vivax. Dr. Felger is a molecular biologist with a PhD in drosophila genetics from University of Tubingen in Germany.

During her first job, she worked for three years at the Papua New Guinea Institute of Medical Research where she established genotyping assays for molecular monitoring in malaria vaccine and drug trials.

So Dr. Ingrid will be giving the first talk on Molecular Detection, Quantification,

Page 185

Genotyping of P. Falciparum in in vivo Drug Efficacy Trials.

Thank you, doctor.

DR. FELGER: Thank you for the introduction.

Good afternoon, everyone. Thank you for coming back after lunch in beautiful summer in Washington.

My talk today will cover three topics -- basically, molecular detection, quantification and genotyping of plasmodium falciparum in in vivo drug efficacy trials. So the focus will be on field work and not so much on the CHMI.

When you talk about molecular detection, the first thing is what people ask -- what about the sensitivity. So for me, sensitivity has two aspects. One, certainly, is the assay. But a major aspect which is always forgotten, this is the relationship of the sensitivity to the sampling methods.

And I would like to point out a few key things here. For example, if we would take a

Page 186

blood -- a whole blood sample on the filter paper, we normally have a very limited amount of material which we add into our molecular assay. In that example here, we have punches -- three punches, three millimeter punches that corresponds about to nine microliters of blood. So if we throw all the punches into a PCR tube, this -- then we have the equivalent of nine microliters of blood in the PCR tube.

However, if we extract the DNA by the Chelex method, which is recommended, we only at -- infect half -- the equivalent of half a microliter of blood. So this is very little and doesn't really compare.

So if we use the finger prick blood sample where we get about 200 microliters of blood, we can extract that with a spin column extraction where it's suspended in 50 microliters. And then we would add about the equivalent of 20 microliters of blood. So this is -- the starting material is really very different.

Page 187

There is this method which has been presented in the White Paper that is the high volume, ultrasensitive method which is based on collecting a venous blood sample. And there, you extract DNA from one milliliter of blood, and you end up with about -- with the equivalent of 200 microliters of blood. So I mean, it is very, very clear that if there is one parasite in that volume of blood, it can be detected by the ultrasensitive method. But it can never be detected by a DNA, which comes from a filter paper.

So these considerations are very, very important when designing a study because the outcome, the sensitivity of the method, really very much depends on the sampling and not so much on the molecular assay. That is just the point I wanted to make.

Now coming to the sub-microscopic infections, do they really matter in a clinical trial? We have -- in a field trial, we have parasite detections requirements at enrollment

Page 188

and at the day of recurrence. So with microscopy, of course, only have a reliable detection if the densities are above 50 to 100 microliters of blood -- of parasites per microliter. This, of course, is very likely sufficient if we have a clinical trial where there is some -- where we start from a malaria case -- uncomplicated malaria case.

There are alternative methods to microscopy -- RDT, PCR, LAMP, quantitative PCR. I'm not going to talk about these because David Saunders later on will cover these topics.

I would like to talk about the alternative methods -- for example, the large volume of venous blood and the ultrasensitive multi-copy marker detection method, or an RNA-based technique where this is applicable in the field.

In these antimalarial drug trials with uncomplicated malaria, we quite likely have a very good sensitivity with live microscopy. There is a complication that in the day of

Page 189

recurrence there might be gametocyte presence. We have heard this already from the human-controlled trials. Already, James McCarthy has also detected those gametocytes. And they might compromise our positivity in the sample. So this is a threat.

So the decision on the method what we will use would very much depend on the study population and the protocol and the facilities at the field site. How can -- what kind of blood sample do we take? Can we take a venous blood? How do we process the venous blood? Or do we need to take blood on an FDA card on a filter paper?

So there is some -- is there -- for us, the question today -- is there a consensus among the experts on the use of the molecular detection in field trials? This is a question we need to discuss later. Do we stay with live microscopy? Or do we introduce the more expensive molecular tools?

So what is the most sensitive assay for

Page 190

parasite detection in a finger prick blood sample? We have two options -- RNA-based detection or DNA-based detection. In both assays, we target the 18S ribosomal RNA -- once the transcript and once the genes. So there are three to five genes per haploid genome in a parasite. But the transcripts are highly abundant. These are millions. So the amplification is tremendous. We have a much -- potentially, a much higher sensitivity. So the limit of detection is quite different in both assays.

So we have used that in a field trial in PNG DNA-based versus RNA-based diagnosis for plasmodium falciparum and vivax. And the result was that the prevalence in those 300 samples doubled when we used the RNA-based detection for both species.

And what's even more important -- if we checked for gametocytes in those samples positive, we also find -- found gametocyte carriers in those who were only positive by RNA-

Page 191

based detection. So we would even also miss gametocytes if we would only look by the standard molecular assay by quantitative PCR. So that argues for RNA-based detection.

When we plotted all our samples, all the results, we had this funny observation that all samples basically declined. And -- but here we have this little neck, and then it seems to trail off. And it -- the curve, really, there is something else happening.

We checked this out, what is happening here, and we identified that there is some -- there are some aerosols which cause contamination. Because the template is so highly abundant, this transcript in the tube that during the RNA extraction, we obviously had problems to contain this in our system.

So we had to decide to use a cutoff like in other methods, in other amplification methods like RNA-based amplification methods. But this is a bit unusual thing. In quantitative PCR, we don't have a cutoff. So that was the condition

Page 192

we found.

When we checked the plasmodium vivax, we didn't see this, right? There was not this trailing off of the -- in the low-density samples. And we figured out that the reason for this is an overall, much lower density in plasmodium vivax compared to falciparum.

So if we compare the two assays, RNA-based and DNA-based, we have the abandoned transcripts in one hand, but we only have three copies which we can target in -- on the DNA-based assay. So there is extremely high sensitivity, which we want. On the quantitative PCR-based -- on the gene-based, we only have a standard sensitivity. But everybody uses that, so we are -- that is standard. And it's already very good sensitivity.

So the disadvantage is that quantification is a little bit imprecise. It doesn't really match very well to live microscopy in the RNA-based quantification possibly because the different parasite cells have different

Page 193

abundance in the transcripts or because the RNA in some samples was mistreated. And RNA is much more fragile than DNA. So much care needs to be taken when sampling RNA. So there are many explanations for that.

On the other hand, the DNA-based assay has a very good correlation with live microscopy. So quantification is certainly possible -- of course, then the contamination issues, which is a drawback -- and there have been no contamination issues, at least what I can see, with the quantitative PCR.

So this, of course, argues for field samples which were collected in the field, which are processed in the field. That is a completely different thing that, when we use the RNA-based detection, the highly sensitive detection in a fully enclosed system where we can contain all these contaminants -- and I think Sean Murphy will later discuss about that because he has the opportunity to have a really safe RNA-processing infrastructure. And in certain settings in

Page 194

central laboratories, this is very possible, in my view.

So the lessons learned from RNA transcripts as a diagnostic marker is that we normally lose a large proportion of infections. They are not noticed by the standard methods. We have to be careful and very cautious and apply tight controls if we use RNA, the ribosomal RNA, as a marker.

It's unlikely field-applicable unless we have a really enclosed system. Quantification is not that -- as precise as like on the DNA-based method. That's -- I think we have to -- that is at least our experience. And the blood volume, of course, matters very, very much because we, basically, detect one -- we can detect one parasite in a huge blood volume because there are so many transcripts in.

So the ultra-low density infections, they also carry gametocytes. We have to carry -- keep that in mind for certain applications. This will matter.

Page 195

So we have developed an ultra-sensitive DNA-based quantitative PCR, basically, two assays. One is based on a telomere-associated repetitive element 2. It has 250 to 280 copies. And the other is based on the var gene acidic terminal segment. And there are about 60 var genes in the 3D7 genome.

So we have checked where this is -- whether they can be used for quantification. And both methods really correlate very well with the standard 18S DNA-based quantification, so a very good correlation. These assays can be used for, also -- despite having multi-copies, they can be used for a good quantification.

So the implication for prevalence in a Tanzanian study where we had more than 400 people was that we gained 16 percent in prevalence. So the -- by combining the two assays, so this already told us that there is much more out than we thought because the microscopy was very low and the 18 -- and still compared to the quantitative piece are the 18S, we still missed

Page 196

some samples.

And also, when we looked at the gametocytes here, in those who have been only positive by the two new assays but negative by the standard assays, still 40 percent carried gametocytes.

So do we need the highly sensitive assays at all in the field trials? On Day 1, the parasite detection at enrollment, I would say no because these are all symptomatic people. I think -- but I don't really think that we need their molecular methods.

However, for validating live microscopy, for example, we could use quantitative piece here. That would be a quality control. It could be an external quality control done in a central laboratory. I would find it very good. And because of blood sample -- or DNA sample is collected anyway for genotyping, we should consider that option.

On the Day X of recurrence, there -- I have two opinions. I mean, no, we don't need

Page 197

that because we have a problem here of gametocytes who are not affected by the drug we have been -- which has been on trial. So we will have false positives.

But I also would say yes because we can much earlier detect recurrent parasitemia. So that is a trait often that also needs a decision. And here, also, we have the chance for quality control by PCR.

These sensitive methods seldomly have room in surveillance and in research. They are absolutely essential. They also just work in malaria research in the times of elimination. They will have -- they will be used. And of course, in vitro drug assays are -- I mean, in the human challenge trials, I think that's the way to go, but not -- maybe not in the field. We should discuss it later.

The next find is quantification. Can we quantify absolutely? I mean, there has always been a talk about discrepancy between quantification by live microscopy or by molecular

Page 198

methods. So I -- these are the different stages. I would like to remind you that not all parasite stages are in the circulation. When we take a blood sample in the field, we primarily have rings -- light rings -- and maybe early trophozoites.

And now, the interesting thing is that the DNA syntheses starts maybe a little bit earlier than that. Or the maximum is about 30 hours. So it's possible that we have a one-to-one relationship. But that might not be really one because there might be some parasites infected by two rings or there might be, also, this little overlap, right, that the DNA synthesis had already started.

So my molecular methods will show maybe twice -- two signals, basically. It would look like two genomes instead of one. So we -- this is a biology, and we cannot resolve this. So it's likely that in the peripheral blood we have 1 or 2 genomes per parasite, certainly not the 30, which are in the schizont.

Page 199

So the essential of -- essentials of quantification by quantitative PCR is that we have to validate this in a trend-line, which is of synchronized ring stage parasite so that we are sure that this is only one genome per parasite. We cannot take a mixture of parasites to evaluate our tests. So it we use those trend-lines, I think we can validate safely.

Then coming to a standard curve, a lot of people, including our lab, use a plasmid -- and as -- instead of a ring stage trend-line because having a ring stage trend-line is a lot of work. And not everybody has it available. So a plasmid is used. When this is in supercoil, how you extract it from the bacteria, then you overestimate the copy number eight-fold.

So there is a restriction digest of the plasmid needed so that then the result is then that the quantification matches that of the trend-line. So many people maybe have not realized this, that this can also be a cause of discrepancy between live microscopy and the

Page 200

molecular quantification.

So in the field samples, the relationship of the density should be, roughly, one to one. If not, we have to consider or think about the DNA stability, which can be compromised. The DNA is nicked. The standard curve, maybe there were not only rings, but also mixed stages, other stages. Or the standard curve, maybe the plasmid was not really digested.

So these are issues which make absolute quantification a bit of problem. However, in a clinical trial, we often have two groups. We have -- you know, we compare two groups. And then this is much less of a problem because, I mean, the -- we have a control group. And what -- in the end, what we do is we don't compare our quantitative results against live microscopy, but Group A against Group B.

So my last point, a few words about genotyping -- there, we are using length-polymorphic markers. And we amplify infogenic (ph) repeats. And these are the three marker

Page 201

genes -- the merozoite surface protein 1 and 2 and glurp.

These are amplified by nested PCR. And the standard is now to use a capillary electrophoresis for absolutely precise sizing. This has replaced the gel-based sizing.

Now, in the past, we have done a couple of experiments where we think we need to revise some of the previous recommendations. For example, we should stop multiplexing the nested PCRs because there is some -- sorry -- because there is some size -- a fragment size bias there.

So the recommendations have been described in this leaflet. There was a meeting sponsored by MMV and by WHO. And it's clear everybody knows that this is a recrudescence because two fragments are the same. Or even if one fragment is the same, this is a recrudescence here. We would see the image -- a gel image of a new infection.

So the achievements in genotyping are that the capillary electrophoresis improved the

Page 202

resolution and the reproducibility of fragment sizing a lot. It permits comparison of alleles between separate runs, which is important. And we can estimate allelic frequencies in the population to determine the probability of a reinfection with the same allele.

But the critical issues in genotyping are the detectability of clones, of minority clones. And well, it's really useful in settings with very low or very high transmission because, in very low transmission, we have clonal population. And in very high transmission, we have just too many examples -- too many clones so that the amplification bias will only (ph) have a role.

The detectability -- I show you here some longitudinal examples -- different msp2 alleles over time. And so we see there is a little gap. And here, there are also gaps. In between, the red dots are the detected dots, and the gray dots are the blood samples taken. So that means that, despite that the parasite is still there, the clone is there, we cannot detect it because it

Page 203

might be sequestered or it might be below the detection limit. It fluctuates.

So over time, we see these gaps, and we have -- this is biology. This is sequestration of a synchronous clone, for example, of fluctuations in the densities. So there is not much what we can do about it.

And here, that is an example of the size bias that, if we mix one-to-one -- in the one-to-one ratio two different alleles, we see that always the shorter allele will be preferentially amplified. So it's still above here, above the detection, the cutoff. But there is an effect of the fragment size.

And here from our (inaudible 00:25:47), there is -- it's only one allelic family. And there is a much dramatic effect, a much more dramatic effect. So this marker needs to be reconsidered.

So as a conclusion, do we need genotyping? I say, yes, we need it because, in an area where there's high transmission, we have

Page 204

no choice. We need genotyping. The protocols are optimized. They exist. And the quantity control is established between the labs. What's needed is to revise the recommendations and reconsider these three markers maybe. And also what's needed is to reassess the usefulness for all different levels of endemicity.

And what is really very much to my heart, that's the quality assurance and external quality control. This must be reinforced.

There is some research needed, also. And the validation on deep sequencing for SNP-based genotyping that is amplicon -- targeted amplicon sequencing. This is on the horizon. This can be used possibly very soon. This might be an alternative to the length-polymorphism. But we can discuss that later. That has certain advantages and certain disadvantages. And we also need to do research on the improvement of the SNP-based detection of minority clones, which we had problems so far to detect these.

So conclusion -- on the molecular

Page 205

detection quantification, I think we have very good protocols. Both in DNA- and in RNA-based, there is the consensus on the epidemiological relevance of these methods. What we need is to build a consensus whether there is a potential application in field trials and, of course, very, very important to reinforce the external quality control for absolute quantification.

Research is needed, certainly. And there comes this digital droplet PCR, which can be used to support this absolute quantification, at least for external quality control. We might use this in future, maybe in some central labs to be able to relate different findings to each other. And also, research is needed on the contribution of gametocytes to the positivity.

So I want to thank my group and my collaborators and you for your attention. Thank you.

DR. BALA: Thank you, Dr. Felger.

We'll save questions to later.

The next talk will be by Dr. Kalavati

Page 206

Suvarna. She's a clinical microbiologist with the Division of Anti-Infective Products, CEDR.

As a microbiologist, she reviews and evaluates pre-clinical and clinical microbiology data submitted in investigation in new drug applications and new drug -- investigation in new drug applications and new drug applications for anti-microbial products, including anti-malarial drugs.

DR. SUVARNA: Thank you.

Good afternoon, everybody.

Thank you, Dr. Felger, for giving that very nice overview and setting the stage for this session.

I'm going to talk about the regulatory concentrations when detection methods are used in clinical trials. The outline of my talk -- basically, I'll give you a very brief background in diagnostic tests in anti-malarial trials. In the setting of the regulations, these diagnostic tests are regulated as devices.

And I will talk about what that means for

Page 207

use in anti-malarial trials; the various tests within each context of use -- the two important contexts of use that we're discussing today are in Controlled Human Malarial Infection trials and treatment trials; and what type of information would be important when you're using an FDA-cleared versus a non-FDA-cleared test; and then provide some conclusions.

So in anti-malarial clinical trials, assessment of parasitological response to therapy is an integral part of efficacy determination. Blood smears have been used. They've been used for the past 100 years and are the gold standard for malaria diagnosis and are currently used for enrollment and monitoring treatment outcomes. However, one of the limitations are that it cannot be used to distinguish recrudescence, which is reappearance of parasites possibly due to treatment failure from reinfection where you have new infections due to new mosquito bites in endemic areas.

There are several diagnostic tests, about

Page 208

200 or so, to detect malaria parasites, but the only to point out that that only FDA-cleared malaria rapid diagnostic test is the Binax NOW. In clinical trials, it basically has been used to enrich patients and enrollment of patients who have falciparum malaria. These tests, however, have to be confirmed by blood smears. Clinically, it's being used, of course, to diagnose patients suspected of having malaria.

So as I mentioned, in vitro diagnostic tests are devices. Here, I have the definition of in vitro diagnostic devices, as it's defined in 21 CFR 809.3. These are reagents, instruments, systems intended for use in the diagnosis of disease or other conditions, including the determination of state of health, in order to determine cure, mitigate, treat or prevent disease or its sequelae and also those that are used in collection, preparation and examination of specimens.

In vitro diagnostic devices are cleared by the FDA Center for Device and Radiological

Page 209

Health, the CDRH. We in CDER, the Center for Drug Evaluation and Research, work closely with CDRH when a sponsor proposes to use a non-FDA-cleared test in clinical trials.

Clearance of a device by CDRH does not automatically render it suitable for use in registration trials. Similarly, lack of submission to or clearance by CDRH for device does not render it automatically unsuitable for use in clinical trials. What's more important here is the context of use and risk to patients enrolled in the trial.

To come to the two contexts of use, tests in Controlled Human Malaria Infection trials are basically used to monitor parasitemia in healthy subjects. If designed to evaluate anti-malaria activity, you have tests also to measure treatment outcome.

In treatment trials, they're used for various purposes in enrichment/enrollment for monitoring the patients, parasitemia in patients and for measuring treatment outcome. Another

Page 210

important use, which we'll discuss more today, is about of use of these molecular tests to differentiate recrudescence versus reinfection.

There's some guidance out there. The ICH E8 document provides some guidance on general concentrations for clinical trials. This talks a little bit about the methods that are used for measurement of endpoints, both subjective and objective. It states that these should be validated and meet appropriate standards for accuracy, precision, reproducibility, reliability and responsiveness.

So what are the types of information when it comes to cleared versus non-FDA-cleared tests? For FDA-cleared tests, the performance characteristics of the assays are described on the package insert. However, if the test is modified from what it's cleared for its relevant context of use in a clinical trial, more information may be needed.

In terms of non-FDA-cleared tests, some of the molecular tests that we heard today, this

Page 211

morning, the performance characteristics of the test in the actual laboratory where this testing is performed is needed for our assessments. Now, the extent of validation information may vary, again, with the context of use.

So for all tests, basically, the context of use and the ability to rely on these tests results for the specific purpose of use is important. Besides performance characteristics are the quality assurance procedures that are implemented are also important.

So today, we'll hear some more about the tests that are used for these two context of use. Dr. Sean Murphy will elaborate more on tests used in Controlled Human Malaria Infection trials. And Dr. Saunders will talk more about tests that could be used in treatment trials.

So in conclusion, blood smears are currently the gold standards for malaria diagnosis. The only cleared FDA test is the Binax NOW Malaria Test. We are -- really want -- we are open to all the new molecular tests that

Page 212

we hear -- are going to hear about today because we believe that this would help with the development of anti-malarial drugs. So we encourage you to submit this type of information.

With respect to the FDA-cleared tests, like I mentioned, the context of use is what's important. And that will determine what additional information is required. With respect to the non-FDA-cleared, we definitely need the performance characteristics of the test within the laboratory where testing is performed.

So we heard today a little bit about the various molecular tests that are used in the session this morning and also in our previous talk and how these methods are evolving and studies that are being done and data that's being collected to understand the characteristics of this test. So we really look forward to your input, scientific input, in how these methods can be used for its various purposes of use in the CHMI studies, the anti-malaria trials and also may need to differentiate recrudescence versus

Page 213

reinfection.

I -- we heard about the quantitative PCR assays and how they are more sensitive and may be very valuable in the CHMI studies in terms of providing rescue therapy and evaluating anti-malarial activity. I guess we -- there's also a lot of interest in looking at genotyping and assays that can differentiate recrudescence versus reinfection and how it could be used in endpoints, outcome measurements and to help us understand the differences and its effect on digested cure rates in endemic areas.

So with that, I'm looking forward to a very rigorous discussion and diagnostic tests. Thank you for listening.

(Applause.)

DR. FELGER: Any questions?

DR. BALA: No, later.

DR. FELGER: Oh, sorry. In the sake of time, we continue to our next speaker. This is Sean Murphy. He's an assistant professor and assistant director of the Clinical Microbiology

Page 214

in the Department of Laboratory Medicine at the University of Washington. He also serves as a clinical investigator at the Seattle Malaria Clinical Trial Center and a medical director of the Human Challenge Center at the Center for Infectious Disease Research. Lots of centers.

Dr. Murphy's laboratory studies malaria diagnostics and malaria vaccine development. Sean completed medical and graduate training at Northwestern University Residency Training in the Clinical Pathology at the University of Washington and conducted his post-doctoral studies with Michael Beban (ph) before becoming assistant professor in 2012.

DR. MURPHY: Thank you very much.

DR. FELGER: Looking forward to your talk.

DR. MURPHY: Thank you for the invitation to be part of today's workshop. I have just a couple disclosures here, some clinical trial support and consulting for Biofire Defense.

And in my talk, I'm going to talk about

Page 215

the main target that's being used in investigational molecular-based diagnostics in Human Challenge Trials; describe some of the tests that are being used at our center and other centers; and then look at how the kinetics of onset of positivity in these tests vary, depending on how you give the parasites and what form of the parasites you give. And at the end, we'll talk about a couple topics that have been broached a little bit earlier about recrudescence and gametocytemia.

So I think it's been clear from the literature and in our own studies, for instance, what we call the demonstration trial that we did in 2009 in Seattle that nucleic acid-based testing, that detection of the biomarkers that are used in nucleic acid-based testing accelerate the time to infection detection as compared to blood smears. And that's shown -- blood smears in the dark line and the nucleic acid test in the dotted line.

And we're working toward understanding

Page 216

exactly, you know, the clinical reliability of this and whether this would be a suitable replacement for blood smears categorically. And so I want to show you a bunch of data that kind of begins to address that. But like all nucleic acid-based tests, there are a number of steps. And often, we focus on the last part of this nucleic acid-based test and forget about the upstream part.

So just to tell you what a test comprises, it involves extraction of whole blood from the patient either to obtain DNA or RNA or total nucleic acids. If you're going to look for an RNA marker, then you have to either do a reverse transcription or do total cDNA synthesis. And if you're going to look for an unspliced target like pfs25 for gametocytes, you also have to destroy the genomic DNA. This isn't necessary when you do 18S ribosomal RNA testing because there are thousands of copies of the RNA to the very few copies of DNA.

And then you go on to what we hear about

Page 217

most, which is the PCR part of this process, where various labs have quantitative or qualitative tests. And amongst those, the most common target is the 18S ribosomal RNA.

And so this is a very useful target, whether you look at the DNA or the RNA target. And it allows you to, I would argue, quantify the parasites with, actually, a considerable degree of accuracy in the bloodstream for P. falciparum. And that's because we know that P. falciparum sequesters in the mature stages where there would be multiple genomes or increased numbers of RNA relative to the ring stage parasites.

So the peripheral circulating parasites are really the ring stage parasites. And in my group, depending on the assay we've used, we find 3,500 to 10,000 copies of the ribosomal RNA per individual ring. And we know from the genome that there are two of the asexual type genes and two of the sexual type genes and a fifth pseudogene. So whether you use DNA or RNA, you can very reasonably quantify the parasites. And

Page 218

we must remember that nucleic acid-based tests are generally viewed with a log base 10 scale.

And so you know, in my lab, we focus on RNA-based methods. And we get a big bump in sensitivity for a given volume because of this biological enrichment of the 18S ribosomal RNA. It's not the only way to do it, and I'll show you what other labs have done as well.

When I reviewed the literature, this is 23 studies that have compared 18S-based methods, be they DNA or RNA, to blood smears. And so this graph shows the time to positivity from the time of challenge with sporozoites until the onset of either molecular-based positivity for the biomarker or blood smear-based positivity in the circles. And what you'll appreciate is that, in all instances here, the nucleic acid-based test accelerates the time to positivity compared to blood smears.

We are very confident in this method in our center, and we've now embarked on studies where we no longer do daily blood smears leading

Page 219

up to infection detection. And so this study on the left shows a trial that we have conducted where our molecular-based test has been the primary endpoint that has triggered rescue treatment in people who have failed the therapeutic that we were testing.

So I'll show you a few tests as they're performed at other major centers doing CHMI studies. And so we conducted an external quality assurance program several years ago and involved all of these centers who, by and large, are doing vaccine studies. Some of them are also doing drug studies. And you'll see that there's diversity in the kinds of testing that people do, even though we all use the 18S target.

So there are people who, like my lab, look at the RNA. And there are more groups that look at the DNA. Within that, you can place your PCR targets either in the sexual or the asexual genes. And so we really have to make sure we're comparing apples to apples when we talk about where our targets are.

Page 220

There are various ways to extract the RNA or DNA, including manual methods and higher throughput methods on automated platforms. And the sensitivities that these tests achieve, fortunately, are generally in the same range. And these sensitivities were designed to be able to test the -- to detect the parasites on or about the day that parasites emerge from the liver following five mosquito bites. And so that sensitivity is probably on the order of 10 to 100 parasites per milliliter.

You can achieve this off of different volumes of blood. And if you use DNA, you need to look at more blood than you need to look at if you use RNA. And so in our group, we use 50 microliters of RNA. This is also the volume of blood that we can place on a dried blood spot. And when we process our dried blood spots in our group, we process them with a laser cutter because, as Ingrid mentioned, there can be a very high copy number of the RNA that could contribute to contamination. And so we've had to,

Page 221

basically, invent a touchless laser cutting system for dried blood spots. And when we process dried blood spots in that method, we completely eliminate any cross-contamination.

We did an EQA comparison amongst these centers knowing nothing about how well the test would compare, knowing only what the claimed sensitivities and quantification of each center was. And what we were very happy to find is that we had really excellent qualitative and even quantitative correlation between centers that placed their targets in different parts of the genes or even in our center that did RNA compared to everyone else who did DNA. And our results put our RNA quantification right in the center of all the DNA targets.

So this was very reassuring. And we know that EQA is needed in this program. And the World Health Organization is working on an EQA program right now that will serve the needs of CHMI centers, of field studies of epidemiologic groups and sort of cut across that need for the

Page 222

field.
So one of the questions I was asked to address is how do we use these tests when we give different parasites or we give them by different modes of infection. And so these are our experiences and my thoughts on this topic.
So mosquito bite versus intravenous sporozoites -- we don't think that this changes the duration of the liver stage by any meaningful measure. We don't think that, based on the biology of the parasite, that there's any indication to test blood during the first five days when the parasite is in the liver and we don't think that it's in the blood at all. And what we've seen in the studies that we've now done both by DVI or by mosquito bite is basically the same onset in positivity, meaning the parasites come out of the liver at the same time.
If we were to do sporozoites and ask how does that differ than red cell infection, I'd like to basically go to the next slide to show you this. This slide basically says that there

Page 223

are three ways to get someone infected with malaria parasites in the red cell stage, which is, after all, the diagnostic stage of this organism. You can give five mosquito bites, a model that's been around for a while. You can give 3,500 P. falciparum parasites by venous injection. These are both going to go into the liver. They're not all going to invade a hepatocyte. But those that do, we think, make 20 or 30,000 merozoites per infected hepatocyte. And on about Day 6, these pour into the blood.
So there's a certain inoculum into the blood at that point. And if -- I've just modeled this up here for you. If there were 10 infected hepatocytes, we're talking about 300,000 parasites in your total number of red cells in your body. So you need a test that might be able to detect 60 parasites per mil in order to find that. And that's on the order of the sensitivity for the test that we designed.
If you take the third route, which is what James is doing in Australia, and infect

Page 224

people with red blood cells, they give 1,800 parasites. And if you put 1,800 parasites into the body, this is too few parasites to detect on the day that he first injects them. It's probably too few parasites two days later. But after the parasites have gone through two rounds of replication, now we're talking about a density that's detectable by the kind of assays that I have shown you here.
So with those tests, we have the option to accelerate the time that we treat people. Historically, we would treat people on the basis of blood smears. And so whether you use a sporozoite inoculum or a red cell inoculum, the previous slide would show you that, because of the liver stage, the parasites in the sporozoite inoculum in the absence of pre-existing immunity will come out on about Day 6 or 7. And they will climb in this saw tooth pattern until you become blood smear positive and you introduce treatment.
In the red cell stage, there's similar growth kinetics. But the onset is after four

Page 225

days because of how many parasites Dr. McCarthy's group puts in. And so in general, you can treat these people by about -- upon blood smear positivity by about Day 10 to 13. And in James's group, you can treat people a little bit earlier because the parasite load is a little bit bigger.
If we decide to treat with the nucleic acid-based treatment threshold, we have the option -- the ability to spare symptoms that subjects generally find uncomfortable and still to obtain quite a bit of really informative quantitative data.
And so an open question is what should those thresholds be because, in a prophylactic study where the goal is to completely prevent infection, you would argue that, in Seattle, there should be no parasites in a person. And as soon as you have a reasonable detection of parasites, one ought to treat that person and clear them with a rescue drug. And so in studies that are designed to do that, various thresholds are now being used. In our center, we're using a

Page 226

threshold of 250 parasites per mL. And I'll show you how we arrived at that number.

In the Netherlands, they're using a threshold of 100. And you can see some other comments about some other centers up here. If you're doing a radical cure study, like James McCarthy's group, in their most recent paper, they initiated treatment -- correct me if I'm wrong -- but at a slightly higher threshold. And at this threshold, the subjects are completely safe.

Most of them are probably asymptomatic. But it allows you to generate a few more data points during the clearance phase to allow you to calculate what the clearance sort of kinetics for that drug are.

In our center, this is how we arrived at our treatment threshold. And what we did was we took our quantitative data, and we compared if we were to treat people based on even the lowest positives for our test. Our test has a sensitivity of about 10 to 20 parasites per mil.

Page 227

And we quantitatively report test results above 20 parasites per mil.

So if we were to treat people on the very first instance of positivity, we would always be treating people before the onset of symptoms and before the onset of blood smear positivity. But as we ratchet that number up, the so-called threshold, eventually, we arrive at a point where that overlaps zero. And there would be no advantage to waiting that long.

And we've now modeled that. And what you can see is that this is how we arrived at 250 parasites per mil. We very confidently can avoid blood smear positives and symptoms, in general, if we use this threshold.

So there are some other considerations about these tests. How often should we sample? At one point, we tested people twice a day, and we now test people once a day because, in this study, there wasn't really an acceleration, a really meaningful acceleration for the amount of work involved, to do twice-a-day testing. But

Page 228

here in our center, the question is infection detection. It's not really dense modeling in the post-treatment phase.

And for prophylactic studies, most of the models really just depend on the density of the parasites on the first day that you're positive so that you can back-calculate how many infected hepatocytes there likely were.

We also -- even at our center with once-a-day testing, when -- this is data on people who broke through and required rescue treatment. And this shows the kinetics of their clearance of our 18S ribosomal biomarker in the three days that followed that rescue treatment. And what you can see is that, within three days, our biomarker goes to zero.

And this is reassuring because we often hear that molecular diagnostics have a positive tail. And that's true if you let people climb to a density where they would be blood smear positive or really sick. But if you treat them with a molecular marker, they resolve to zero

Page 229

very quickly unless, as in the case of this one subject who had the very highest parasitemia, you detect gametocytes.

In James's group, they do more-dense sampling. And he's explained why earlier. And that's so that they can more adequately model the kinetics of clearance in these radical cure studies.

So I'm going to present just a little bit of data on recrudescence versus gametocytemia and expand just briefly on what Dr. McCarthy had commented on. This is data from a paper they published earlier this year, which showed that, in some subjects, there was a recurrence of the 18S ribosomal RNA marker that was shown to be asymptomatic gametocytemia. And this gametocytemia can persist in the absence of treatment with primaquine. And so they followed subjects who received no primaquine or two different doses of primaquine and followed the resolution of the pfs25 target down to zero.

And so in our center, we haven't seen

Page 230

this much recurrence of the 18S ribosomal RNA target that could be either a recurrence or asymptomatic parasitemia. And we believe that this is because we're treating people at a much lower density. And so there's less -- fewer cycles to generate gametocytes, and the overall parasite density is lower.

Obviously, Dr. McCarthy presented earlier this very nice data that uses a gametocyte marker, Pfs25, and a ring stage marker to differentiate between asymptomatic gametocytemia and the additional presence of recurrent and, eventually, probably symptomatic asexual recrudescence using that ring stage marker.

My last comment is about what we need in the field -- in the malaria field. And that is, for these tests, we recognize that harmonization and reagent availability is very important. And there's no commercial source of standards. Most labs generate infected whole blood. And this is okay, but it's not the way a commercial test would be run. Nobody -- no commercial test ships

Page 231

BSL-2 material around as part of the ingredients in their test.

We also recognize that, beyond standards, what we really need are calibrators. And so our group has generated some plasmids that we linearize. And we have also created plasmids that contain both the asexual and sexual type gene together on one plasmid. So we have a plasmid that, for instance, contains the targets of six different CHMI centers so that we can distribute this to DNA testing facilities for a one-to-one-to-one comparison between centers.

And just this week, I also took delivery of a full-length 18S ribosomal RNA as a custom Armored RNA. So this is a 2,000-based parapesa (ph) RNA encapsulated in a verion (ph) that would contain the entire sequence of the 18S and would be stable from RNAsis (ph). So we hope that this will also be a resource. And then as I mentioned before, the WHO is working on an EQA scheme that will also help all of us.

So in summary, the most common target is

Page 232

the 18S. These tests that target the 18S are in use in a number of centers. We think that these are useful in a variety of CHMI studies, and I touched on a number of issues that we think will help to harmonize and pull the field together.

I'd just like to thank my group. And especially, I'd like to thank the collaboration we've had with the other CHMI centers who have been very open to harmonization and quality assurance, despite the fact that we all have different tests.

(Applause.)

DR. FELGER: Thank you very much, Sean.

So we are moving to our next speaker. This is David Saunders. He is a clinical pharmacologist and internist currently stationed at the U.S. Army Medical Material Development Activity.

DR. SAUNDERS: All right. Well, thanks very much. I'm honored to be the last speaker today. And I'll try to keep things punchy because I know people are probably a little

Page 233

sleepy by now.

But so I'm just going to talk about some practical considerations for detection methods in clinical trials, field trials, and expand on some of the points from my colleagues earlier this session.

So we'll just look at some of the detection methods as they apply to field trials. We will consider how they're used for enrollment, and then I'll talk a little bit about how we might use it -- how we use them to measure outcomes and, really, in three areas. One is the use of PCR to correct results of microscopy.

The second is how we could use molecular methods to look at parasite clearance and, finally, how we can use PCR to adjust the treatment outcomes. So I use slightly different terminology there. And that means differentiating new infections from recrudescence. And I'll talk just briefly about considerations for P. vivax, even though this is focused on P. falciparum. In areas where vivax

Page 234

is co-endemic, there's some important considerations there.

I don't have a disclaimer slide. But I should say that these views are my own, and the U.S. government is free to disavow them if I say anything that they don't agree with.

So in -- here's my sort of bottom lines up front as far as using these methods in field trials. The first is that RDT use, really, is pretty limited. We use it mostly for screening potential subjects, but, really, it doesn't have much of a role. And I think this follows on to, you know, Kalavati's point earlier that, really, RDT results have to be confirmed by a blood smear anyway. And for that reason, we don't really put a whole lot of stock in them.

Microscopy is still the gold standard. And it has several advantages. Of course, it's less sensitive than PCR. So it probably -- these days, it's almost routine that we use PCR methods to interpret the results of microscopy because it's very sensitive and specific.

Page 235

The limitation, of course, with PCR in the field is that onsite use is fairly limited. There's not too many centers that can actually do PCR in real time making it clinically meaningful or -- and producing actionable results.

The good news, I guess, is that, you know, PCR can pretty much quantitate parasitemia as well as microscopy now. And so that may provide some advantages, particularly when you're looking at parasite clearance for resistance studies.

And then finally, I think it's also pretty much become the de facto standard that the results of trials in a field need to be PCR-adjusted to determine whether the recurrence that you see represents a reinfection or a true recrudescence. And this is important because reinfection rates vary quite a bit. It may be less than 10 percent in low transmission settings like Southeast Asia or Latin America to more than 50 percent in some settings. And this can have significant impact on the interpretation of

Page 236

efficacy.

So there's -- starting with RDTs, there's a huge variety available. WHO has a table. There must be, you know, at least 100, 200 tests in there. Only one is FDA-approved. That's Binax NOW. It's not necessarily the most sensitive or specific among them. Most of these are lateral flow immunoassays. And the sensitivity of some of these is really approaching that of microscopy, although specificity is not necessarily as good.

The limitations here, really, are in red, though. They're not useful for follow-up because they remain positive after the patient is even cleared clinically. They don't give you a quantitative result. They don't give you a permanent specimen result. So you can't go back and read an RDT like you can with a microscope slide which you can stick in a box and look at it 10, 15, 20 years later.

You also run the risk when you use RDTs of ending up with treating people based on false

Page 237

positives, which can ultimately lead to invalidation of trials. And that actually did happen in one U.S. Army trial in the past.

And then for -- you know, so bottom line is we really -- they're pretty much unsuitable, I think, for clinical trials. And particularly, we're talking about, you know, regulated trials that you would submit to support an FDA licensing application. I think they probably do have a role in academic trials and, you know, therapeutic efficacy trials that the WHO does, but probably wouldn't stand up to allowing you to make a GCLP submission.

Microscopy -- it really is still the gold standard. It's probably the most widely available method in the field. It gives you real-time actionable results. You can identify species in parasite stages. You can look for gametocytes. You can have your results, usually, within 30 minutes to two hours. And it's relatively inexpensive, a low-tech method compared to PCR.

Page 238

Now, that being said, it is not necessarily a simple thing to have a cadre of adequately trained microscopists. It can take several years to train these folks. There -- you need to have a good training program. You need to have a really solid set of SOPs. So not every center is capable of doing microscopy to a standard that would support a regulated trial.

In the hands of an expert microscopist, they might get down to a density of 10 parasites per microliter. But that's really only sort of the most skilled and the most patient readers. But the WHO actually offers a very good external competency assessment exam program. And when your microscopists take that, it -- they will actually get a report that estimates what their personal sensitivity and specificity is. And so you can go back and, I guess, do some, you know, post-talk analysis on your data based on those estimates.

The other thing with microscopy, you really need to have, in my view anyway, at least

Page 239

three readers to look at it. And the readers should be blinded to each other's results. And then you really need some expert C -- what we call C-level readers that do blind over-reads whenever there is a non-concordance between the A and B reader.

So that, you know, logistically, is challenging. You need to amass a sufficient number of microscopists to be able to get through a trial, particularly if you're talking about a large trial.

Okay. So that's microscopy. Now, molecular methods as far as enrollment goes, it's tough because if the test is not available onsite or in real time, you're pretty much going to be limited to microscopy to determine whether or not to enroll somebody.

If you do have microscopy onsite -- sorry -- molecular methods onsite, then it does -- they do offer the advantage that they're more sensitive -- usually, several logs more sensitive than microscopy -- highly specific. So this can

Page 240

be used to a particular advantage when you're looking at -- in very -- you know, you're trying to enroll subjects with sub-clinical infection. And there are special methods for gametocyte detection if you're interested in doing transmission blocking.

But I think the thing to say about molecular methods is it really requires a pretty significant infrastructure and, you know, good training, good quality control. And it's very expensive if you're going to try to do it onsite. We only recently at AFRIMS tried to do this, and it required several years of running -- setting up the lab; training everybody; developing the SOPs and making sure that, you know, for the most part, we were able to produce reliable results, avoid -- you know, handle situations if there was contamination and so forth -- to make it clinically useful.

But overall, you know, qPCR has come a long way. And you can see there's, literally, probably hundreds of publications on this. This

Page 241

is a method that we developed, you know, very similar other methods looking 18S RNA. But you can see that there's a really nice correlation between the, you know, controlled numbers, or specific dilutions of parasite genomes with the number of cycle thresholds that have to go through before the RT-PCR test becomes positive. There's good assays for general plasmodium falciparum and vivax.

So this is a fairly well-established system. And this also compares nicely to quantitative microscopy and quantitative PCR. If you compare samples in blinded fashion using both methods, you actually get fairly good concordance of results to the point where it would be reasonable to use these tests to follow out parasite clearance in studies where you're particularly interested in resistance and the rapidity with which your drug is clearing the parasite.

So bottom lines on enrollment -- microscopy is still the gold standard, and it's

Page 242

rarely going to miss clinically impaired infections. It may miss sub-clinical infections. RT-PCR is very good, but it's often not available -- very rarely available to use in -- for enrollment. And RDTs, really, are used, I think, just mostly for enrichment of patients and initial screening. And often, you know, patients will come to you from local -- public health facilities with an RDT. But these always really need to be confirmed by -- really, by microscopy.

Okay. So I'll switch quickly just to talk about how do we use these methods to measure outcomes. And there's three important roles that molecular tests are increasingly filling. And the first is looking at parasite clearance. And we're starting to use PCR to quantify parasite clearance. But we're also using it to confirm the results because, often, when a patient comes back with a microscopic recurrence, the parasite densities can be very, very low.

And because of that, it can be easy either to miss or to miss a mixed infection or to

Page 243

call the wrong infection, so thinking that someone has falciparum when, in fact, they have a vivax infection, which is very common in areas where vivax is co-endemic. So this is what we call sort of PCR -- or I call, at least, PCR correction of the microscopy result. And it's useful, I think, post-talk in the trial to assess final outcomes.

And then finally, molecular methods are useful to distinguish recrudescence from reinfection and all the other possible things that can happen as an outcome of a malaria trial.

So PCR correction, microscopy, I think it's really becoming recognized as a critical factor for ensuring that you have accurate outcome measurements. And this is because, oftentimes, recurrences are detected only sub-clinically. Patients have no symptoms. They have no fever. They may have a very low parasitemia. And so PCR can really help distinguish whether it's a true P.f. reinfection, whether it's P.v., whether there's a mixed

Page 244

infection, whether there's actually no infection and it was a -- it turned out to be a false positive -- and so useful to evaluate your outcomes after the trial is done.

Now, one of the things that it opens up, though, particularly if you're following, you know, patients over the course of a trial with serial blood smears, is it's going to detect a lot of sub-microscopic infections. And because of that, you know, potentially, you can open up a whole new level of issues that you have to deal with when you have, you know, sub-microscopic infections persisting after the patient has become clinically well.

Again, the major challenge of PCR correction is that it's rarely available in real time. It's usually done after the trial. And the clinical significance, particularly a persistent sub-microscopic parasitemia, is going to be debatable, I think, in some cases, whether it affects the patient's health. And it really sort of has to be died back to what your

Page 245

objectives are when you undertake your trial. But it's certainly -- I think, for the most part these days, it's going to be part of a post-talk analysis of just about any trial that's going to be done.

So useful also in measures of parasite clearance, particularly with resistance studies -- and you know, there's some -- you know, microscopy is certainly the standard for measuring parasite clearance. But it can be inaccurate because microscopy is often -- parasite density is often calculated based on formulas.

And the formulas -- sometimes we'll just substitute a standard, you know, white blood count, for example. So if the readers are counting based on a number of white blood cells and they don't know the patient's particular white blood count that day, 8,000 is often substituted. But that can lead to wide variation in the actual parasite densities. So in some sense, PCR may even be a little more accurate

Page 246

because it's just measuring the overall parasite burden.

Now, and you can see here in these figures this is -- and sorry. It's kind of hard to see. But at the bottom of the screen here, you can see these bars. These are the, you know, PCR results where we're looking for parasite DNA. And then you can see in the red line here is the microscopic parasite burden. And you can see by Day 3, Day 4 -- or in this case, Day -- yeah, Day 3, Day 4, the patients have cleared microscopically, but there's still this persistent parasitemia that goes on. And in this case, both of these patients went on to recrudesce.

But you know, how is this handled in cases where patients don't end up recrudescing? Obviously, now you're looking at a much more sensitive assay, and this could have implications for how you interpret your study and how you define parasite clearance -- so some things to keep in mind as these tools are employed.

Page 247

Finally, we have the issue of PCR adjustment of trial outcome. So here, you're really trying to figure out what actually happened to this patient. Did they come in with P.f. and then end up with the same P.f. that was affecting them, which we would call recrudescence? Or did they come in with P.f. and end up with a different P.f., which we would call a reinfection?

So if it's a recrudescence, we hold that against the drug in terms of efficacy. If it's a reinfection, the drug gets a pass because most of these drugs are suppressing the blood stage. And we wouldn't expect them to prevent a new infection from another mosquito bite. So there are -- this is -- it's important to assess this in the end.

Now, another possibility is the patient comes in with P.f., and then they develop P. vivax. And so what do we call that? Is that a cure? It raises some questions. This is a common occurrence, particularly in Southeast

Page 248

Asia. We'll see almost one-third of patients in Southeast Asia where vivax is co-endemic have a blood stage P.v. infection after they've been treated for P.f. And you know, it's thought that the drugs precipitate a relapse of P.v. And tropical P.v. relapse is fairly often, sometimes as often as every month. So you know, how do we interpret that?

And then you know, more complexities -- patients that come in with mixed infections of P.f. and P.v. then end up with P.f., we still want to know is that P.f. a reinfection with a new P.f.? Or is it a recrudescence? And in cases where we have mixed infections and the patient then comes back with P.v., is it -- was it a relapse of the P.v., a reinfection, a recrudescence of the blood stage P.v.? So things get fairly complicated very quick -- fairly quickly.

So it's important to be able to evaluate, you know, what actually happened. And this is generally used by, you know, parasite genotyping

Page 249

methods. And I think Ingrid went into the details.

I think, for the most part, the current standard is to use the, you know, msp1, msp2 and glurp. Those three endogens seem to be fairy reliable. I think there is some -- you know, maybe some limitations. But for the most part, they seem to do a fairly good job in helping us identify patients that come back with either a recrudescence or reinfection.

Now, you know, one of the challenges, though, is when we genotype, we often see that there are polyclonal infections. And these polyclonal infections may be represented disproportionately in the recrudescence versus the original infection.

So things do kind of get complicated. But it's important to interpret things. You know, in Africa, up to 50 percent of your, you know, recurrences of malaria could be a reinfection. And that's going to have a major impact on your efficacy if the crude efficacy

Page 250

could go from 50 percent up to 95 or even close to 100 percent once you adjust the results from PCR.

And here's just some examples. You know, we published some of these. And you can see sort of, you know, here's one pattern of -- here's one subject, and here's their pattern of msp1, msp2 and glurp. And you can see how, over time, this stays fairly consistent. And then at the day of recurrence, the same parasite appears.

And -- but at the same time, you can see this case. This patient had this pattern of msp1, msp2 and glurp. And at recurrence, they had that. But then they had a new organism as well. So where did that come from? Did they get a reinfection on top of the recrudescence? And then some other examples where, you know, a patient had, you know, a multi-clonal infection at baseline and then only one of the variants reappeared.

So doing this also, you know, gives you some insight into what are -- you know, what are

Page 251

the dominant variants, what would -- what's responsible for the resistance. So this is useful data to have beyond just adjusting your efficacy result. And then you can see here an example of a new infection where this patient had one pattern of msp1, 2 and glurp bands at baseline. And then at -- reinfection had a totally different set.

And then just to say with vivax -- and I know we're not here primarily to talk about vivax trials -- but in trying to distinguish vivax, we took a crack at this. It's pretty complicated. You can see here these are all patients -- you know, patient numbers. And you can see what they came in with and then what they looked at -- what they look like on recurrence using various microsatellite markers. And it's very complicated to the point where we can pretty much say each patient is sort of their own snowflake of outcomes. So trying to sort all this out in a vivax trial could be -- prove to be very challenging, indeed.

Page 252

And this was one poor, unfortunate individual who relapsed three times during a cohort study that we're doing. You can see each time they had a different basket of microsatellite variations -- so very hard to track what's actually going on with vivax in terms of efficacy during a trial.

So just to reiterate and sort of go back to the bottom lines, RDT is limited use. Microscopy is still the gold standard. But PCR is increasingly becoming a critically important factor, or method, to be used for several roles in trials. And I think, you know, there's going to be -- as the technology progresses further and gets more sensitive, there is going to be, really, I think, a need to determine how these results are used in a field trial and how much is required and how much is a sort of a nice to have.

So thank you very much, and I appreciate the opportunity to talk to you today.

(Applause.)

Page 253

DR. FELGER: Thank you very much, David.

So I think it's time now for a coffee break. So we have a 10-minute coffee break, and we'll reconvene then. We have to be on time because some people must leave early. So only -- that's why only a 10-minute break.

(Brief recess.)

FEMALE SPEAKER: Good afternoon. After completing the talks, I think the next step is to have clarifying questions for the speakers. But before we do that, I want to give opportunity to our colleagues from CD8 and well as CDER to introduce themselves. Maybe we can start with Noel.

DR. GERALD: Hello. My name is Noel Gerald and I'm a biologist and reviewer in the Center for Devices and Regulatory Health. I'm in the Office of In Vitro Diagnostics.

DR. CHATTOPADHYAY: Hello. I'm Rana Chattopadhyay. I am in the Office of Vaccine in the Center for Biologics. And in another life, I was a malaria researcher for 25 years. That's

Page 254

it.
DR. BALA: Thank you. So at this time, any clarifying questions for the speakers from the panel? Yes?
DR. MCCARTHY: I'd like to make two points of clarification. The first is about the expenses of QPCR. I think that's -- always I've talked about. But when you think about it, the cost of maintaining a high quality microscopy service that's got all the staff available to do -- whether it be CHMI or a randomized clinical trial -- I think, greatly underestimated is the cost of keeping the staff trained and making them available.
And at our center, the QPCR is logarithmically less expensive and more convenient. And I think that we really need to consider the opportunity cost of having high-quality microscopy routinely available in terms of these clinical trials and recognize that logic problems of executing a trial where you're infecting, particularly in the CHMI setting,

Page 255

we're infecting people to be able to do, in my case, twice daily QPCR and have a reliable and reportable and reproducible data back within four to six hours in cohorts of eight to ten people is much more feasible than trying to run a microscopy service.
In my hospital, I wouldn't rely upon the ability of my pathology department to reliably diagnose malaria because they see it so rarely. So I really think that we need to put that into consideration when we weigh up the pros and cons of QPCR versus microscopy.
The second point I'd like to make goes to the point of residual DNA and persistence of DNA I hear quite commonly talked about that volunteers in clinical trials or subjects in studies endemic settings where people have high level of parasitemia and parasites after treatment, that that is representative of a residual free-floating DNA, DNA that are associated DNA, who knows where, but doesn't represent viable parasites.

Page 256

We've had the opportunity in a clinical trial with a company that was doing an experimental study where people start out with extremely high levels of parasitemia. And what was apparent when we did analysis of their blood, right at the enrollment period, they had gametocytes present in their blood and the drug wasn't killing the gametocytes.
I had consistent positive PCR with their asexual parasitemia. And everybody was saying oh, look at the drugs failed or there's free DNA around or we can't associate one with the other. But when you went and did Psf25 PCR for gametocytes, what you were seeing was all their asexual parasites being cleared by the drug, and you had a persistence of gametocytemia.
So I think it's a gross over-estimation of the situation to make a claim that persistent DNA signal after cure of treatment represents anything other than persistent parasites, and more often than not in this situation, it's persistent gametocytes. So I don't know if ours

Page 257

would come in on that, I've had experience. But I think it really gets in the literature and tends to compound people's thinking that what we're seeing is really somehow or other something other than viable parasites. And therefore, cast aspersions on the reliability of the QPCR and clinical trials.
DR. BALA: Thank very much, James. This is really spoken from my heart because I have encountered these reports and meetings, conferences and so on as well. And I always commented on gametocytes and people have not done tests for gametocytes on RNA level and I think we have to watch out for that.
And eventually, if we are reviewing papers and stuff, point it out because that is brought out in the scientific community some doubts and some people are puzzled.
So I think we really need to make a strong statement about that. But also for us, it has, of course, consequences because we encounter this eventually also in field trials, so we have

Page 258

to consider this.
And coming back to my point, if we want to apply the QPCR in a field setting, we might pretty well detect gametocytes. And then what do we do then? How do we treat it? So that's why my argument would be not to go to the ultimate sensitivity level but stay with microscopy and then, either you see the gametocytes or you can ignore it.
I know David is thinking possibly along different lines. But I think we could still use it, as he suggested, as sort of a quality control at the end of a trial if you have doubts about the microscopy because we keep the blood spots, and that is very easily done.
DR. MCCARTHY: And to add to that, the other possibility is to give a small dose of primaquine that will clear the gametocytes, then that would take that off the table.
DR. MURPHY: So I have a comment and a question for Dr. McCarthy. So the first thing I want to say is I would like to go with what Jim

Page 259

McCarthy said, which is that these tests, though they're often called to be expensive, have been actually very cost-effective in our center.
For instance, we used to domicile all the subjects in a hotel. The hotel phase is a well-known feature of human challenge studies, historically. And because we now treat people on the basis of molecular tests at low densities where they're at asymptomatic, we have no need any longer to spend two weeks basically of hotel costs in every study for every subject. And this is a tremendous savings.
It also is much better for the subjects who start the trail thinking it will be great to be in a hotel with a pool and, two weeks later are going crazy, basically.
And so now they come to the clinic every morning and they go about their business for the rest of the day. So this has been very cost-effective. And these kind of molecular tests are used in HIV trials all the time.
We've learned a lot from the HIV

Page 260

community. And they don't have microscopy to look at the virus so this is how it was from the beginning for them and we think it's very effective.
My question to Dr. McCarthy has to do with recrudescent. And what I'm wondering is, if you were to treat people at a low density and go to zero with a molecular test quite promptly, is there a certain number of days beyond which you would be very unlikely to have a recrudescent?
That is, if you had three or five or seven days of negative molecular tests, would it be more likely that in the field, if someone came back with malaria, that it's a new infection rather than a recrudescence. Do you have any data on that?
DR. MCCARTHY: I don't have any data on that. I think the key issue there is the drug half-life.
As soon as you get below what would be considered to be an inhibitory concentration of drug, you're going to then be in a situation

Page 261

where your parasites are going to begin to multiply. So a very short half-life artemisinin then you're going to quickly see recrudescence.
At that's certainly been the experience with one of the ATPA four inhibitors that we had some experience with that we saw very rapid reappearance of parasites, early recrudescence where a drug such as mefloquine or piperaquine for example, when we used in it low dose, we saw that it took a couple of weeks before the recrudescence took place.
I think we could model that, but I think, experimentally, one would be very cautious to take somebody out of the study with a long half-life drug saying that they have been cured, unless you followed them up for quite a period.
DR. BALA: Thanks very much, Sean and James.
DR. NAMBAIR: Any other questions from the audience?
PUBLIC COMMENTER: I have two questions, and actually, I'm going to read them. One of the

Page 262

most important things in clinical trials is selection of the candidates.

I was a little worried this morning that we wouldn't get to this point of enrollment but I'm glad that we got here. I wonder if in selecting candidates, if two important tests would be one, the selection test; for example, a PCR or microscopy. And from what is being said, I guess the PCR is going to be the more effective test for screening.

The other test I suspect that we might need is an immunological test to determine whether or not the subject has antibodies to one or the other parasites, one of the other species.

I wondered has anybody looked at the possibility of Duffy in respect to identifying candidates with P. vivax. That's number one.

The second question is, in mixed species infection, when you're doing a PCR, if you have a very high count in one of the species, for example, falciparum, does it mask a low infection of vivax? Have you seen that?

Page 263

Thank you. Those are my questions.

DR. MURPHY: So I think those are all good questions. I think your first point of whether you should use PCR microscopy, I think the point I was trying to make is that PCR for enrollment is rarely available in real time, and you're probably going to be limited to microscopy in most center.

Immunological test of antibodies are notoriously not helpful in choosing candidates to enroll. Because most of the tests that we use don't tell you about functional immunity. They can tell you maybe about past exposure but they don't necessarily tell you how immune somebody is, per se, to malaria at that moment.

And they also do not necessarily correspond to a clinical response. So those have been challenging to quantify and use in a clinically meaningful way.

And then masking mixed species infection, mixed species infections can mask one or the other and often confound microscopy. It's not

Page 264

necessarily easy to tell a mixed infection by microscopy, particularly if one of the species is a very low density compared to the other. So I think that's where PCR really is essentially, at least in your post-talk analysis to figure out what the patient actually had at the time.

I'm not sure I understood the question about the Duffy antigen though.

PUBLIC COMMENTER: Yes. You know Duffy affects plasmodium vivax. So I'm wondering if it's important in determining whether somebody has vivax or had vivax, if it would be important to check to see if there were antibodies for Duffy. Duffy antigen is a receptor for --

DR. WEINA: So I can try to answer that question. My only experience in this is some studies that Ruben Wang did in Colombia to look at immune responses in subjects to vivax and to falciparum, and they categorically tested people for Duffy and split the data along the lines of Duffy positive and Duffy negative individuals.

So yes, it's true that if you were

Page 265

looking at P. vivax, you would probably want to know whether people were Duffy positive or negative. So yes, it's true that if you were looking at P. vivax, you would probably want to know whether people were Duffy positive or negative.

I was going to comment on your second question which was about mixed infections. So for instance, in our group, we built our test to have a P. falciparum specific channel, a pan plasmodium channel that detects the 18S not just from the human species, but also from Simenon and Myriam species. So it's a real bona fide pan plasmodium target. And when we do mixing studies, we can detect one part of P. falciparum in the presence of 10,000 parts of P. vivax. So we felt that that was an important thing to be able to show.

Obviously, if it's P. falciparum as the overwhelming component, we can't find one part P. vivax in that setting because the pan target is overwhelmed by the P. falciparum but we could

Page 266

find a needle in a haystack when it's falciparum in the presence of something else.

PUBLIC SPEAKER: Thank you.

DR. BALA: All right.

PUBLIC COMMENTER: How good are we at distinguishing reinfection from recrudescent infection?

The reason I'm asking the question is that if somebody has initially a polyclonal infection and is a minority species, can we find that early on? Recrudescence could be the minority species popping up at some later point in time, as opposed to get a new mosquito bite, new infection. Do folks have insight into that or is there data that helps to address that issue?

MALE SPEAKER: Well, I think there's some and part of the problem is often, you know, recrudescent infections -- recurrent infections have very low parasitemia. So it would be possible to miss, you know, very low minority variance if they were to occur.

Page 267

I think for the most part, it's fairly useful, fairly predictive. I don't know, though, that anyone has really gone to the trouble of quantifying exactly how use it is. And I think that might actually be a little bit challenging to do.

DR. FELGER: May I comment on that quickly? I would say it has been quite robust technique, despite the fact there are polyclonal infections and that is the rule for P. vivax normally. And plasmodium falciparum in African samples, there are infections, about five co-infections. So when you compare the pretreatment and post treatment sample, we don't need to find all the genotypes in both samples. If we see one, which is the same, then we would say this is recrudescence. So we don't need to redetect all the clones, right. So that is the definition.

Normally, if that is a resistant parasite, it would come up. It would have selective advantage very often and will expand because not all clearance are -- not all baseline

Page 268

clones would be resistant. So you can expect to see that one. Of course, there are new clones coming in, which will be competing. But I mean, it has been a very, very good, very robust methods so far.

There is much advancement in the methodology. We have started by gel electrophoresis where you could hardly really -- the two bands have the same height. But now, I mean, really, with a couple of electrophoresis, we can size it to one base pair and it's very precise. So there has been a huge advance and I think we are still improving because we are learning more.

The field is moving and thank God the field is moving. It just shows that we make efforts to optimize. So I think we can now, for example, stop multiplexing reactions. It is a little bit more expensive, but we reduce the competition between clones very much. So that simple, really simple method can sort out the problem to a certain degree but there is always a

Page 269

biological handicap. I mean, because the biology of the parasite is quite amazing with the sequestration and absence in the peripheral plat of a cell clone. We cannot overcome that even with the best method, we cannot because we only can sample 200 microliters maximum. That's the problem.

DR. BALA: With that, I think we can move to questions for discussion here. The first question is please discuss the detection methods to be used in CHMI studies, when infected by different routes, or with the different state of the parasite such as bites with the infected mosquitoes, injected with the sporozoites intravenously, or infected erythrocytes.

Please discuss the assays, their performance, and threshold for positive findings to identify patients that need rescue therapy.

MALE SPEAKER: So my talk attempted to kind of provide some data and some perspective on what other centers are doing. I do think that there is not agreement over -- amongst the

Page 270

centers. There is some disagreement about whether we should endorse a specific threshold or whether thresholds should be specified within each clinical trial protocol.

So for instance, some centers advocate getting more data points in order to model to look for things like partial immunity. In our center, when we do mostly prophylactic studies, the goal is to prevent infection emerging into the blood stream, virtually in every subject that we've ever seen, there is really -- once the parasites are in the blood stream, they are free to multiply, even if the therapeutic or the drug was intended to block something upstream of there.

So in our sense, this means that these shouldn't be there and it's time to treat the patient. And so I showed the data on that and we've selected a threshold that is not rated at the limit of detection, so we're not sort of struggling with the test at all. Not so high that the patients are symptomatic. But that is

Page 271

not a view that is held by some of the centers, some of which are not represented here today.

So there could be some disagreement in the field over that. But I think for prophylactic studies, at least in our center, we've endorsed this. Gradually, we've seen the implementation of a threshold because if you're going to do a molecular test that's quantitative, you must have a threshold if you're not going to treat at the very first positive.

For instance, one of the centers that was on the slide is the NIH Clinical Center, which has a very good assay, but it's a qualitative assay. They know the approximate limit of detection. And for them, their threshold is two positive tests because they can't describe a specific quantitative value. And so they similarly can avoid most but not all of the symptoms in that setting.

So for them, two positives equals rescue treatment. At the moment, that's what we have is a bunch of different thresholds that hover around

Page 272

each other. And if your goal is to mitigate symptoms and declare people failed for liver protection, that's reasonable. I guess one of the questions along these lines that I have for Dr. McCarthy has to do with if you're testing a radical cure, what are the most important parameters?

Obviously, you have to be safe, but in order to adequately challenge a drug, is it enough to have a few days of exposure and clearance to zero, or do you want a biomass that is 10 or 100 times higher than the threshold we're talking about in order to be a little closer to what really symptomatic patients who are coming into the hospital are like?

Are you trying to mimic symptomatic disease or do the curves, you showed earlier, two curves that had the same slope. If they have the same slope, might you treat earlier or do you need to go later?

DR. MCCARTHY: First of all, it's a statistical issue in terms of getting enough data

Page 273

points to be able to draw a line between data points, but that's not particularly reliable. So the more data points you get, the better off you are. I don't think we have a good understanding of what the symptom threshold is in falciparum that I see enormous variation in symptom in my volunteers and some are actively collecting data on that some volunteers can be symptomatic in what I would consider to be trivial levels of parasitemia.

DR. CHATTOPADHYAY: Right. If the sponsor of the trial they are saying we will be using PCR, if that country's accommodation is no, you'll have to -- whenever a person has a fever, you'll have to first do a blood spear with RDT. So they kind of go by that also. So it is kind of, you know, like depending on what the trial is. So we sometime look into those things, too.

DR. MURPHY: I'll just make a comment about our own application from the biomarker qualification program.

The FDA has a program called the Drug

Page 274

Development Tool program. It's through CEDR. And my group has submitted a -- we submitted a letter of intent and then an additional briefing package.

And the focus of this -- our project is to qualify the that 18S ribosomal RNA and/or the ribosomal DNA as a biomarker to replace microscopy for whatever the context of use that was specified.

So the initial context of use that we're hoping to submit later this year has to do with replacing microscopy in CHMI studies in non-endemic regions like in Seattle. And then it's our hope that if we can proceed with that, we might extend that eventually to other contexts of use, for instance, CHMI in the endemic regions and potentially down the road, you know, more like field acquisitions. Then each of those benchmarks would have different questions.

Obviously the non-endemic study in Seattle is the most highly controlled and most of the questions have to do with, you know, the

Page 275

validity of the biomarker as evidence of infection and the performance characteristics of the test.

As you move to the field, there's obviously issues of strain diversity, reinfection and recrudescence. And so those are things that we might have to deal with in the future.

It's my understanding that the qualification program is not a categorical approval of any one test. It's a qualification of the target. But along those lines, if we qualify the target, then there would be test characteristics that are required to meet that qualification that one would have to meet.

Now, that could mean you could use our test or you could use another test that meets those qualifications. We thought this was a good pattern because recognizing that there are a number of centers that have different and good tests that this may be a way to streamline things and yet provide a guidance.

DR. WEINA: And in general, too, as we

Page 276

gain experience with different types of tests and approaches, we can memorialize those, too, in guidance documents. So you sort of see sort of the progression here over time.

DR. MCCARTHY: Well, I mean, the main reason I bring this up is kind of just thinking through the whole thought process and direction of the conversation because, you know, we're using one of these tests and you spoke about thresholds.

If you have a threshold that's too low, you know, picking up, you know, the idea of moving the product forward is to, you know, see how it's going to be used in real life. I mean, that's, you know, part of the argument that we have for doing the phase threes, right?

And if you're always picking somebody up before they even have any kind o0f symptoms whatsoever, so the idea is to try to stay as safe as possible hanging back, are you really giving them a fair trial because you haven't -- the drug a fair trial because we're treating before the

Page 277

patient's even being symptomatic, where in the real world the patient feels crappy for a day, maybe two days, maybe three days before they even bother to come in, and then you've got to have a clinician actually being astute enough to recognize what's going on and test them.

Next thing you know, it's three or four, maybe even five days of parasitemia and being symptomatic before the drug even comes onboard. So now you've got a huge biomass as opposed to what you were testing in -- originally and exposing it to in which there was -- biomass was so small because the person was asymptomatic recognizing, of course, there's tremendous variability among patients. And some of them are going to be pretty wimpy and come in early and other ones are going to be John Wayne and have 10 percent parasitemia before they even start to complain, so.

DR. MURPHY: I'll just comment on our approach to the (inaudible) and I suppose approval process for our qPCR in conjunction with

Page 278

MMVR approaches being to validate RSA, to develop a strong quality system. It's well documented with all the appropriate tests that one would use; work with our local regulator in Australia to have our tests registered under our local regulator and then participate in an EQA program with other centers doing CHMI and also doing a qPCR for clinical trials.

And I suppose with that platform, although we've not tested it with a regulator in terms of a drug registration process, we're hopeful that all our efforts won't be in vain.

DR. FELGER: I mean, we could also add -- it's just an idea -- but at some additional external quality control that would define certain center which would perform a highly-alkaloid assay, for example, digital droplet PCR which could then process 10 percent of all samples from a clinical trial just to validate that against something which is unbiased because a digital droplet PCR doesn't need a standard, internal standard.

Page 279

It's just basically gets a very alkaloid quantification independent of some external standard trend line or whatever. So this will take out some of the viability and that could be maybe one idea to have one center who could run this for clinical trial just to be on the safe side if there are concerns on quantification.

But I think your concerns were more on is that relevant, is -- that very low threshold, is that very relevant at all? This, I mean, from the lab side we cannot really -- I mean, that needs to be discussed.

MALE SPEAKER: An additional issue I think for the performance of these assays is when we do start, you know, and when we're evaluating these drugs, some of them are kicking out higher parasitemias than others.

And it looked like, you know, from your data, James, for example, the quantification of those gametocytes may have been higher than the actual parasitemia, and that is likely due to the transcripts of the gametocytes perhaps being

Page 280

greater than the asexual form. But we don't know that definitively. And so investigating that further, I think will be, you know, very helpful for the centers as we monitor those gametocytemias, you know, post rescue therapy.

MALE: I mean, it's a good point. We have been talking about developing a standard because we have thoughts about having copies of pfs25 where there are per gametocyte, and we have a process in place where we're sharing samples at the moment.

I think those numbers are really based upon transcripts numbers per mil against a standard curve and not the number of gametocytes present.

DR. MURPHY: James, can I ask a quick question about the gametocytes? When you look at the 18S for gametocytemia, it is always a fraction of the 18S content that was the maximum of the asexual, right? The 18S never rises above what the max was in the asexual stage.

DR. MCCARTHY: I think the curves were

Page 281

reversed and that was an issue related to standard curves. I think our gametocyte curve on that particular graph was higher than the 18S graph. And I think that's just an artifact of how we set the standard curves and not the fact that we've got more gametocytes present than we have.

PUBLIC SPEAKER: So in well, you're doing DNA. We find the RNA content of the gametocytes is about four times higher than it is for rings, which isn't surprising. They're a little bit larger. Okay. Thanks.

DR. FELGER: Any other questions from the audience? Can we have the next question?

DR. BALA: The next question would be that we discuss --

DR. FELGER: Just one moment. I think we have someone else.

DR. BALA: Oh, sorry.

PUBLIC COMMENTER: Hi. This question is about regulatory strategy. Okay. So assume the -- as I understand it, the thick blood smell is

Page 282

the gold standard, right? That being so, if I develop a drug and I use the gold standard and have an in-house developed tests, which I correlate with the blood smell and I submit to CEDR, and through discussions the product is approved based on my tests, (inaudible).

Now, if after approval I went to market that test, for market purposes, what is CEDR going to say about that? Is that a lab-developed test or what is it?

DR. GERALD: So yes. In general, if you wanted a separate application for marketing your test now for in vitro diagnostic use in the US, that would be a separate application to CDRH.

DR. COX: Yeah, and it is possible to do both. And one of the things we talk about is that, you know, a clinical trial and, you know, the patient specimens that are obtained in a clinical trial, you know, with appropriate consent when planning ahead of time, it may also be an opportunity to study and/or develop a diagnostic test. So it can happen, you know,

Page 283

concurrently.

DR. FELGER: And then the other thing that I would mention is that, you know, for CDRH, for diagnostics, everything is focused on the intended use and how you define that. And so the parallel here that we're talking about in this workshop is, you know, performance characteristics of the context of use.

And so being very specific with your context of use, and when we talk about issues of, you know, performance characteristics for quantification, you know, you're going to need to know, you know, just as an example, you know, in CDRH, you know, the accuracy of your test itself, the imprecision of the test itself becomes important if that's near the clinical decision points of what you're going to use that for.

So it all, you know, has a lot to do with the intended use in CDRH, and parallel considerations come into play with context of use.

DR. BALA: Thank you very much.

Page 284

Now we are moving to the second question. We are going to discuss whether the molecular assays are a tool for enrolling subjects in a field trial and the different section of recrudescence from new infection and the ability to differentiate multiple strains including those present in low density.

I think we have quite discussed a little bit on that, but we haven't reached a conclusion on the first point. Is it a tool for enrolling subjects in a field trial?

We have concluded that on the spot we have light microscopy, which clearly needs in some instances a confirmation by molecular speciation, molecular methods. I mean, I think we all agree on that. There could be cryptic vivax there and that is a problem in some areas.

But yeah, I don't know. I mean, are there any further opinions here on the panel or in the audience on that topic? Yes, please?

MS. HIGGINS: I have a question about recrudescences and reinfections and when they're

Page 285

typically discovered in a clinical trial.

Dr. Saunders, I noticed in your talk it looked like they were found pretty much near the end of a trial, let's say if the test of cure was Day 28. And in that case, I'm a little less concerned about using an adjusted cure rate as opposed to if a new infection was found early on and then the subject was treated for that new infection such that it might suppress the recrudescence that they couldn't be captured.

So would you say that most recrudescences and reinfections are found at the end of the trial?

DR. SAUNDERS: Yeah. I mean, it's fairly rare. Because of the incubation period, if a new infection is unlikely to occur until after about two to three weeks, that's the earliest we would see it. And I would say, you know, again, there's no hard and fast rules here, but the recrudescences also tend to occur usually a few weeks later, so Week 3, 4, 5, if you're doing a six-week follow up. That's when the majority are

Page 286

seen.
You know, the way things are defined, if somebody occurs -- recurs earlier than that, you know, within the first week, that's usually called an early treatment failure, and oftentimes they've never really, truly cleared, and they may have submicroscopically -- they may have cleared down to a submicroscopic level. But the clinical infection becomes apparent within a couple of days.
But, no, I think for the most part they tend to occur later in the follow-up period.
DR. BALA: Thank you. I have a question for Dr. Saunders. Did half-life of the drug have anything to do with the time and recrudescence would occur?
DR. SAUNDERS: Yes, it has -- it can have a lot to do with it. It just depends on, you know -- and that's highly variable. I mean, it depends on what drugs you're using and what combination and what the properties are and so forth. So I think that can have a significant

Page 287

impact.
DR. BALA: And the second question I had was regarding the msp2, msp1 glurp markers which you used, did you do them sequentially or were they done -- all three were done on -- which is baseline at the time recrudescence occurred?
DR. SAUNDERS: Yeah. No, you have to take parent samples and run all three tests on them at each time point.
DR. BALA: So all three were done.
DR. SANDERS: Oh yes. Yes.
DR. BALA: Okay.
DR. FELGER: Can I ask a follow-up question about your recrudescence data?
So when you say recrudescence, I think you mean microscopic recrudescence. Is that correct? At Day 28 or 56, those -- because in order to detect msp1 and these things, there would have had to have been molecular detection at all of those time points in between.
DR. SAUNDERS: Right. And generally, because the molecular methods are not used in

Page 288

real time during the trial, recurrences are, if they occur, are only going to be discovered microscopically.
PUBLIC COMMENTER: Sure.
DR. SAUNDERS: So, you know, generally speaking, in field trial, we don't hunt for submicroscopic recurrences. But now because increasingly PCR is being used to correct the microscopy result, we discover after the fact that, you know, there was a persistence of microscopic infection.
The other problem, though, is, you know, at the level where you only have submicroscopic infection, there is often not enough DNA to do an adequate PCR or adjustment using msp1 or msp2 where that is convincing.
And even sometimes in very low parasitemia recurrences, it's challenging to actually adjust the results. So there are occasionally, you know, recurrences that we cannot genotype adequately and determine.
FEMALE: Maybe a comment to genotype, to

Page 289

detect cones, which are at very low density. This is quite possible if there is one clone only, which is extremely low density and we perform next the PCR on that. You have no problems to detect it.
So this is also only a problem in competition with other clones, of course, and currently methods are underway and the idea is that you don't use length-polymorphic markers anymore but markers which have a stretch, which have a lot of single nucleotide polymorphisms in a stretch of about 300 bases.
And then these fragments are sequences with next-generation sequencing, and there it should -- there should be no bias anymore, and the detection of that part -- of a particular minority clone would only depend on the depths of the sequencing, right, so how many fragments, individual single fragments are sequences.
And, of course, the depths of the sequencing depends very much on the costs or affects the costs. If we want to do it cheaply,

Page 290

we multiplex 100 or 200 different samples in one run because this is quite expensive, costs more than $1000 one run. So it's only feasible with many, many samples, which are run at the same time with sort of nucleotide identifier index so you can deconvolute individual samples later on.

So there is a possibility but this is currently under evaluation whether this method, the amplicon sequencing method will be better in determining the minority clones because we also learn from HIV, again, that has been in HIV exactly the same question, the minority clones, and they also try to address this with next-generation sequencing.

So the methods are in development and I am pretty sure we will see that later. But this would clearly be, you know, done in certain centers which have the bioinformatic expertise. This is not something what can be done anywhere in the world.

Right now what we do so far, there are many places who do recrudescence typing all over

Page 291

Africa and in Asia, South America. So that possibly will be restricted because there the challenge is certainly the bioinformatic and the analysis.

DR. FELGER: Any other questions from the panel or the audience? So maybe then we can move on to the next question.

What did these two information should be collected besides genotyping to confirm resistance to the drugs in an endemic area?

DR. SAUNDERS: Well, I suppose it depends on what resistance you're concerned about in that endemic area. But, you know, I mean, there's pretty well-defined markers for most drugs now. And so those markers are obviously helpful and pharmacokinetic data is helpful.

You know, someone brought up unity data. You know, we still don't completely understand the interactions between unity and apparent resistance and drug resistance. I think maybe that's less so. But those, you know, known markers of resistance, pharmacokinetic data I

Page 292

would say those are two essential pieces.

FEMALE SPEAKER: I would like to add something along the lines of the targeted amplicon sequencing. Our hope is that we could there include, because we are multiplexing highly and indexing each sample, that we would include amplicons of, for example, Kelch 13 or, I mean, we could -- depending on the drug we are interested in or the drugs we are using, we could use known molecular markers of drug resistance. We could use all of them without any additional costs.

So then we would gain the currently available information and drug resistance markers, blast the genotyping and maybe other things. So we have multiplexed 10 different fragments already and we can deconvolute them later without any problem, so it is feasible.

But I mean, it's really a question should -- because we can do it, should we do it all?

DR. SAUNDERS: Well, I think the other thing to say is it's a rapidly evolving field.

Page 293

You know, K-13 just a couple of years ago was thought to be the (inaudible) and resistance marker and now we realize maybe that's just a setup for other markers. So the understanding of resistance, particularly with the has changed very rapidly.

One other thing I would mention, I think when you're looking in an endemic area, one of the issues -- one of the big issues -- and this is often overlooked in trials -- is preexisting use of antimalarials by the subject. So that's often an exclusion criterion for trials and one that is notoriously unreliable based on clinical history.

So identifying what drugs a patient may have taken in the last 30 days or longer that may still have some anti-malarial activity or may have influenced the resistance of the parasite that you're treating now are critically important.

And there's a couple of ways you can do that. There is -- you can take the patient's

Page 294

plasma and incubate it in vitro or, you know, ex-vivo against known parasite clones to determine whether there's anti-malarial activity in the blood before you treat the patient. So it's important to get a baseline sample.

There's also, you know, pharmacokinetic methods that have been worked out, bioanalytical methods that can scan a patient's blood sample pretreatment and look for a series of known antimalarials, I think up to 14 or 15 in one run. That was published by the Swiss Tropical Institute several years ago, a very helpful thing to do to see what preexisting antimalarials the patient may have taken because that could, one, you know, influence the results of the outcome of your trial, could exclude the patient, you know, based on what were the stated enrollment criteria in the study.

And it could certainly influence variables like parasite clearance and so forth, and it could inform, you know, particularly inform the results if you have a clinical failure

Page 295

by, you know, indicating, well, oh, gee, that patient actually had taken that drug already and if we had looked at their blood we would have known that.

MALE: So a much more low-tech answer to your question, so before genetic resistance markers are identified or validated, prophylaxis failures in travelers, treatment failures as well can help detect your drug resistance in the endemic area.

PUBLIC COMMENTER: Just a brief comment, which I think reaches the same conclusion as the other comments here, and that is one tends intuitively to say to oneself if you could grow the parasite from that blood sample when the patient had their recurrent illness and then test that in the lab, maybe that would be optimal.

And in our experience, that's really not been the case because so many of these infections, especially in Africa have multiple clones. And if you use your markers carefully, you usually see an attrition or loss of clones as

Page 296

you go from the initial specimen to what grows in culture.

So what comes out of culture will often give you a less accurate idea than what the panelists have been talking about, which is using the genotype approach.

MALE: Yeah. I actually don't think the genotyping as we were describing it is particularly useful. It's certainly not the primary variable for resistance. I think it's just sort of a crude adjustment of your efficacy overall, but there can be many factors contributing to that lack of efficacy and not just resistance.

PUBLIC COMMENTER: Talking about low-tech technology providing that we merge with the microscopy or PCR seems to be something that we should not forget, especially when we don't know which marker we want to look at. This has been, at least in the way we have detected falciparum resistance.

DR. BALA: Any other comments, questions?

Page 297

No?

So with that, I turn it back to Dr. Cox.

DR. COX: All right. Well, I want to thank everybody for a very productive day and a chance to, you know, discuss clinical trial issues, methods of detections.

And really I think, you know, the reason that everybody is here is really to, you know, move forward the field of therapeutics for malaria, recognizing the patient needs out there.

I also, too, just want to reflect back to and, you know, the many folks that had this, you know, as an idea and something we ought to do, I want to thank in particular Wendy Samhain (ph) for helping to push this along and also the folks from MMV for all their participation, folks from industry academia, the government colleagues and Sania Shukla (ph) from our office, too, who also was key in planning all this.

So we thank you all for your efforts and for all that was done to make the opportunity to get together here so productive.

Page 298

We recognize there's a tremendous amount of work that goes on in preparation for these workshops and all that was done, and I think that been apparent in the discussions today and really the fine presentations.

So you know, clearly, you know, this is an area of drug development that's important. I think the workshops provide an opportunity for us to get together and understand, you know, the current state of where the field is with regards to clinical trials and drug development and also areas for additional development and questions for the future. But I do think it's a great opportunity to push things forward, and I look forward, as we all do -- our colleagues in CEDR, our colleagues CEBR (ph), our colleagues in CDRH -- to talking about development of new therapies, whether they be drugs, diagnostics and/or vaccines.

So with that, I'll thank you, wish everybody safe travels, whether you're going near or far, and look forward to future opportunities

Page 299

to meet with folks and continue to push forward the field of anti-malarial drug development.

Thank you all. Have a good day.

(Whereupon, at 4:02 p.m.,

The public meeting was adjourned.

was concluded.)

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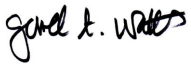
Page 300

CERTIFICATE OF NOTARY PUBLIC

I, GERVEL A. WATTS, the officer before whom the foregoing deposition was taken, do hereby certify that the testimony that appears in the foregoing pages was recorded by me and thereafter reduced to typewriting under my direction; that said deposition is a true record of the proceedings; that I am neither counsel for, related to, nor employed by any of the parties to the action in which this deposition was taken; and further, that I am not a relative or employee of any counsel or attorney employed by the parties hereto, nor financially or otherwise interested in the outcome of this action.



GERVEL A. WATTS

Notary Public in and for the

State of Maryland

My Commission Expires: June 7, 2020.

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