UNITED STATES FOOD AND DRUG ADMINISTRATION

PATHOGEN REDUCTION TECHNOLOGIES (PRT)

FOR BLOOD SAFETY

PUBLIC WORKSHOP

Silver Spring, Maryland

Friday, November 30, 2018

PARTICIPANTS:

Welcome:

C.D. ATREYA, Ph.D.
OBRR, CBER
Food and Drug Administration

SESSION 4: Emerging Innovations Relevant to Pathogen Reduction Technologies and Alternatives:

STEPHEN WAGNER, Ph.D., Moderator
American Red Cross

Blue Light Inactivation of Pathogens in Platelets and Plasma: A Pilot Study:

MICHELLE MCLEAN, Ph.D.
University of Strathclyde

A Nucleic Acid Binding Photosensitizer With Flexible Structure for Pathogen Inactivation in Red Cell Suspensions:

STEPHEN WAGNER, Ph.D., Moderator
American Red Cross

Pathogen Reduction in Blood Products: Refrigerate and Use PRT:

COLONEL ANDREW CAP, MS, M.D., Ph.D., FACP
U.S. Army Institute of Surgical Research

Panel Discussion:

MICHELLE MCLEAN, Ph.D.
University of Strathclyde

COLONEL ANDREW CAP, MS, M.D., Ph.D., FACP
U.S. Army Institute of Surgical Research

PARTICIPANTS (CONT'D):

SESSION 5: Funding Opportunities for Future Pathogen Reduction Technology Research:

SIMONE GLYNN, M.D., MPH, Moderator
NHLBI
National Institutes of Health

Panel Discussion:

ASHLEY CECERE

MAJOR BRYAN KUJAWA, M.D.
U.S. Army

SESSION 6: Summary Presentations:

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SIMONE GLYNN, M.D., MPH, Moderator
NHLBI
National Institutes of Health

Session 2:

BILL FLEGEL, M.D., Moderator
NIH Clinical Center

Session 3:

RAYMOND GOODRICH, Ph.D., Moderator
Colorado State University

Session 4:

STEPHEN WAGNER, Ph.D., Moderator
American Red Cross

Concluding Remarks: Insights for Future Research and Development:

PAUL NESS, M.D.
Johns Hopkins Medical Institutions

PARTICIPANTS (CONT'D):

Closing Remarks:

NICOLE VERDUN, M.D.
OBRR, CBER
Food and Drug Administration

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P R O C E E D I N G S

(9:00 a.m.)

DR. ATREYA: Good morning, everyone. I hope you enjoyed yesterday's sessions very well and this is welcome to day two of the workshop. And I am looking at anybody else in the hallways to come inside and then, when we start as soon as possible. And hopefully, we'll have very pleasant discussions today, and it will end around 1:00. And then, after that you guys are free. Thank you.

Steve, you want to come here and do it after -- you have anything to say your words, then we can keep on that point.

DR. WAGNER: Good morning. My name is Steve Wagner. I'm with the American Red Cross and welcome to session four which is emerging innovations relevant to pathogen reduction technologies and alternatives. And our first speaker today will be Michelle MacLean from across the pond, as you will. And it's entitled "Blue light inactivation of pathogens in platelets and

plasma; A pilot study."

DR. MACLEAN: Okay, thank you.

DR. WAGNER: Wrong one. We'll get it eventually, yes. Okay. You're good.

DR. MACLEAN: Okay, thank you very much, and first thing, I'd just like to, again, thank the organizing committee and C.D. for inviting me here today. It's an honor to be able to come here today to tell you about some of the academic research which we've been doing at Strathclyde University in collaboration with C.D. and Monique here at the FDA. And I'm going to talk to you a bit about the work we've been doing looking at blue light for inactivation of microbial pathogens within platelets and plasma.

Now just to give you a bit of background initially about myself and the team that I work with; I, myself, am an applied microbiologist and bioengineer. And I work at the University of Strathclyde which is in Glasgow in Scotland. And the area that we work in is very much associated with the development of novel technologies in both

optical and electrical engineering technologies for infection control applications.

And one of the main technology areas that we have worked on over the last quite a number of years now is the use of violet-blue light for antimicrobial applications. Now in terms of germicidal light, it's well known the germicidal properties of ultraviolet light. And over the decade or so there's been a growing awareness of the antimicrobial properties of light in the kind of violet-blue region.

Now the peak antimicrobial efficacy we found through a number of studies which we've conducted at the university, but the peak antimicrobial efficacy is found to be in the region of 405 nanometers. So we're looking at wavelengths down towards the kind of cusp of the ultraviolet region of the lower end of the visible spectrum. These violet-blue light regions have been found to possess some quite broad spectrum antimicrobial effects.

Now in terms of the use of these

wavelengths, it has a number of benefits. Although it is less germicidally effective than ultraviolet light, and this is due to the fact that it's low-energy wavelengths. That also has a benefit in that these wavelengths can be used at levels that permit safe application for exposure to mammalian cells and people. So this safety benefit has opened up the interest for this technology for a variety of application areas.

And two of the kind of most prominent areas that are being investigated currently are the use of these blue-light wavelengths for environmental decontamination. Now this is an area that we have worked a lot on at the University of Strathclyde. And to give you a bit of background about this, we have developed lighting systems, broad spectrum white light systems which contain high output of light in the kind of 405 nanometer range. And these lighting systems permit continuous environmental decontamination in areas where there's people present.

So the fact that you can have this decontamination effect being safely applied 24/7 in areas where there are inhabitants has proved very beneficial. And we're working a lot for this for development and commercialization within the healthcare sector. This has, over the last year or so, been commercialized as a separate application.

But there is also a growing interest and other research groups have been looking at it for wound decontamination. Again, the problem of antibiotic resistance is growing, so the development and emergency of new technologies which can help limit the spread of infection are being investigated. And there are a number of groups, particularly in the US, who have been looking at the development of lighting systems for exposing wounds for wound treatment. So these are some of the areas that are going on looking at the antimicrobial effects of these light wavelengths for practical application.

To give you a bit of background about

the antimicrobial effects of light and how the actual mechanism of inactivation works, I have a diagram here which displays it. But ultimately, it relies on a photodynamic inactivation process. So within organisms that are exposed, organisms contain these molecules, porphyrin molecules, and these molecules have an absorption maxima in the region of 405 nanometers, so typically between 400 and 410 with peak around about 405.

And when these organisms are exposed to light of these wavelengths, the photons are absorbed by the porphyrin molecules, and this results in the photoexcitation of the molecules. And once these molecules have become photoexcited, they can then react with elemental oxygen, or with components within the cells to produce a range of reactive oxygen species. And once these reactive oxygen species are developed, they can then work throughout the cell to cause a range of nonspecific damage. So this can include things like membrane damage, DNA damage, and also lipid damage.

So the fact that it is -- doesn't rely on DNA-specific damage, it's very much nonselective. So essentially anywhere within the cell that has this ROS come into contact you'll ultimately get the damage. So this is ultimately the mechanism that we've been finding and there's a growing body of evidence surrounding this in the scientific literature.

What we want to mention as well is that so far we have found these light wavelengths to have very broad spectrum antimicrobial effects. Within our group at the university, but also wider groups across international research groups, there's a lot of work now looking at the antimicrobial of these wavelengths. And ultimately, what we've found has been that it's got broad spectrum efficacy against a wide range of gram positive and gram negative organisms, also against yeasts and fungi, and we've done a bit of work looking at viruses as well. And I'm going to come back to talk about viruses in a few slides. Because with viruses, although you get an

inactivation, it's very situation-dependent, so I'm going to talk more about that.

But currently, from what we have found, we're yet to find an organism that doesn't show susceptibility to inactivation through this mechanism. So it does demonstrate in great broad spectrum application.

So through the work that we were doing at the university, there's a large range of advantages of these light wavelengths which I can talk about. So I mentioned the broad spectrum antimicrobial efficacy. So this was opening up various application areas, but in addition to this, a key aspect is that these wavelengths, because they are longer wavelength than ultraviolet, it does mean that they have greater penetrability. So they can penetrate into materials and into substances to a greater depth than shorter wavelength energy.

So this, again, helps look at different applications areas. And additionally, I mentioned that the non-requirement for photosensitize are so

-- the photosensitizing agent in the case of this technology is actually molecules which are within the microbial cells themselves. So there's no necessity for the addition of additional chemicals or molecules into the inactivation and treatment.

And again, operator safety, operational safety, so the fact that these wavelengths are from within the visible spectrum means that there is increased safety and improved safety; it means, again, it opens up a variety of different application areas. And we've also done a lot of work looking at the effects of these light wavelengths on polymers. Some light wavelengths are associated with the breakdown of polymers, but from the work that we've looked at, the effects on polymers are negligible.

And again, these application areas altogether, these advantages led to discussion with colleagues C.D. and Monique at the FDA, and we've opened up the potential that this might be an option for looking at the treatment of blood products. So this is how the, kind of,

application area came into investigation.

So the key objectives of what we start to look at -- there was a range of different stages we had to go through. So first of all, it was, essentially, investigating the potential for antimicrobial efficacy of these light wavelengths for decontaminating contamination, microbial contamination within blood products. Now we have specifically focused on platelets and plasma, and again, this is a lot to do with the transmissibility of the technology. Whole blood and red blood cells are red in color and they're very opaque and they don't have -- allow the degree of penetrability of light that we would require for an application in this area. So we're very much focusing on platelets and plasma in terms of what we're looking at today.

We also wanted to look at the potential for decontamination of blood products within blood transfusion bags. So the penetration and the penetrability of these light wavelengths means that it could pass through the material of the

blood bag themselves. And it allowed the potential for using these light wavelengths to actually decontaminate blood which is already pre-bagged within the transfusion bags, therefore minimizing the handling and processing risks.

And importantly, an aspect which was picked up very strongly yesterday is the fact that we have to determine whether these light wavelengths can actually obtain the antimicrobial effects whilst retaining the integrity of the blood components themselves. And this is, obviously, a really important aspect for any PRT that's being developed.

So our current areas of investigation following on from these three points, in terms of antimicrobial potential, we're looking at inactivation of microbial pathogens, both in terms of bacteria and viruses. We've been evaluating energy levels that are required for decontamination. And also, as I mentioned, we're looking at decontamination within sealed bags. So these are all aspects which we were investigating

in terms of looking at the actual potential of the antimicrobial technology.

For blood product quality, it was really important for us to start looking at evaluating the quality of the platelets and plasma post-exposure. And a key aspect of what we're trying to currently determine is the upper and lower threshold limits that we can use for this technology. So we're going to try to determine what the lower level of treatment that's required in order to obtain the effective antimicrobial dose, but we also need to ensure that we establish what the upper threshold is so that we don't cause unnecessary damage to any of the blood components.

So these are all areas which we are currently working on, and I can show you some of the data which we have on this today. A final aspect which I'll touch on later in the presentation is the prototype development.

So the laboratory, the research group that I come from is an interdisciplinary research laboratory. We work. There's a combination of

physicists, electrical engineers and biologists all working together. And the element of what we're looking at together with the antimicrobial and the biological effects on the cells is looking at the development of a prototype which could potentially be used for trialing some of these antimicrobial processes on. And I'm going to get into some details about that as well.

So as I mentioned, the first thing we had to establish was the potential for antimicrobial efficacy when organisms were held within the platelets and plasma suspensions. And I have some data up here which has been taken from some of our publications, and the photograph, you can see, is essentially looking at some bacterial inactivation. Now a lot of our early studies looked at small volume samples and high radiance light. And what you can see here is an inactivation curve. So we've got low population by dose and we can see inactivation curves.

So this initial curve, which I want to highlight, is the inactivation of staphylococcus

aureus in a salt, saline solution, in a phosphate buffer and saline. And here we also have two curves looking at the inactivation of staphylococcus aureus in both animal plasma and human plasma. So we wanted to establish the efficacy, first of all, could the inactivation be achieved in plasma, and also comparing it against inactivation in a substance such as inert saline solution helped us evaluate how the transmissibility of the plasma effects the inactivation potential.

So we looked at some key organisms, some key bacterial organisms and found that inactivation could be achieved albeit at higher doses that are necessary for this. What other aspect I wanted to highlight here is to come back to the viral inactivation.

From the work that we have done in our research group, we've looked at inactivation of a virus in different situations. Now because of the inactivation mechanism relying on the presence of porphyrins within the microbial cells, this wasn't

going to prove successful for viruses because they don't contain these endogenous molecules. So what we looked at was actually seeding plasma with the viruses. And what we actually found was that you actually got a good inactivation effect. And the likely explanation for this is that the plasma itself contains photosensitive molecules which can actually absorb light in the appropriate wavelengths. And this causes a photodynamic oxidative effect from the outside of the virus rather than internally as was the case with the bacterial yeast cells.

So as you can see, this is an example of a norovirus surrogate that we've used for this in plasma. So the data is showing that there is potential for viral inactivation. This is something we need to look a lot more into, but again, the mechanism is slightly different in that it's relying on the presence of components within the blood components themselves. And there will be elements that we have to really investigate quite thoroughly to make sure that this isn't

actually causing any damage to the plasma or the platelets themselves as well. So this is all EDS that we're looking further into just now.

So what I want to move on now is actually looking at the antimicrobial efficacy and compatibility with plasma in terms of looking at it in terms of within the blood bags. So I mentioned about the penetrability of the light and the fact that you can actually get light, adequate light penetration through the blood bag material. And this image that you can see here highlights the transmissibility of light through the blood bag material. Again, we're up at the 405 region; as you decrease with shorter wavelengths then the penetrability decreases significantly.

But the ability of the light to pass through this blood bag meant we had the opportunity to investigate whether this could potentially be applicable for in situ decontamination of platelets and plasma within the bags themselves. And we published work a couple of years ago and this is some of the data from it.

So to do this, we would essentially set up a light set-up which used LED arrays and it's typically 405 nanometer narrow band LED arrays that we use. And we have to model the irradiance profile to ensure that the bag is getting an appropriate irradiance across the entire surface, and then artificial seeding of the plasma bags was undertaken. And you can see the successful inactivation that was achieved over the time periods.

Again, in another of the studies that we've done, we've used fairly low-level contamination with a view to trying to inactivate the low density contamination that is likely to appear within the blood supplies. From the previous slide you saw, we did work, initial work, on much higher population densities. So we're able to decontaminate using high -- decontaminate high contamination levels. But for all the work we're doing currently, we're looking at the low levels to see what energies are required.

So this example that you can see here is

what we found with the plasma and I'll show you in later slides that the inactivation capability within platelets was actually very similar. So the similar kinetics were achieved in both instances.

So that was specifically looking at the quality of -- sorry, the antimicrobial efficacy of the light for decontamination. But as we mentioned, it was really important for us to look not just at that, but at the quality of the blood components and if there's any changes in these areas. So we set up some studies fairly recently to look at the quality and looking at key indicators within plasma to try to determine what's happening, and also to try and help us establish some threshold levels that we want to try and start working towards for a more practical application.

So for these experiments, we would look at exposing different samples of plasma to different durations and different intensities of 405 nanometer light. And following this exposure,

samples were then analyzed using SDS gel electrophoresis and also Western blotting. And the gel electrophoresis allowed us to look at general changes in the protein quality and contact, and then the Western blotting would help us to look at specific markers that we had selected to investigate.

So we set up, initially, two levels of inactivation kind of processing. So we wanted to look, first of all, at the high irradiance levels, 100 mW/cm2 irradiance is what we used and this is a very, very high level exposure which we selected. And for these tests we exposed the plasma from one hour to five hours at this high irradiance light, and then we analyzed the plasma using the gel electrophoresis. And what you can see is that after two hours of exposure to the 100 mW/cm2 light, there tended to be changes becoming evidence in the banding pattern. So that helped us to establish the kind of upper level of an hour at that irradiance level.

We also looked at Western blotting, and

specific key markers we selected were two immunoglobulins, IgA and IgG. And we also selected fibrinogen and human serum albumin as well as two key markers. The results from this varied from protein to protein, and again, these are very preliminary results. We're working on repeating these within the laboratory currently.

But generally you can see changes. Everything that's highlighted in the red was starting to show changes from the control samples which had been left sitting not exposed to the violet-blue light for the same time periods. And ultimately, it varied from marker to marker, but the lowest was changes an hour becoming evident with the IgA. So again, taking these results together, this was suggesting that using a high irradiance of 100 mW/cm2 one hour would be the typical kind of maximum duration that you would want to expose this to.

We then went on to do similar analysis with a much lower irradiance of light. So in this case, we're using 10 mW and simply sp -- 10 mWs

exposure were conducted for up to ten hours in this case and differences in the banding patterns were observed after about five hours of exposure to the light.

And with the Western blotting, the same procedure was carried out, 10 mWs/cm2 exposure up to ten hours. And again, highlighted are the points where some breakdown was becoming apparent within the proteins. And three hours seems to be the minimum time both for the IgA and the human serum albumin. So together, this then helped us, again, establish something of a lower threshold. So three hours using 10 mW/cm2 was helping us to kind of establish a low exposure.

So the next stage of what we want to investigate was looking at dose dependency. Now with a lot of energy and light-based technologies in particular, there is likely to be a difference in the affects you see in biological cells depending on how you apply this energy to them. So from the results, we were finding that about one hour at 100 mW/cm2 and this gave a dose of 360

Joules. This appeared to be a high-level threshold that we didn't want to apply more than.

But with biological cells, these can be quite sensitive. So we wanted to investigate whether if you applied this dose level, but you apply it in different ways, for example, using much lower irradiance over much longer time periods, do you actually get a difference in how the cells themselves, and the components, the protein components are affected because this has a lot of influence on how, ultimately, you would want to deliver a particular dose.

So for this we conducted a range of treatments, all of which equaled a dose of 360J/cm2. And the maximum, which we mentioned, was one hour at 100 mW down to using ten hours exposure at 10 mW/cm2. And we wanted to evaluate the efficacy of these. And what we found was that regardless of how you applied this dose, the inactivation efficacy was apparent across all the different dose regimes.

We did tests conducting inactivation

efficacy using seeded contamination levels as it was 102 up to 105 CFU/mL within the plasma. And at each of the dose regimes, the five different dose regimes, we were able to achieve significant inactivation, and this ranged from between 92 and 99.99 percent inactivation, so up to -- full of reduction in the majority of cases.

So the established that this dose, if applied in different ways, it was still proving effective for the antimicrobial properties. And the point that we're at just now is actually looking towards how the dose being applied in different ways is affecting the actual components. And what we have here is just an example of one of the gel electrophoresis that we have conducted, and from these results, it's quite difficult to see, but the initial results seem to be showing that the hour at 100 mW seemed to be causing some noticeable changes in the protein structure, whereas the other regimes aren't causing this to the same degree.

Again, this is just the first run of

this experiment which we've conducted. So we have a lot more investigation to do. But it does show that there is potential to apply the same dose but in a much more gentle fashion to allow compatibility with the blood products themselves.

So that was very much focused on the plasma. I'm just going to touch slightly on what we've been looking at with the platelets.

So again, a lot of our initial work looked at the antimicrobial efficacy of the light treatment within plasma and platelets. And the result that you can see here is platelets that were seeded with staphylococcus aureus contamination, and we conducted a range of different treatments of the sealed blood bags ranging between 3 mW and 10 mW/cm2 radiance. And what you can see here is a typical inactivation curve which we're achieving of the treated sealed bags. So typically, the inactivation with increasing dose we're achieving complete inactivation of the contamination.

Some of the aspects that we were looking

at for this work was to look at the effective agitation. With platelets, the standard treatment conditions and storage conditions are under agitation. So we were looking at the inactivation under the same types of condition and results were promising. Results is the inactivation capability is enhanced by the use of agitation because the light -- this helps any contamination in the platelets and plasma to actually be exposed, probably more, to the light than they would be if they were just sitting static. So the use of agitation in standard storage is compatible and actually helps improve the antimicrobial efficacy.

Now also some work was conducted, some immune tests were conducting using platelet samples which had been exposed to the light. So what was -- we wanted to evaluate whether the light treatment had any effect on the recovery of the platelets. And this was done using scid mice as the model. And for this, the platelets were treated for eight hours and irradiance of 10 mW/cm2. And these were then infused -- transfused

into mice.

And control platelets, which had been non-exposed, were also transfused into a mice model. And as you can see from the data, the light exposure didn't have a significant effect on the recovery of the exposed platelets in the mouse when compared to the control platelets. So this, again, was very promising and looking at the compatibility of the technology with the blood components themselves.

Okay. So I mentioned briefly in one of the initial slides about the fact that we're interested as well from a university perspective in trying to develop a prototype both in terms of helping us with our research, and also looking at the potential of this as an actual pathogen-reduction technology that might be of use, of interest to the industry.

But one of the stages we're at just now is trying to decide what the best route and best way that this technology might be applied. So we see that as being two, kind of, routes of

potential application; the first being a rapid treatment application. So it could be that the technology could be applied as a high-intensity short duration treatment early in the processing stage in order to decontaminate platelets or plasma before the storage of the components.

But also there is a potential for continuous decontamination more in the consideration of platelets. With platelets, there's potential to have very low irradiance, violet-blue light during the storage period. And what this would do is, in addition to helping decontaminate, it would help maintain any low bioburden within the platelets themselves. So over the five-day period of storage, there is a potential for low contamination levels to actually replicate over this time. So the potential of having a blue-light treatment which could actually be incorporated into the storage conditions themselves is something that might be of great use in trying to minimize the contamination.

So got some pictures here just for

showing the stages, again, our initial prototype and what we're trying to build and evaluate within the laboratory to help us with our research in order to help us control the environmental conditions in which the blood components are exposed. And again, some of the work that we've been doing, just highlighting using the cabinets that we've been developing, again, just confirming the inactivation efficacy of the pre-bagged platelets and plasma within the systems.

And just to finish up, as we mentioned, these are very early stage results, very preliminary results. But they are starting to establish that there could be potential for use of violet-blue light in the -- as a pathogen-reduction technique for platelets and plasma. Preliminary analysis has demonstrated that decontamination can be achieved at levels that appear to be non-detrimental to the proteins and the plasma, and also the survival and recovery of light-treated platelets and untreated platelets showed similar trends. So these were all showing

great potential.

But again, much is still to be understood. Light interactions of biomolecules are a very complex area, and there is a lot more information that needs to be understood before this could be developed further. Things like assessing broad-spectrum antimicrobial efficacy, particularly in terms of antiviral properties are of great interest. But these are all things that we're looking to evaluate and investigate over the coming period.

So I thank you very much for your time. And I'd also like to thank my colleagues back at Strathclyde and also colleagues here at the FDA for the work that we've been doing. So thank you for your time.

DR. WAGNER: Okay. Thank you. I'm going to be talking about a technology that we developed many years ago between 2004 and 2006. So you would not believe how surprised I was to field a call from C.D. a while back saying that you wanted to hear about it. But I guess

especially that it was in emerging technologies.

But I guess there's not been a lot of emerging technologies between 2004 and now, and I think the work that we did was probably ahead of its time. I should mention that I'm in the inventor of a patent on this and I guess the good news is if you're willing to wait a few years, not very long, it's going to be off patent, and so you can use it for free if you so wish.

So this is a slide that basically describes what happens when you add photochemicals to solutions of blood. And what we've seen for about 25 years is a cartoon which is represented in the right part of the slide that shows basically the specific interaction that results in the inactivation of pathogens by the association of a photochemical with DNA, and its illumination producing an excited photochemical that produces photochemical reactions that damage particular DNA. But this is really not what happens.

This is not really the full story. This is a cartoon, because in real life, for any of you

who have ever worked with microscopy, if you add a dye to a cell, a suspension or a tissue culture, you're going to get the dye-labeling where you want it to label, but almost all microscopists spin down the preparations in something called the cytospin which removes the free dye from solution because it's not -- it doesn't all go to the nucleic acid.

And this dye that's free in solution can undergo photochemistry just like the dye that's bound to DNA. And it undergoes photochemistry either by singlet oxygen-mediated reactions which in themselves can change and morph to making other reactive oxygen species such as hydrogen peroxide or hydroxyl radicals or super-oxide. But through electron transfer, for example, with the psoralen molecules, you can make dimers. That is obvious that it occurs in solution. And the reason it makes dimers is that these molecules tend to be flat and planar and hydrophobic and they tend to stack on top of each other into solution and make dimers. And actually, the dimers that they make

are exactly like DNA bases that are adducts, for example, in psoralens.

And this is demonstrated if you do HPLC of the material in a solution, for example, psoralens, you'll see these dimers. And you'll also see for psoralens some yield of singlet oxygen. The particular psoralen that was picked by the company that involved -- that's involved in the current licensed product does make some singlet oxygen. But it's been selected to reduce the amount of singlet oxygen.

Now if you make singlet oxygen from a photochemical, that can diffuse and it can diffuse basically about 100 angstroms. And so any singlet oxygen molecules that are close to the membrane, you're going to see membrane damage from the single oxygen that's produced in the solution. And if it happens to morph into other things such as hydrogen peroxide, which is much more long-lived than singlet oxygen, which only has a lifetime of microseconds, you're going to see long-lived damage and peroxidation of membranes.

In addition to that, photochemicals don't just bind -- don't just live in the solution or bind to nucleic acids, because of their chemical properties, they interact with cell membranes. And that's because cell membranes are generally slightly negatively charged, and most of the photochemicals that are used to inactivate RNA or DNA in pathogens have a means on them and have a positive charge. So they can interact ionically with the phosphates of nucleic acid. And they're hydrophobic because they inter-collate between the bases of nucleic acids which they are themselves hydrophobic.

So you have this hydrophobic core and then on the outside of the molecule you have an amine group which is going to interact with membranes. And if you make singlet oxygen in membranes, you're going to have a lot of membrane damage. And if you have a psoralen, for example, on membranes, that makes -- goes by electron transfer, then you're going to have electron transfer that occurs in membranes as well.

So Dana Devine gave a very nice talk yesterday about the types of things that happen in blood cells when you interact -- when you do photochemistry with platelets, and these things also occur in red cells. And so you'll see changes in the in vitro properties of red cells and platelets with the treatment with the photochemicals. For red cells you see potassium leakage, you see hemolysis, and there are in vivo changes where you may see changes in the 24-hour recovery or survival.

And the same is true of platelet damage. You'll see changes in activation of platelets. You'll see changes in the metabolism, speeding up of the metabolism of platelets. You may see changes in aggregation if you look at aggregation response. And this -- and you'll see it in vitro and you'll also see it in vivo with 24-hour recovery and survival. And you'll see it with corrected count increment in platelets as well. And all of these things have been documented in studies.

Now around the time that Larry Corash and Lily Lin were busy in their lab investigating 8-MOP before they started looking at other photochemicals, this paper came out by Kathleen Specht and Robert Midden. And they were able to show that fatty acids that make up the lipids of membranes, the unsaturated fatty acids have double bonds, and when a psoralen interacts with them, they put adducts on the fatty acids, and so you end up with these fatty acids with the psoralen adducts on it.

And this is true, actually, even with amotosalen as you can see in the bottom of the slide, about a third of the photoproducts that are produced by amotosalen bind to high-molecular lipids. So what happens -- lipids are really important for platelet receptors. There are boundary lipids which are important for signal transduction. And here you are -- and they're very sensitive to the fluidity of the membrane. And so if you're adding adducts that are spinning because they're in phospholipids, that's going to

change the fluidity of membranes.

And so the basic point I'm trying to make is that there's no free lunch. If you're going to get inactivation, you're also going to get some damage to the components that we care about.

Now all of these photochemicals that I've spoken of up to this point are rigid molecules made of fused rings of conjugated bonds, whether they happen to be psoralens, whether they happen to be acridines, or phenothiazines, and basically, the photochemicals are normally in ground state. And when you expose them to light, they're excited to an excited singlet state. Once in the excited singlet state, they can fluoresce and give up their energy, or they can go to the triplet state.

And it turns out interestingly in life ground state oxygen is in the triplet state. And so there can be an interaction with the ground state oxygen in the triplet state and the triplet state of the dye and that's what make singlet

oxygen. And singlet oxygen is really reactive. It's about 42 kilocalories per mole more reactive than regular oxygen. And so you have this tremendous amounts of reactive oxygen species that potentially goes and damages the things that we care about, the cells. They react with targets.

Also in the triplet state, you can obviously decay and get phosphorescence going back to the ground state. But these rigid molecules don't really go back to the ground state directly because they're rigid. They can't rotate with heat to release their energy. And so there's no real way to get back to the ground state by bond rotation.

And so the idea that we had in the lab was what if there was a photochemical that was flexible? If that was the case, then you would be able to rotate about a single bond and release the energy from excited triplet state to the ground state. And so if you were -- if that molecule was in solution, it shouldn't participate in photochemistry. Now if it was originally bound to

a substrate, for example, DNA or RNA, and it was held in a planar confirmation, then it could undergo photochemistry.

And so this was a way that we thought we might be able to introduce more specificity for inactivation where the molecule could only be a photochemical if it was bound -- rigidly bound to nucleic acid. But it wasn't a photochemical if it was out in solution. So unlike the microscopists who are spinning down cells to remove the molecules from solution in the cytospin, you don't need to do that.

This is a molecule that we began to study in 2004. It's called Thiazole Orange. It's actually used for scanning of reticulocytes in both red cells and platelets. And as you can see, it can rotate about a single bond and dissipate its energy. But you can imagine if it was held fixed in nucleic acid in a planar state, then it would act as a photochemical. And it also fluoresces in that state.

This is just proof. These were stained

virocells that we didn't wash the virocells. So you can see outside of the virocells there's no fluorescence. So the molecules -- if there's no fluorescence, the molecules can't act as photochemicals, but if there is fluorescence, they can. And as you can see, they stain both the nucleus and the cytoplasm, the RNA in the cytoplasm.

So another problem that people run into with photochemicals is that, as I mentioned before, the dyes interact with cellular membranes because the dyes are all positively charged and the membranes are slightly negative charged. And they're also amphiphilic or hydrophobic-like molecules at the core.

And so this has been a problem over the years. And so when we've studied hundreds of photochemicals in our lab, and when we studied them, we always did assays to see how well they bound to blood cells. And so basically, what you do is you add the dye to cells, for example, red cells. And then you spin the cells down and look

at the supernatant and you see how much dye is in the supernatant. And then you do an identical experiment where you add the dyes to the supernatant without the cells there. And you can calculate what percentage of dye is interacting with the membranes.

And in this case, Thiazole Orange, about 20 percent of the dye interacts with membranes. And in our hands, with almost all of the photochemicals that we studied, of the 100 or more that we've studied, usually the amount of dye that's bound to the membrane is usually around 60 percent or so, about two-thirds of the dye are bound to membrane.

And so we thought, gee, this looks like it has some advantages where it's not interacting with red cells as much as what we're familiar with. And so these were the experimental conditions that we did.

We had the dye at 80 micromolar. We didn't add any quenchers. There's no glutathione. There's no antioxidants. There's no nothing, just

the dye.

We exposed the cells and the suspension to oxygen, because, after all, if you're try -- you saw that the photochemistry for singlet oxygen requires oxygen. And if your red cells have all the oxygen and there aren't -- there's no more oxygen in the suspension, you're not going to get inactivation of the pathogens as readily. And so we added some oxygen.

We did this in petri dishes. You know, this was not ready for prime time. We were just studying it in the laboratory. And then when we were done, we would pull the material from the petri dishes after illuminating them with cool white light. And we studied how well the red cells were and how much inactivation we got.

So these are inactivation curves of three model virus; vesicular stomatitis virus which is the model for HIV, and pseudorabies virus which is the model for HBV, and obeen (?) virus, diarrhea virus in red cells. And you can see that you get four or more logs after eight or so joules

per cm2. So what happens in red cells under these conditions?

Well, not yet. Let's do some more. What's the mechanism? So M13 is a bacteriophage. It's non-envelope that I used many years ago when I was doing Sanger sequencing as a graduate student in the eighties. But and so we treated M13 and we looked at inactivation, and we also isolated nucleic acid from the M13 and transfected that in, and lo and behold, the virus inactivation kinetics were the same. So what does that tell you? That tells you that nucleic acid of M13 is the target here. It's not the protein capsid.

We also looked at HIV inactivation and we saw inactivation -- robust inactivation in both extracellular HIV and intracellular HIV. We saw inactive -- we worked with Lisa Cardot. I don't know if any of you ever remember Lisa Cardot or not. And she looked at leishmaniasis and T-cruzi and we saw inactivation of both of those. So it looked like it was pretty robust.

I did some work in the lab and we looked

at bacterial inactivation because that was something easy that I could do. And it inactivates bacteria. It's a little odd the way it inactivates bacteria. It's species-dependent. And it doesn't go with gram negative or gram positive, and it depends on the species. And I still don't understand why there are differences.

Ray is sitting there shaking his head yes. And we looked at red cell storage. And we were able to store the red cells out to 42 days. And we saw some nice talks by Dr. Cancelas yesterday with riboflavin that only gets out to 21 days. And we were able to get out to 42 days. And I might add, if you take the cells that have been treated, and you wash them to remove the dye from the supernatant, and then you store the cells, the hemolysis is even less. It's basically about.2 at day 42. So the degree of hemolysis can be managed and it's pretty low.

We looked at potassium leakage, and no surprises there. We saw a rapid potassium leak and, you know, of the hundreds of photochemicals

that I've studied in the laboratory, or we've studied in the laboratory over many years, I've always seen increases in potassium. The rate of increase of potassium is two to threefold greater than basal rate, and that's very similar to what you see with gamma radiation.

And with gamma radiation, we know that the 24-hour recovery is slightly less than that of untreated red cells. And they're only stored for 28 days. So until you do the recovery and survival experiment, I don't know what to expect, but I would be -- I would think that might indicate that there is some damage. But it's a lot less than what we've seen for most of the photochemical -- well, all the photochemicals we've studied over the years.

For ATP we heard Dr. Cancelas say that ATP levels were predictive with riboflavin on the survival and recovery. And as you can see here, we didn't really see differences in ATP levels. And in fact, the ATP levels were close to 4Mmol per gram of hemoglobin which is actually quite

good. So we really didn't see that lesion in what we were -- in our studies.

So unfortunately, I'd like to be able to give you more information on this, but the project and our work with pathogen reduction was terminated. And we really haven't been able to study this for probably about 12 or 13 years. And so unfortunately, there's no end to the story. We did go out to talk.

Ray, you know, I came out to talk to you about this many years ago, and I went out to Cirrus to talk to them, but they were very busy in what they wanted to do. And so no one really picked up on this technology. And so there it stays.

So the conclusions, all photochemicals used for pathogen reduction have secondary reactions that damage non-target molecules. These secondary reactions are responsible for some of the damage to blood components that are observed both in vitro and in vivo.

Use of a flexible photosensitizer that

only undergoes photochemical reactions when rigidly bound to target can reduce damage to blood components from photosensitizer free in solution. An example of such a flexible photosensitizer is Thiazole Orange which can inactivate a number of viruses and bacteria in parasites in red cells with the maintenance of several in vitro properties during 42-day storage.

And I'd like to thank the people in my lab at the time who were involved in the work, Andrey Skripchenko who now is at the FDA and I wish him well; Helen Awatefe; and Dedeene Thompson-Montgomery. Thank you.

DR. ATREYA: And so to end the session, we have Dr. Cap and he's going to talk to us about pathogen reduction in blood products; refrigerate and use PRT, and that sounds like an order.

DR. CAP: All right, let's see if I can get the right slides up here. Okay, great. I want to thank the conference organizers, our colleagues at FDA for inviting me to speak here

today, and I look forward to the discussion after our talk.

You know, we heard some alternate approaches to pathogen reduction here this morning from our colleagues here, and I'm going to remind you of another alternative that's perhaps a blast from the past; talk about refrigeration. These are my disclosures. What I'm going to tell you today represents my own personal views and should not be construed as official policy of the Department of the Army or Department of Defense.

So the DOD is interested in pathogen reduction like everybody else, but with a caveat. And that is that our primary role in military medicine is to support the warfighter and our main mission is combat casualty care. And so that means treating bleeding patients.

And so whatever we deliver in terms of blood products on the battlefield has to be able to provide a hemostatic resuscitation. So we really focus on that when evaluating technologies for storage or pathogen reduction or what have

you. Now that said, of course, we want to deliver a safe product to our soldiers, and, you know, so we're interested in basic risk reduction like everyone else for platelet bacterial growth in particular. And we're concerned about the short shelf life of platelets.

We deploy troops to environments sometimes where there's endemic risk, where the risk profile that the soldiers face is very different from what we have here in the United States. And for short shelf life products like platelets that means collecting them downrange, and sometimes when we don't have enough blood products, we use whole blood collected from our walk-in blood bank. And those emergency collections, of course, might expose recipients to whatever endemic diseases are in the area.

Another thing that forces us to think hard about pathogen reduction is what I'll call the Zika scenario. So we had an urgent requirement for new testing during the recent Zika epidemic. And our blood system is relatively

small compared to the civilian, you know, overall blood supply. However, it's very geographically dispersed. And so for example, the Zika testing requirement really hit us hard in platelet availability in certain locations.

For example, we have troops in the Western Pacific, and based in Okinawa we have a blood collection center and getting samples tested at participating laboratories in IND back in the United States meant collecting and shipping back and waiting for results and, you know, we had a problem with platelets expiring before we ever got the results of the testing back. So this could happen again, and so this is a major concern for us.

And then lastly, there's always the unfortunate reality that we may be faced with radiological injuries. And having a technology that might allow us to provide white blood cell inactivation in far forward locations in treating those troops to avoid graft versus host disease in heavily irradiated soldiers would be potentially

useful. So those are sort of the broad spectrum issues that we think about with regard to PRT.

So getting into platelets, I'll just remind everybody, we really do have a problem with this product. So the platelet dose study, the PLADO study led by Sherrill Slichter and colleagues identified a dose-dependent increase in transfusion-related adverse events. And not surprisingly, fever was the big problem here. So keep that in mind. Platelets dose-dependent increase in, we'll say, potentially infectious toxicity, and of course, this topic has come up a million times at BPAC and every other forum including this one.

Conversely, the PLADO study did not identify a dose response effect on bleeding. And so you can double the amount of platelets transfused and there's no change in bleeding or overall transfusions. And there's a similar bleeding risk across a range of 10,000 to 80,000 which suggests that we may have a problem with efficacy with this platelet product that we're

using.

And then we all know about the platelet storage lesion. The PLADO study showed here, again, no dose-response effect on bleeding, but if you look at the effect of storage time of platelets on bleeding, there was not a statistically significant difference because most patients don't receive fresh platelets. But you can see there that it seems to be a trend that if you get fresher platelets, you might have lower bleeding. So I think the platelet storage lesion is real in that room temperature storage leads to loss of function, along with, obviously, an increased risk of bacterial growth due to the higher temperature.

We saw this in the PROMMTT study. So this was a 10 center observational study of trauma patients across the US and Canada that evaluate a number of different things. And one of the outcomes of this study was that we found an association of older platelet age with total increased adverse events but also sepsis. So

again, there's a signal here that we really have a problem, and we saw some numbers yesterday, you know, 1 in 30,000 platelet units may be at risk for bacterial growth.

And it's hard to wrap your head around those numbers, but, you know, but the reality is that when you look at patient outcomes you actually see this reflected. So I think, you know, there really is a problem with bacteria in platelets.

All right, so to summarize all that we have a short shelf life, hard to maintain inventories for everybody, it's really bad for us, and we have to deploy units downrange. We can't ship to forward locations. We're doing downrange collections where we're using untested units, by the way, with no bacterial testing available to us in those locations. Limited donor pools plus the platelet storage lesion, and that's a problem.

Now for us in treating bleeding patients, we're sort of held to the, you know, room temperature problem of storage because of

recovery and survival, but there's no evidence that that matters in hemostasis. So we have a real issue with this relatively high risk product that's not delivering, kind of, what we really want.

And I'll just point out that that's a problem for many people in the United States. So if you look at the map on your left that has sort of a few dots, those are level one and two trauma centers. And you notice they're pretty sparsely distributed across the country. And if you look at level three, four, and five trauma centers which are really not trauma centers, they're kind of concentrated in rural areas. And then if you look at what are called critical access hospitals, again, tiny little hospitals that do see trauma out in the rural communities, none of these places have platelets, folks.

And so 50 percent of the US population lives greater than an hour from a trauma center and basically has no access to platelets, whether they're going to get fresh platelets or platelets

that are old and have storage lesion and bacteria in them, it doesn't matter if there's no platelets. And it's been documented that there's high rural trauma mortality in the United States. So you need platelets in trauma. And in case you don't think you need platelets in trauma, there's more and more evidence emerging from military experience, but also civilian experiences. There's data from Mitch Cohen's group in San Francisco showing that in level one trauma admissions, 46 percent of patients have platelet dysfunction on admission.

Percent of them develop it early during their ICU stay, and if you look at the panels on the right, if you have poor platelet aggregation response to these various agonists, you have worse survival. So when you bleed and you're in shock you need platelets is the bottom line. And if you can't get them, that's a problem.

Luckily, we have a low-cost technology that may help us with this. So here we go; cold storage of platelets. It's been an option for

many, many years just not implemented due to short shelf life.

In case you're skeptical that refrigeration is really going to solve this problem with platelet bacterial growth, you know, I'm sure most of you keep your milk and fish and steaks and other highly perishable items in the refrigerator. You can do this with platelets as well, and we did the experiment here to look at platelets versus platelet poor plasmas as seeded with bacteria, in this case, Acinetobacter. And you see that at 4-C on the left nothing grows.

What was really fascinating about this, though, was that the bacteria -- the platelets -- actually the platelet-containing products seem to accelerate bacterial growth. So if you look at the panel on the right, the top two curves are platelets with bacteria seeded in them grown at room temperature. And the bottom curves are just plasma from the same donors grown with the platelets in them.

And you know, there's a lot of

literature out there that says that platelets contain antibacterial peptides and so forth and so on. But what's interesting is that the platelets facilitated the growth by four logs of Acinetobacter. So this is really interesting and we pursued this in a broader range of bacteria. And you can see here on the left we looked at Acinetobacter, E. coli, Pseudomonas, Staph aureus, and Staph epi.

And Acinetobacter, Staph aureus, and Staph epi are all facilitated, we'll say, by the presence of platelets compared to plasma alone. E. coli and Pseudomonas are just fine at room temperature, of course, but they don't need the platelets to help them out. In further experiments we determined that this was due to the lactate production. So some bacteria really like three-carbon sugars instead of six-carbon sugars and we'll preferentially use them. And of course, all this can be obviated by putting them in the cold because metabolism is pretty much not happening. You're not consuming glucose as you

can see here. So this is all in press and in transfusion right now.

So we've known the platelets -- so getting back to the hemostatic piece of this, why we want this, well, you know, 1973 Becker and colleagues showed clearly that cold platelets actually do work and both aspirinated volunteers and in thrombocytopenia bleeding patients.

So what's needed to make this a reality? Well, we can already do cold storage of platelets. And FDA a few years ago granted a variance for doing this in apheresis platelets as well as whole blood derived platelets all stored without agitation. I'll show you some data looking at platelet additive solutions versus plasma. And I think it supports use for either one of those and we've stored them out to 21 days and they look pretty good. And we've recently worked with FDA on trying to develop a variance for 14-day cold-stored platelets. And I'll show why that's supported.

But for us what would be really helpful

is 21-day cold storage because of transportation issues to get fully tested products from the United States to our deployed locations. And I'll just point out it's not as crazy as it sounds. I just said 21-day cold-stored platelets, right? That sounds like a long time, right, five to seven-day storage now. So if you think about it, we store liquid plasma out to 40 days, right? And red cells out to 42 days. These are refrigerated products. So we're talking about just a half of that storage duration. It's not like, you know, a tremendously long storage duration in terms of bacterial growth and things like that.

And then the other thing is suppose they sort of peter out and don't work that well at 21 days. Well, then you're basically transfusing either liquid plasma or maybe liquid plasma with some additive solution in it. At either rate, if there's any efficacy of the platelets at all, you're still doing better than what you currently have which is nothing in many locations.

So keep that in mind. But how well do

the platelets really work? So here's some rheometry studies looking at clot strength; fresh versus current standard of care, five-day room temperature, and then stored out 14 days in the cold in plasma in this case. And you can see that the clot strength is better maintained by cold-stored platelets.

Todd Getz, when he was in our group, is now at Red Cross, Steve -- did this work on aggregation responses in additive storage solutions in platelets. And you can see on the gray bars that platelet aggregation response is well-maintained out to 22 days in this case to a variety of different agonists. And I'll just quickly show you the dual agonists kind of behave the same way compared to room temperature in the black bars that drop off pretty quickly.

We've tried to figure out exactly what's going on to -- that maintains this hemostatic function. So one of the things we looked at was mitochondrial function in the platelets, basically thinking that all these shape change and

aggregation responses and release reactions and all that requires ATP. So the platelets have to be metabolically active. Platelets use both glycolysis and mitochondrial respiration. But we figured that probably mitochondrial respiration was more vulnerable to the platelet storage lesion over time.

And indeed, that's what we found. So these are oximetry studies showing routine respiration on the left and then oxidative burst on the right. And you can see that function is better maintained in the cold than in room temperature which drops off after five days of storage pretty dramatically.

Mitochondrial dysfunction is often associated with induction of apoptosis and so we studied that as well. And we can see in panel A increasing mitochondrial depolarization. It happens, you know, sort of across the board. There's no free lunch as Steve said. But it's worse at room temperature than it is in the cold that's associated with caspase activation. Loss

of membrane integrity is determined by fluid and staining of actin that shouldn't be exposed if the platelet membrane is intact and then microparticle formation. So bottom line is that we do think that the mitochondria are sort of driving the platelet storage lesion just due to increased metabolic activity and the room temperature compared to the cold.

There are some drawbacks to storing platelets in the cold. So you know, as Dana told you yesterday about PRT kind of activating platelets, we all know that from quite a few studies that there are some activation, sort of a pre-activation stage of cold storage, and that causes some aggregation in the bag. And if you look at platelet counts over time you'll see them decrease.

Interestingly, if you store them at room temperature they don't do that. We have, again, this is work that Todd Getz did when he was with us, showed that if you store them in an additive solution, interestingly, this clumping problem

goes away. And to make a long story short, just in the interest of time, we'll say that this is basically driven by fibrinogen binding and you make -- the more fibrinogen you have in the bag, the more binding opportunities there are. And so you'll make small aggregates of two and three platelets put together, and we've imaged these. And they're still smaller than a red cell, so it's not really a concern in terms of what it's going to do when it gets into the patient. If there are larger aggregates that don't break up when you rewarm the platelets, those get caught in the transfusion filter and don't really affect function afterwards.

So we think this is really a non-issue. Most blood bank technicians, of course, see aggregates in the bag and they think contamination, but that's not what's going on here. This is just a little bit of fibrinogen binding in the bag that can be mitigated by storing in additive solution.

What about clinical function of the

cold-stored platelets? So our colleagues in Norway worked with us to develop a pilot -- sort of an early phase study in cardiac surgery. And so basically, this is an intervention where they took additive-stored platelets either at room temperature in the cold for out to seven days, and patients who were found to be bleeding after reversal of Heparin and coming off of bypass were transfused whatever they were going to be transfused, red cells and plasma and platelets, and they either got warm platelets or cold platelets.

So we looked at aggregation responses and there's some indication that there's slightly better aggregation response in patients receiving cold-stored platelets. Blood product usage was overall similar, you know, kind of trending towards lower in the cold stored, but the important point is that the 24-hour test tube output was actually lower in the cold platelet arm. Now I just want to caveat this by saying this is a small study, 20 patients per arm, and,

you know, it isn't really designed to demonstrate that any particular platelet product is superior to another.

But it's just to try to reestablish, if you will, the biological plausibility of cold-stored platelets being hemostatically active because we have 30 years of transfusion medicine textbooks that say they're dead, and they don't work. And I hope I've convinced you that through any number of in vitro studies they are alive. They do work. The mitochondria function. Their membranes are intact, they aggregate. And oh, by the way, when you put them in the patient, they actually can stop bleeding. And so even though they are cleared faster, from a surgical bleeding standpoint, a trauma bleeding standpoint, they have clinical relevance.

And if you storm out to 14 days you get kind of the same results here. So this is an extension of the Norwegian cold-stored platelet study in the cardiothoracic surgery patients where they sort of did an adapted trial design and added

another arm to the study storing them out to 14 days in the cold. And you can see that they still work. So it's consistent with the in vitro data that I've shown you so far.

And here is the aggregation data, again, in patients getting platelets stored out to 14 days. And you can see that, generally speaking they're going in the right direction pre and post-transfusion. Not every time, not every patient, disease, or individual patients, but you've got to remember, too, these are actively bleeding patients getting a resuscitation that contains all sorts of things; red cells, plasma and so forth.

So again think about this as like a biological plausibility study. Are these platelets doing something? I think we can say yes. They're doing something and it's positive for hemostasis.

What about whole blood? I told you that we collect whole blood in theater. So here we studied the hemostatic properties of

Mirasol-treated whole blood and this panel is a little confusing to look at, but basically, if you look on the left panel, that's platelet aggregation response. And the top curves are cold stored. The bottom curves are room temperature stored because there was actually a thought that maybe we would store whole blood at room temperature for a short period of time after it had been treated with Mirasol.

And we just took that out over 21 days to see what that would look like, and obviously, it does not look good. So that's not really an option. But if you compare the top two curves, the top curve in non-pathogen reduced, the bottom curve is pathogen reduced, or Mirasol treated I should say. And there's no real difference between the two curves. There's a little bit of a drop with the Mirasol treatment but it's not significant. And if you look at thromboelastography on the right you basically see the same thing. And I'll just point out to you that clot strength is preserved to 21 days of

storage. So even though you lose aggregation function, you still get some pretty decent contribution to hemostasis. If you just did red cells and plasma and you looked at the TEG MA it would be 20, not, you know, between 50 and 60.

So again, there's no free lunch. There's a price to be paid both for duration of storage, and for use of PRT, but certainly it's better than nothing. And at least it improves the margin of safety.

Now I have up in the title there why aren't we doing this now? I think it's interesting that we have data, in vivo data, from the AIMS study in Ghana showing decreased transfusion trans-minimal area. From the standpoint of, I think, military use of a product like this, we send soldiers who have been multiply screened for transfusion-transmitted disease. Many of them are blood donors to start with, but if they're part of a unit where we're going to depend on a walking blood bank, they are screened. The donors are tittered for anti-A and anti-B.

They multiply deploy. They're multiply tested. We know that they're not getting Hepatitis B and HIV in theater.

But if they're operating in a malarial zone, they could get malaria. They're supposed to be taking their prophylaxis. They usually do, but, you know, sometimes they don't. And so as a risk reduction measure for at least malaria, I think this is a reasonable alternative based on the data we have now and something to consider. And it doesn't compromise hemostatic function to the point that I would be concerned about.

Now what about intercept on the platelet side? So these are the preliminary data, but basically what we have here is Trima collected, stored in plasma, and either intercept treated or not; all stored in the cold, okay? So there's not a room temperature arm here, and here you're looking at aggregation out to 21 days. And you see that they're basically the same.

And here you have ROTEM on the left showing clot strength and clot lysis. A little

bit of a possible decrease in clot strength with the intercept-treated platelets, but it's not huge and I think that we need more data to be sure about what's going on there. But clearly, at least out to 14 days, there's no major difference.

And if you look at thrombin generation on the right, there's basically no difference. So the US Navy has actually implemented intercept in its treatment -- in platelet collection programs, and I think this is going to be particularly important in our very geographically dispersed areas where, you know, we have problems with testing turnaround in a Zika-like environment. But in addition, there may be endemic transmission of disease which we worry about, and also if we can store them in the cold, which clearly, I think we can without compromising hemostatic function, that would allow us to deliver the platelets to where they need to go.

So I think this also holds potential as a way to improve our ability to deliver safe component therapy that has hemostatic function far

forward. So if cold is good, how about frozen?

That not much bacteria growing in frozen platelets at minus 65, cryopreserved platelets have been around for a long time, how's their hemostatic function? Well, they don't really aggregate much. As you can see here this was worked on by Lacey Johnson and colleagues in Australia. They do shorten the TEG R time. They generate plenty of thrombin. They do contribute a little bit to clot strength. They make a bunch of phosphatidylserine-positive microparticles which contribute to that thrombin generation. How do they work clinically?

Well, we don't have much in the way of RCT data, although we do have a phase one led in part by Dr. Cancelas in which we didn't see really any increased adverse events. We did see some good hemostatic function but in addition to that, from a standpoint of combat casualty care, and bleeding patients and trauma, the data that we do have comes from the Dutch military where they were supplying blood to one of the areas in Afghanistan

for quite some time. And they looked at their massive transfusion protocol and the pre and post-introduction of cryopreserved platelets. And what they found was if you just resuscitated with red cells and plasma, if you look down at that bottom left panel there in terms of patient outcomes, when you introduce the cryopreserved platelets you see a decrease in mortality.

So it's not a randomized trial, but it does suggest that these may be beneficial to stop bleeding as well. Okay, if frozen is possibly an option, how about lyophilized? So here we have a picture that some of you have seen several times from Mike Fitzpatrick.

You can allude -- in this case you have shrimp larvae producing trehalose to protect against dehydration. If you put trehalose in platelets and freeze-dry them, you can make a product that has quite a bit of shelf life and stability, which is great. The process does include a heat treatment step to -- I can't remember if it's 60 or 80-C but, you know, perhaps

some measure of pathogen reduction, and perhaps other pathogen-reduction technologies can be applied prior to the freeze-drying.

Those products just, by the way, do also have in vitro evidence of hemostatic function as well as animal data that shows that they reduce bleeding. So that's also a potential alternative for the future. We'll see how clinical development plays out with that product.

So bottom line is I'm showing you a relatively low-tech approach to pathogen reduction that I think works pretty well for platelets and for whole blood which is to use cold. It's been around for a long time. I think if you look at dollars per quality adjusted life year it's going to be cost-competitive. And importantly, I think, maintains hemostatic function which, at least from the military standpoint, and I think from a standpoint of most people treating trauma or surgical bleeding is a critical thing to consider.

Cold platelets are being used by the Department of Defense in theater right now. Mayo

Clinic has a program. There's another -- I'm thinking about investigating this, and then cold-stored whole blood fully tested and distributed for trauma care as used by the DOD, shipped from the United States downrange, as well as by the Norwegian military. And I have a couple of major trauma systems listed here, but the list has now grown to, like, 25 programs that are using both cold-stored whole blood in both the pre-hospital and in-hospital setting.

So I think this is a trend that is catching on. And with that, I'd be happy to take your questions. Thanks.

DR. WAGNER: Okay. So we're ready for the panel discussion. Yes? Ray?

DR. GOODRICH: I don't want to monopolize the microphone here, but I had several questions. Maybe one I'll ask the panel members to address, but one comment I think, Dr. MacLean, the target molecule that you were describing in porphyrins, actually in that actinic range between 4 and 500 nanometers, there may be other agents,

cytochromes, alloxazines, other types of compounds that may absorb in that range. So it might look a little broader.

My question is relative to just storage of products today. Your data seemed to indicate that just exposure to light, and in that 400 to 500 nanometer range, you will get that from even the fluorescent lights that are in this room. Has anyone evaluated things like the storage of plasma in a liquid state or the storage of platelets without any additives, without any components, and what impact do you think you would see as a result of the exposure of light in those settings?

DR. MACLEAN: Yes. In terms of the light output that you will get that is contained within your normal white light spectrum, the levels that we're using are much higher. So that's why you get the amplified response, but you would need -- to get an effect from normal while lighting you would need to expose it for a significant length of time. From the work we've done we've -- and with the platelets and plasma,

we haven't specifically looked into that, but from the work that we've done in our other microbial work comparing to controls with normal white lighting, then you would really need to give levels and durations that are probably not compatible with the techniques, certainly, for the blood applications. But those wavelengths are part of your normal white light spectrum, certainly.

DR. WAGNER: Steve?

DR. KLEINMAN: Yes. From the perspective of transfusion medicine, those of us who are not expert biochemists or biophysicists, I think over the years when we've looked at the technologies for PI in platelets, we've tended to focus on do they add a photochemical. You know, is amotosalen different from riboflavin, different now from no photochemical in the THERAFLEX procedure.

But what I'm learning here is that it seems to me we should pay more attention to the differences in the wavelengths of light because

they're different in the three systems, which I think we kind of knew, but also the energy exposure. The degree of energy that goes into the system may have, I guess, and that's my question, may have an effect on the functionality of the component.

So I'm wondering with, you know, three experts up there, if you could kind of address that issue of how important is the wavelength in the ultraviolet range into the visible range. How important is the dose of energy that each technology requires for thinking about how that'll affect function? Obviously, we have to do the studies. The data is important, but sort of from a theoretical viewpoint.

DR. WAGNER: Okay. I think it matters. Proteins tend to absorb at around 280. Nucleic acid absorbs at 254. When you get out to the 400s, really what you're talking about is endogenous photosensitizers. Riboflavin and the flavins absorb out that far, but other things do as well.

And so it does make a difference what the light wavelength is. I think some wavelengths are more damaging, for example, to platelets. Visible light, I think, tends to be less damaging to platelets. And normally, there's a law in photochemistry that says that if you deliver the light faster but give the same amount of light versus delivering it slower, there should be no difference. And we've looked at that in the laboratory and found that not to be true in blood.

And so I don't really understand why. But if you -- we had -- we were using LED lights when LEDs first came out, and they delivered a much higher Fluence rate and found that there was more damage to the cells that we were studying in blood than if we'd delivered it slower. And I think it's because it -- in just in solution chemistry it's all very simple. Of course, you just have your buffer and you have whatever you're studying. But when you get -- and you're studying blood, there's so many different molecules and there's so many different things happening that

all the rules that you learned as a graduate student in this field don't necessarily apply.

And so it really requires experimentation in the lab which is what they did to tease out whether there is a light Fluence rate effect. So from theoretical grounds, I really can't give you any information.

Jim?

DR. AUBUCHON: Dr. MacLean, thank you very much for sharing your very interesting work, and wonderful Scots as well. Do you have data of the content of treated plasma, or by individual procoagulants, and also the effect of treatment on platelets in terms of their response to various agonists?

DR. MACLEAN: No, again, we're still at quite early stage research. So the majority of work we've been looking at has been very much artificial seeding and spiking with the bacteria, and we've just really started to start to delve into the impact of other things that might be in the plasma and the platelets. And that's

something we really need to do a lot of work on because to find out if there is going to be changes between different additives or different dose regimes, indeed, then we need to look a lot heavier into that. I'm afraid that it is still very early stage.

DR. AUBUCHON: Thank you. My second question, I don't know if you can answer, or perhaps Dr. Benjamin can answer, it's been a long time since my high school physics. How is the amount of energy delivered in the systems that you're developing compared to the amount of energy delivered in the intercept system?

DR. MACLEAN: Okay. So in terms of the light, or visible light, we are working at much higher energy levels. The principles and the workings of ultraviolet light, these energy levels are much lower because the photons are much more energetic. So for anything involving longer wavelengths within the visible light spectrum, it is much more higher energy that is required. Again, Dr. Benjamin will be able to expand.

DR. BENJAMIN: Richard Benjamin, Cerus. Just to confirm, I think we added 3 J/cm2 when you were at the 100.

DR. MACLEAN: Yes.

DR. BENJAMIN: So it's a big difference. How do you deal with heat?

DR. MACLEAN: Heat, in terms of the systems we're building, we have very good thermal management. It's all mathematically calculated to get the right heat-seeking and fan operations. So it is a big consideration with all energy delivery systems, but it's carefully monitored throughout it.

DR. BENJAMIN: So it is heat controlled basically?

DR. MACLEAN: Yes, very much, yep.

DR. BENJAMIN: And is that at 4 degrees or room temperature or --

DR. MACLEAN: We've done most of our work at room temperature. So the platelet work that you saw there was at room temperature, and what we're currently building is a system to

control that within the 20 to 24-degree range to make sure that everything's held at the correct conditions.

DR. BENJAMIN: Thank you.

QUESTIONER: Did you try 4 degrees?

DR. MACLEAN: We've done, actually, some antimicrobial work, not with blood, but the light inactivation potential is significantly enhanced when the light is applied at refrigeration temperature. So the combined stresses -- bacteria tend to be much more susceptible when you can hit them with multiple stresses at the same time so.

QUESTIONER: Yeah, my question is for Dr. Cap. So if we -- so you're talking in the military sector, but in the civilian sector where we're using platelets for both trauma patients, surgery patients, as well as prophylactically in hem-onc patients, would you see an evolution to a dual inventory, both a cold-stored inventory for one patient population, and a room temperature inventory for another population?

DR. CAP: I'll be ambitious and say I

think you'll see that as a transitional phase until we get rid of room temperature platelets completely. But those studies remain to be done.

Nevertheless, yeah, I think so. You know, we reintroduced whole blood, as I mentioned, in trauma care recently. And it has taken off. We have found that we are able to deliver a more hemostatic product more quickly to bleeding patients, and time is everything in bleeding patients. I think if you have a dual inventory of cold platelets and you put them where they need to be in the emergency rooms, and, you know, actually, in Mayo Clinic they're putting them on helicopters believe it or not.

And you know, we'll have to see how much, you know, what data come out of those experiments, but -- or experience, but the reality is time is everything. You've got to get the hemostasis happening immediately, and the only way to do that is to have functional platelets as close as possible to point of injury.

QUESTIONER: Just as a follow-up

question, and this is terminology, I mean, the usual whole blood product, when we have it, is cold stored. Obviously, we store whole blood and red cells in the refrigerator. So I'm wondering why you're emphasizing cold-stored whole blood; is this in distinction to what went on years ago when people said, well, we're going to use fresh whole blood and not even put it in the refrigerator?

DR. CAP: Right. So in the military context when we're doing collections from a walking blood bank, it's usually an emergency scenario where there is no blood available, or we ran out of platelets, for example, and we need to provide platelets to a bleeding patient. And so that's -- we consider that warm, fresh whole blood.

I mean, really, you know, we're out of the donor into the patient and that, obviously, has some implications from a pathogen risk. I mean, in our population it's very low, but in a broader population it might be higher. But the other point about the whole blood that's been

collected in a normal, you know, under typical blood collection settings and fully tested, and so forth, is that you would store it cold as you said.

And by the way, what's in there is cold platelets which we've been taught don't work. But actually they work great. And it's a very hemostatic product.

QUESTIONER: Yeah, no, and I get that part. I guess is this because some years ago, at least in military setting, people were saying warm whole blood is better? Somehow not putting it in the cold is better? I seem to remember hearing that at meetings, and are you sort of trying to react to that by saying cold-stored whole blood?

DR. CAP: No. So there's no question that warm, fresh whole blood right out of the donor is going to be your best product from a fully functional standpoint, hemostasis, oxygen delivery, everything. However, there are trade-offs. So one, you have a constrained donor population to collect from. I mean, you just

don't have, at any given time, large numbers of donors available to give blood. So it's a limited-supply product.

Number two, it's not fully tested. And so what we've said is okay, how can we get most of the benefit of whole blood in larger quantities and have it fully tested, and potentially, pathogen reduced at some point. And to do that, you have to store it, obviously, so you store it in the cold. And then the question is, if you store it in the cold, are you still going to have a hemostatic product, and the answer is yes.

So there's always a price to be paid, as I said, for either storage or pathogen reduction; the longer you store the worse the function. There's no getting around that, but you can mitigate that in the case of platelets by putting them in the cold. And in the case of whole blood, it's not that the cold-stored whole blood is better than the fresh whole blood, it's that it's available. Whereas, you know, you have very limited supplies of fresh whole blood.

DR. WAGNER: Ray?

DR. GOODRICH: I'm going to go ahead and ask my two other questions. First of all, Steve, I wanted to make the comment if I didn't say it that when we talked 14 years ago I'll say it now, it's brilliant chemistry. My question to you was did you ever do the experiments where you would add the dye, separate it out, then do the treatment? And if you haven't done it, what would you expect from it?

DR. WAGNER: You mean spin stain with a dye and then spin the red cells down and then reconstitute and -- no, but I don't -- Andre, I don't recall we ever did that, yeah. I was always concerned with the degree, at the time, of membrane-bound dye. You know, if 60 percent of the dye is still bound to the membrane, for example, we were doing a lot of studies with methylene blue and dimethylmethylene blue, et cetera, et cetera.

You start adding those, and if most of them are bound to the membrane, you can wash until

the cows come home, but, you know, you're still going to have a problem. And so we, over the years, with some dyes started using dipyridamole which seemed to bind to red cell membranes and prevent the binding of the dyes. So it was a competitive inhibitor, and that was interesting, and we saw less damage.

But then you get into the problem of adding two substances to the blood supply, both of which you don't want to add. And so we never really spent a lot of time on it.

DR. GOODRICH: Interesting.

DR. WAGNER: Okay.

DR. GOODRICH: And my other question was for Andre (sic). You indicated that lactic acid production in the platelets was responsible for promoting bacterial growth in some of the -- if I recall the slides correctly. I'm assuming that that might be consumption in the Krebs cycle where you're getting production or growth of the bacteria or metabolism of that lactic acid.

Have you ever looked at the effects of

acetate and what does that say about platelet additive solution if it could promote the growth in bacteria in those cases?

DR. CAP: That's a great question, Ray. We actually have not looked at acetate. You know, we've done studies where we've taken platelets, I mean, plasma without the platelets and seeded them with bacteria and added lactate back and recapitulated those growth curves. So we know the lactate will do it. It's a good question whether acetate would do it. It might. I mean, I don't know why it wouldn't. It could be a problem.

DR. GOODRICH: And one comment earlier on a question, that the question about energy, it's E=H\nu where nu is the frequency or the wavelength, the inverse of the wavelength. And so if you're in the visible light region, you may deliver lower energy photons but you may need to deliver more of them to be an equivalent energy.

That doesn't really matter when you're talking about photosensitizers because it's the absorption characteristic of the compound that

determines the chemistry, not necessarily the energy of the photon. If that photon is not absorbed by the compound there will be no chemistry. So it doesn't matter what its energy is.

DR. WAGNER: Do we have questions from the phone or online?

QUESTIONER: Yes. So we have two questions. The first one is Dr. Cap. The question is we have whole blood for trauma containing hemostatically actively platelets, why do you need cold platelets for hemostasis in trauma?

DR. CAP: Well, that's a great question. So from -- some of this is a military-unique set of circumstances. Our blood system is essentially self-supporting, so all of our donors are collected on federal facilities, and essentially it's mostly active duty military giving blood to other active duty military. And so if we have a requirement to supply components, for example, to hospitals that we operate in the United States and

elsewhere, and we also want to produce whole blood, you know, there's a tradeoff there.

I mean, you can't take a unit of whole blood and have whole blood. I mean you have to choose, it's either whole blood or components. And so we can shift that balance a little bit, but at some point, we run into some barriers in getting as much whole blood as we want. So that's one thing.

Second thing is, you know, in some environments, it may be more convenient to have components available. You could imagine a scenario in which say if you don't have pathogen-reduced whole blood, but you're operating in an endemic zone, and you really are concerned about disease transmission. If you can ship red cells and plasma from the United States and even if you have to collect platelets locally, if you used a pathogen-reduction technology then you could supply safe platelets.

So I think at the end of the day, there is a role both for components and for whole blood

in trauma management. I mean certainly in the ICU, sometimes you have to fine tune things. I think in early resuscitation whole blood's hard to beat. But you know, you could still have bleeding in the ICU after the initial resuscitation where you might want to give, you know, just a platelet unit. So that's how we kind of balance all that.

DR. WAGNER: And there's one other question.

QUESTIONER: This question is for Dr. MacLean. So the question is have you tried to inactive spore-related bacteria or bacterial biofilm?

DR. MACLEAN: Yes, not in terms of within blood, but back in the university we've got quite a large bank of antimicrobial information which we've published. We've got data published on the inactivation of bacterial endospores. The energies required for these are significantly higher than for vegetative cells as you'd imagine.

We've also looked at fungal spores and germinating fungal spores. And again, the

energies required for inactivation of dormant spores are significant. But once you initiate the germination procedure, you do get increased susceptibility. And we've also looked quite significantly at biofilm inactivation. And again, excellent inactivation capacity there so it's --

DR. WAGNER: Thank you. I think if there aren't any other questions for -- we have a break now, C.D.? And we should be back at 11:10. That's correct? Okay. Thank you.

(Recess)

DR. ATREYA: Hello everybody, now we are ready for the session five which is funding support for future (inaudible) research. Marion White, Dr. Glen to be here on the podium, thank you.

DR. WHITE: So, good morning and I'm going to invite Ashley and Bryan to join me at the table. So, what I thought we would do is first introduce one another and let you know a little bit about the programs that we are supporting currently that might pertain or solicitations that

we may have and then after that open it to the audience for questions. So, because of the way you sat down, Bryan, do you want to go first?

DR. KUJAWA: Absolutely, good morning, my name's Major Bryan Kujawa and I'm known as a battalion surgeon assigned to attend special forces group in Colorado Springs and, kind of, as a major role that I do as a special forces physician is I supervise the training and advice for all the special forces medics who are really our front lines for the initial transfusion treatments for point of injury care that is being prioritized right now.

So, I do want to mention that I am not a researcher. I'm probably the only non-researcher in the room and even thought the title of this talk has funding in it, I have no ability to authorize contracts, which I am sorry. It is probably very disappointing to many people here.

So why am I here? Really, I think it is a three- part answer. The first part is special operations command is very interested in one,

trying to improve the safety profile for transfusions for places that are very austere and in remote locations and second, to increase maybe the donor pool potential that we would have when we are -- for deployed. The second part of that answer would be to see the, kind of, the ground truth of what is happening for PRT research right now and if it can be applied for a special forces mission in the future going forward. So, often times things that are working in the lab and eventually are being able to be utilized in a hospital setting won't translate to a beneficial technology in a deployed remote environment. And the third answer to that question is when special forces command buys you a plane ticket and tells you you are going to the FDA you get on the plane and you go and see what's happening at the FDA.

So, I see myself, kind of, as a representative of the end-users; those being the medical personnel that are doing transfusions for deployed as well as the recipients of the blood products. So, really I think it's helpful for

frontline researchers to know what's happening on frontline military medicine and the paradigm has really shifted from the golden hour ride evacuation within 60 minutes as the biggest determiner of decreasing mortality. And it's probably better said that it is time until initial transfusion that is the most important thing and a lot of goodness has come out of the golden hour but now we are shifting our efforts for immediate point of injury transfusion capabilities.

So, special forces operates, of course, at very austere and remote environments. There is not an ability to access blood banking abilities or a lab facilities. We might not have access to cold chain storage along the way, so it's not feasible and realistic that we can divide whole blood into blood components. And even if it was, we have to look at what PRT technologies that are currently available. INTERCEPT being just one example and right now as the machine exists, it's too large and cumbersome for us to really deploy with. Space is a premium in helicopters and

vehicles, and unfortunately the size would be a limiting factor. So, remembering again that really whole blood is lifesaving, we need to look at how we can facilitate rapid transfusions in the austere environment and really at that point of injury which is where the special forces medics are working.

So, their operating procedure, as Dr. Cap briefly mentioned, is right now we give quick, fresh whole blood and the donors really are team members or support staff from other service members. So, it's impractical to perform pathogen testing at point of injury, of course. And as Dr. Cap mentions, the military has a generally healthy population that's pre-screened prior to any deployment. Of course, that doesn't count again, as Dr. Cap mentioned, any possible exposures during a deployment and special forces specifically operate with very small man teams; usually around 12 plus or minus support staff. So, a donor pool is an incredibly limited resource. It'd be very nice if we could use local

population or perhaps partners, but as we are operating in high-risk geographical locations those personnel obviously would fail any donor screening questionnaire we might administer them. So, it'd be very nice if we could utilize PRT technologies to be able to provide this ability both safely and quickly.

So, one of the main things that special forces is looking for: Proven technology for its primary application, again which Dr. Cap mentioned, really is massive hemorrhage from catastrophic combat-related trauma and then second, the equipment is very, very important. We need something that's light, easy to use, able to get dirty, operate in extreme temperatures, and durable to survive any rough transport that it might go through. So, technology such as Mirasol certainly look promising, especially as Dr. Cap mentioned, for malaria endemic regions, but we need to field test such equipment to make sure that it would operate in the areas that I mentioned.

So, it was suggested several times throughout the workshop that one approach probably is not the only answer and for a military application, perhaps, a combination approach for that specific idea for deploy transfusions, would be the best avenue for us to look at.

So, of course, in the ideal world; perfectly, again, ideal world we could utilize PRT without any concern for pathogen testing for local population or any partners that we're working with. So, for better or worse military, and specifically wartime, has the tendency to drive innovation for both patient care and for medical technologies. And with the increased emphasis on early transfusions I think this does have the possibility to drive innovation. Again, in PRT, not really from the hospital-cost benefit point-of-view but more for a military application.

Thanks.

DR. GLYNN: So, Ashley, yeah, if you want to --

DR. CECERE: Good morning, my name is

Ashley Cercere. I am a interdisciplinary scientist at BARDA. I am sitting in for Dr. Mary Homer who was unfortunately unable to attend in person today.

So, a little bit about BARDA. So, we stand for Biomedical Advanced Research and Development Authority. We fall within the assistant secretary for preparedness response within the health and human services. So, we are charged with doing -- supporting advanced research and development and also potential procurement of multiple medical countermeasures that fall within multiple threat areas.

So, our blood products portfolio actually fits within our radiation and nuclear countermeasures division primarily to support in the event of a radiation or nuclear event, patients that are affected by acute radiation syndrome or the associated trauma of the event. Since these patients are expected to be neutropenic and thrombocytopenic, these patients are more susceptible to infection and -- as well

as sepsis and graft versus host disease.

So, I've been asked to give a little bit of information on the programs that we're currently supporting which you heard about a little bit yesterday.

So, we are supporting the Cerus INTERCEPT program for the S303 red cells. So, we're supporting an efficacy clinical study that was briefly mentioned yesterday in Puerto Rico as well as other areas in the continental U.S. have the potential to be impacted by the Zica virus.

In addition, we are supporting -- or plan to support -- the studies have not initiated yet, two phase three clinical studies in acute and chronic anemia trials in the United States. We're also supporting the second-generation system for the red blood cell.

In addition, we have a contract with Terumo BCT. We're primarily supporting their MIPLATE trial which was also mentioned yesterday in which they're using the Marisol pathogen-reduced technology system to assess their

clinical effectiveness of Marisol-treated platelets compared to standard platelets in hyperproliferative thrombocytopenic patients as well as supporting additional in vitro work.

As far as our open solicitations we have an open broad agency announcement which means at any point and time you are able to apply. This can be located either on our website at medicalcountermeasures.gov or on the Fed Biz Ops.

Our radiation and nuclear group is listed under area number four for that. Remember, specifically blood products at 4.2 in which we're looking at products that enhance our ability to respond to mass-casualty events such as radiation and nuclear event.

That's about it.

DR. GLYNN: All right, so my name is Simone Glynn and I am the branch chief for the clinical therapeutics branch in the blood division of NHLBI. So, NHLBI is one of 27 institutes and offices at NIH and it is responsible for the NIH supporting the researching blood transfusion,

blood safety, blood availability.

So, the research that we support goes all the way from basic research to translational clinical research and implementation research. The main tool used to provide funds to researchers is the RO1 grant and this is the one that I would encourage you to apply for if you are interested, certainly, in conducting basic research, translational research related to any development.

If you are a small business, we, of course, have a small business research program as well so you can apply for a specific type of grant applications if you are a small business. And if you are interested, again, I encourage you to let me know and then I can guide you to the right program officer who knows all specific about those particular grant applications.

In terms of clinical research, NHLBI has undergone some, I guess, reorganization of how we solicit clinical research applications. And by clinical research we divide both, of course, between observational studies and clinical trials

and I'm talking primarily about the clinical trials.

So now when you have a clinical trial application in mind, please contact us because we will guide you depending on the phase of the clinical trial application, whether it's single-center versus multi-center towards different solicitations that have different requirements. And the whole reason for making these changes in NHLBI were that we wanted to assure that the reviewers would not only review an application for its scientific value but also for the feasibility of the application so that if a clinical trial was going to be funded, chances were that it was actually going to be doable and feasible which is quite important to address the important scientific question you have in mind.

And then just also to remind everyone that we have training grant applications which I think is also very important. So, if you have a good mentor, again, you can apply for a K-type of application and again, we do have some program

officers who specialize into those kinds of applications.

So that's, kind of, in a nut shell and I think now I'll open it to any question that anybody would have. The one thing that I forgot to mention is that we do have a memorandum of understanding between our agencies so that we actually do have routinely meetings between us so that we can discuss potential applications and try then to guide the researcher towards one of our agencies depending on what the application is about. So, I think that's been quite helpful. We've had that in place for about two to three years now, I think.

So, I don't know if we have any questions. The other thing -- and it's outside, I think, also is a handout. I did provide a handout that, kind of, lists some of the major solicitations that you might be interested in looking at for NHLBI. It's available outside and I think it will be available after the meeting.

So, nobody is interested in getting

funded.

(Laughter)

SPEAKER: I'll ask a question.

DR. GLYNN: Oh, thank you, Rick.

SPEAKER: You may not like it when I ask it.

(Laughter). I think a comment that was made to me is about bringing new technologies forward in this space. Obviously, the precedent has been set that the amount of investment that's required to turn these into reality is enormous. I -- estimating that combined we're probably looking at more than a billion dollars over the period of time that this was first conceived where they're actually getting into routine clinical use. What do you say to someone who has a new idea that wants to come forward to bring a product like this into existence

with that kind of a track record and what kind of funding sources are we talking about that might be available to do that?

DR. GLYNN: Right, thank you for the question. So, several things that come to mind is it would be great, I think, if we could encourage, again, researchers to try to think about innovative ideas that hopefully then can come up -- come to fruition.

So, the first thing, in terms of the -- a lot of the pre-clinical work to look at the research hypothesis in both kinds of thing, that's really RO1 amenable and -- so there we can try to help with that certainly at NIH.

The question afterwards -- I think, the hard part, right, is when you have a -- you know, something that is ready to go into your -- into human beings. So, you can do a phase one clinical trial and that, again, we can provide some support there in terms of either as a small business application or, again, under the kind of clinical

trial applications that we can support at NHLBI.

In terms of a lot of pre-clinical, the big animal model work, then I'm going to let Ashley -- because we usually turn towards BARDA at that time.

DR. CECERE: Well, I mean, as of right now there's only product or one device that's out there that's approved for one indication. So, I think that we still are continuing to see what our possibilities are. In addition, BARDA has always believed in not having a single point of failure, so I think we are always open to understanding what technologies are out there. I think there's been a lot of discussion about having a device or a way of treating that can be done on all components and so I think there's still a lot of progress to be made.

I did want to highlight that on our website we have the opportunity for companies to ask for tech watches. We are continuously interested in understanding the landscape for all of these -- for all of the products that we work

on. It helps us in our decision matrix on moving forward with programs. And we're also very open to providing advice. We have experts that are ex-FDA, clinical/non-clinical CMC and all that stuff. And so, when we do work with our partners, whether it's through official contracts or just through communications, we really view it as a partnership in helping not only the products that we are supporting to get to licensure but additional products as well.

And I forgot to mention, we usually pick up at TRL 5 for radiation and nuclear. So, to have at least had a pre-IMD meeting with the FDA and have, kind of, a clinical path or regulatory path forward, obviously we like to see clinical data when possible.

SPEAKER: I'll just chime in and say that, yes, I agree that DOD has -- make it clear we have a great working relationship on all these things and, sort of, take a whole government approach, I guess you could say, to developing these various technologies. Great working with

you all.

DR. BENJAMIN: Richard Benjamin, CERUS and I want to reiterate how appreciative CERUS is for the funding support we recently received from BARDA, but there is another funding source that, perhaps, we can consider would help here, and that is if you create a market for a product more innovation will come in to fill that space and, you know, the length of time it has taken after inactivation to become a reality in the market and the length of time it then takes to actually get the U.S. market to buy it and to -- you know, that is an impediment, but I really want to recognize the FDA for having this meeting because it's -- it shows their commitment and I think a renewed commitment towards pathogen inactivation because things like the bacterial guidance that we are -- adopt guidance we're expecting help to create that expectation that pathogen inactivation is what is needed in the marketplace. It helps to create the demand. It creates the physician who's actually seeing the patients and the actual patients who

benefit from this to learn about the technology and start to demand the technology because once a product becomes successful in the market the money will come from industry to drive the innovation we are looking for. The second and third and fourth generation products will not be there without a successfully marketed first generation product.

So -- and then we can try and stoke the fire or prime the pump with research funding but that is what you're doing. Ultimately if the initial products don't succeed in the marketplace the subsequent products probably will never arrive.

So, I just wanted to recognize the work you've done over 20, 30 years, but also the FDA, for what we see as a really renewed interest in this area in helping us to move forward, and this meeting is just a great example of that so thank you.

DR. GLYNN: Thank you. So if there are no other questions, please remember never hesitate to contact us. That's what we are here for; to

try to guide you and help you try to, you know, get to the next step of what you want to do, so that's a major thing.

DR. ATREYA: Okay, if there are no further questions, we will move to the next one; that is session six. Let me get the slides. So, session six is the summary presentations by each moderator. Roughly it is -- add on 15 minutes for each moderator to speak. First is Simone Glynn again and I might try to brief you for that.

DR. GLYNN: So, hello everyone. So, my session -- well, my session; your session number one was titled Blood- Borne Infectious Agents and Their Impact on Blood Safety.

So the first session started with Dr. Busch presenting another view of the risks to blood safety from infectious agents, and in his presentation he reviewed the evolution of responses to established emerging and re-emerging transfusion transmitted infectious diseases and highlighted the ongoing surveillance for and the systematic responses to emerging infectious

diseases up to (inaudible) with sensitive metagenomics, multiplex NAT and serological testing strategies in Sentinel global donor populations.

So, in his presentation, Dr. Busch showed that over the past five decades; so, 50 years, so that's why he took a little bit longer than his allotted time. For serological assets targeting virus-specific antibodies and antigens that were implemented proved effective for screening our donors who are chronically infected with a classic transfusion transmitted infectious diseases. So, we're talking about syphilis, HPV, HIV, HTLV, HCV and T. cruzi. And then the goal of closing the pre-seroconversion infectious window period led them to progressive implementation of NAT screening for HIV, HPV and HCV over the past 20 years.

So, NAT screening, as I think we all know, has proven quite highly effective in introdicting [sic] the window period of donations and reducing the residual risks for these major

agents to -- as we heard yesterday about 1 in 2 million in the U.S.

Now in addition, NAT screening has also proven to be the preferred option for detection of many emerging and re- emerging transfusion transmitted infectious agents that cause acute transmitted infections including parvovirus B19, HEV, babesia and West Nile Virus and most recently Zica. Such infections are effectively introdicted by NAT and serological testing would not work in this case and would result in loss of high rates of seropositive donors would have result infections.

So, the other thing is that he told us was virus discovery using metagenomics technologies has also led to identification of transfusion transmitted pathogens that warranted interventions but also to detection of contaminating virus sequences. So, we heard about XMRV, non-pathogenic (inaudible) human viruses such as (inaudible) viruses and the known transfusion transmitted viruses.

We then heard from Dr. Kleinman in our second presentation who reviewed policy issues pertaining to pathogen reductions. So, Dr. Kleinman noted that pathogen inactivation reduction should be viewed in the context of shifting the blood safety paradigm from one that is reactive to one that is proactive thereby providing insurance against known and unknown pathogens that may enter the blood supply or are currently underrecognized or not recognized.

So, assuming that therapeutic product efficacy is maintained and cost issues can be addressed, the goal is to have all blood components or whole blood treated by pathogen inactivation which could then allow for a relaxation of redundant donor lab screening, modified donor questioning deferral, hopefully, and simplified handling of post-donation information.

A fully PI-treated blood supply would then shape the response to threats from new emerging infectious agents in that there would be

less pressure to give up new lab rat rescreening assays.

So important considerations in evaluating the role of PI and blood safety policy are that one, not all infectious agents are inactivated. We know we have a problem with nonenvelope viruses and sometimes (inaudible). And then the second problem is that each manufacturers' process must be independently evaluated for quantitative levels of inactivation of numerous known pathogen as well as for its therapeutic efficacy of the treated component and potential adverse effects in the recipient.

Also, Dr. Kleinman noted that the healthcare reimbursement system is to be able, of course, to accommodate the cost.

So, this other view of policy issues was then followed by a presentation from Dr. Snyder who reviewed the current status of pathogen-reduced platelets in the U.S. Dr. Snyder mentioned that currently the only PI manufacturing system approved by the FDA in the

U.S. uses Surolan, a UVA light-activated photochemical as the agent of inactivation.

Approval is limited to a collection using one of two apheresis devices and stored (inaudible) a platelet additive solution or an autologous plasma depending on the apheresis device used for manufacturer.

Both PR products have a five-day shelf life right now at room temperature and other manufacturing systems are also under varying degrees of development. So, we have the -- we heard about the riboflavin one and the one that uses the shorter wavelength for UV light or UVC.

Dr. Snyder then told us about the major benefits of PR platelets include -- including a multi-log inactivation of most blood-borne pathogens as well as the inactivation of lymphocytes thus protecting against transfusion as (inaudible) graft versus host disease.

He also noted that despite FDA approval and the acknowledgement if it's of a technology of a medical field has been slowed to adopt and

integrate platelet technology -- PR technology into day-to-day hospital operations for several reasons which he went over. So these included, in particular, concerns over the reports of lower post-transfusion corrected count increments in PR platelets versus conventional platelets; reports of lower hemostatic efficacy of a PR platelets risk of these transfusion associated GVHD because irrigation is not recommended; unknown potential for toxicity from repeated administration of Surolan especially if this is a worry in neonates and children; the possibility that the PR platelets might increase the incidents of transfusion reactions; the skin rashes in neonates that are exposed to blue light therapy for hyperbilirubinemia; the lack of long- term data on the effects of repeated use of Surolans in adults and children, especially neonates, and the increased costs associated with the use of PR platelets.

So, the FDA to date, he told us, has provided draft guidance that has stopped short of

encouraging adoption of PR technology. Thus, the use of PR technology is left up to the individual hospitals as to whether they embrace or abstain from use of these products.

So, he then went on to say that currently the major ongoing credible threat to the nation's blood supply comes -- in talking about platelet products, comes from the potential for bacterial contamination and (inaudible) a new viral or other known bacterial agent threaten the national blood supply, the time to ramp up adequate PR manufacturing infrastructure to meet the threat would likely take quite some time. So more widespread adoption of the PR technology now would do much to ameliorate this concern if this scenario occurred.

So, overall the use of PR technology is slowly increasing and they are addressing many of the above-listed concerns have been published. However, the lack of published data, especially in pediatric and transplant patients coupled with the lack of strong FDA endorsement of the technology,

and finally the increased cost of this technology, has hampered widespread acceptance of these platelets.

So, the possibility of another blood-borne threat to the safety of the national blood supply seems inevitable and how well we then mitigate that threat may well depend on how these issues regarding PR blood products are resolved.

And then he ended by saying that it's really critical that early adopters of the technology in the U.S. Make sure that they publish their experience (inaudible) with the utilization of platelets that have been treated; especially their pediatric experience.

Finally, the last speaker for this session, Dr. AuBuchon, reviewed the current status of pathogen-reduced plasma in the U.S. and he noted that available pathogen- reduced plasma products are safe and effective despite some content reductions. There may actually be a reduction of some of a known infectious adverse event risk associated with their use. However,

given the current level of safety of frozen plasma, Dr. AuBuchon told us that he thinks that there is little impetus to adopt pathogen-reduced plasma at this time in the U.S. and, therefore, widespread adoption of pathogen- reduced plasma will likely require licensure and adoption of systems for all of the blood components.

So that was a summary for the first session.

DR. ATREYA: Oh, you're here? Okay, I have the slides (inaudible).

DR. FLEGEL: So, your second session was entitled Implementation of Pathogen Reduction Technology for Blood Products in the U.S., and for that purpose I mercilessly pilfered the slide set of the speakers.

So, we had five presentations. The first one was on -- by the American Red Cross showing the experience of the introduction of the technology in this largest blood service; the second presentation, the introduction at the NIH Clinical Center at a hospital setting with a

smaller blood donor service; a third presentation on the effect on the quality and -- of the platelets; the fourth presentation was on an alternative pathogen-reduction technology for plasma, the SD treated plasma, and the final presentation on the health economics considerations.

So David Reeve presented the experience of the introduction of the technology in the American Red Cross which was first implemented in Puerto Rico in March 2015 using two different blood collection apheresis devices, Trima and Amicus, which differed slightly in the guard bands that can be applied for those systems such that a decision was made to move to the Amicus blood form which was then introduced U.S. Stateside in July 2016 and is used since. There were mitigation needed to make the production possible in the larger scale. So initially the American Red Cross primarily used duel storage kits; one-third was large volume kits and the small volume kits were hardly used. After the mitigation, however, the

small volume was used in two-third [sic] of the cases. A large volume remained with one-third and the stool -- duel storage is hardly used.

The conclusion is that pathogen reduction of 100 percent of the product is not practical based on the current guard bands would then imply that, perhaps, one could work on expanding those guard bands if possible. The mitigation required to meet the guard bands was feasible but labor- intensive and time-consuming. Again, if the guard bands would be wider than one would reduce the labor and the time and make it more feasible and, in particular, less expensive. The implementation of the pathogen reduction technology will require adjustment of set points and collection parameters on the apheresis devices.

These conclusions are exactly mirrored by that -- what we experienced at the NIH Clinical Center when we tried to implement it there which was actually implemented at a hundred percent of our apheresis platelet collections in January 2016

and we are supplying the NIH Clinical Center with our products -- a hundred percent pathogen-reduced since. We still do use non-pathogen which use platelets for those platelet products that are -- that need to be imported because physically we cannot supply a hundred percent of our patients with our own product.

In striking difference to the American Red Cross, we are using dual storage kits only. The implementation into this production took about one year and as I said it can be done more quickly but one should consider if one wants to implement it one probably should consider a good year to do that. So, if an emergency would arise it's not possible to implement it quickly. One needs to consider a certain longer timeframe.

The experience when we went live at our NIH Clinical Center Hospital was that -- such that we needed to educate and notify the nurses and physicians ahead of time. Since this was done the acceptance was very straightforward and as is (inaudible) note the introduction of the

pathogen-reduced platelets at the NIH Clinical Center overlapped with the occurrence of (inaudible) in the U.S. which actually helped with the acceptance by the prescribers.

So the task on the home stretch in the time of the introduction of the product in January 2016 was to inform and educate the clinicians, nursing staff, external customers. The current situation is such that we had to adjust our collection parameters quite a bit and it took us awhile to get to the point that we actually reached the aim for the loss due to the guard bands of less than one percent. So at this point, three years later, we actually hit that target but it took quite a while to get to this low loss due to the guard bands.

We are continuously evaluating at the quality assurance of the platelet with tension rate in the back. Obviously, platelets are lost during the process but more than 90 percent are retained and we approve that continuously with testing a large number of the platelets and a very

positive aspect is that we cannot only eliminate irrigation for those products, but on top of it the quality of the teasel inactivation for avoiding transfusion associated graft versus host disease is actually better improved by that technology. The education and notification was done, again, to the external customers, prescribers, and the nursing staff.

Which brings me to the third presentation presented by Dr. Dana Devine from the Canadian Blood Services with the impact of the technology on platelet quality count and clinical implications.

Dr. Devine reminded us that it is expected that the quality parameters changed. The treatment must balance between killing pathogens and killing the transfused cells. The risk mitigation must consider both infectious risk and the risk to product efficacy.

And she noted that it was published since that the reduction in blood component potency has been postulated two percent greater

risk than benefiting countries with low risk of transfusion transmitted infections. This, kind of, addresses the risk benefit balance and we certainly should have a eye on that topic beside -- perhaps, cost efficiency considerations.

What we knew at the beginning, Dr. Devine has stated that pathogen-reduced platelets show a 15 to 25 percent decrease in survival and recovery in normal volunteers. Licensing trials that were done, obviously, to get it approved here in the U.S. also showed the impact of treatment and this is a tradeoff for increased safety.

There is a clinical assessment done of the pathogen- reduced platelets showing that patients with cancer had an increased platelet or effectiveness and platelet transfusion requirements. However, and probably no effect on mortalities, severe bleeding or serious adverse advents.

Also, descriptive studies did not identify a significant problem in bleeding patients. Dr. Devine noted that further studies

to that effect are really -- would really be helpful. Entire quality evidence would be helpful to understand whether or not using bleeding patients is a real concern.

The fourth presentations was on an alternative of pathogen-reduction available for a long time focusing on the experience at the University of Minnesota presented by Dr. Claudia Cohn. So it's Octaplas is as treated plasma frozen at 200 ml bags. It can apply it in an ABO blood group specifically. It's pulled from 600 to 1500 donors. It's U.S. Donors only and this should be the first point here; it's FDA licensed.

The randomized control clinical trials shown here did not provide any evidence for a difference in efficacy. It was, however, noted that all of these five studies had very low numbers. So they aren't really designed to necessarily prove a difference of efficacy.

Also, hemovigilance data, primarily from Europe with really large numbers of blood bags and transfusion events, showed that there is no TRALI

report at all. Dr. Cohn noted that obviously this hemovigilance data are passively collected and may no -- not reflect every incidence but one is left with the conclusion that the product is very safe in regards to TRALI which otherwise obviously is one of the number one concerns with this blood product as a very serious, potentially lethal side effect.

So in conclusion, Octaplas key consideration, viral screening for enveloped and nonenveloped viruses is provided. The effect of pooling plasma and solvent detergent treatment contributes to (inaudible) of side effects and there is a long history of use worldwide since almost a quarter of a century. So this product offers another approach for plasma to blood safety and pathogen-reduction technology.

The final presentation by Dr. Brian Custer was on health economic considerations for pathogen-reduction technology and he pointed out that there are quite a number of interesting and important operational gains that will eventually

offset the investment costs. However, he noted that cost neutrality will be difficult to attain. So there is something here on the right lower hand left that, perhaps, will not be recovered and would require a net investment for blood safety even if the initial cost for implementing this technology is overcome.

Not shown on the figure are additional cost savings like prevention of test introduction to emergent pathogens when used with transfusion reactions and a potential for simplified inventory management. I would note that at NIH and other places for quite a long time we will have added cost for additional inventory management due to the dual system that will be required for life but very long -- longer term that this may actually come true and would get to one inventory only.

He -- Dr. Custer noted on the health economic summary, broadly speaking, that pathogen-reduction technology for plasma would cost around 800,000 to $1.2 million per quality adjusted life year regardless of the technology

that would be applied to that plasma. The number for platelets alone are best because we have the highest risk of -- with the bacterial contamination and the platelet units such that a quality adjusted life here has the price tag of a quarter of a million dollars. This might be approached and this can be considered if all bacterial contamination is considered and the culture is discontinued. So that's the best figure for the pathogen-reduction technology that he could calculate. If one combines this technology for platelets and plasma then the numbers are somewhere in between those for plasma and platelets.

The summary is within the blood safety context. The technologies are relatively cost effective despite the numbers that he showed and were shown on the last slide. As they are no less cost effective than other widely adopted interventions.

A budget gap is likely to remain until pathogen- reduction technologies are available for

whole blood or red cells. That's a very important consideration that additional research and development is required to bring this to the red cells which, after all, the number one blood part product and will remain so. And he noted that -- Dr. Custer noted that the reimbursement remains the key limitation in the U.S.

We then had a productive panel discussion with quite a number of questions and I think that those questions and the answers will shift into the summary that will eventually be published for this very interesting symposium. I have to say that I learned a lot and it was very worthwhile to come here for those two days. Thank you.

DR. ATREYA: You don't have any slides now?

DR. GOODRICH: I don't have any slides. I'll just give out an overall summary from the presentations that were made during the session.

Third session was pathogen-reduction technologies for whole blood and red blood cells.

I was the first speaker in that session. My presentation basically described some of the issues that we originally envisioned, might be present in the development and implementation of these technologies into the future dating back to a time in the early 2000s when most of these technologies were in their early development phases.

We discussed through that presentation how some of those characteristics or some of those issues have been resolved, how others remain. In many regards the observations that have been made with the platelet and plasma systems are very similar with red cells and that changes do occur as a result of these treatments. The clinical trials are currently in process with the technologies that are in development to determine whether or not those in vitro or other changes that are observed have significant outcomes relative to the clinical results and the clinical utilization of those products in a standard treatment setting.

The message from that initial presentation, my presentation, was that innovation around existing technologies is likely to be the most straightforward and likely path forward given the amount of investment that has already been made in this field in those particular areas. What those modifications may look like is yet to be determined.

Dr. Benjamin followed with the presentation of data on the technique utilizing amustaline which is a chemical method for inactivating pathogens in red cell products primarily, but it is -- it does not involve the use of light. The primary focus, though not exclusive, is on red cells. He detailed extensive studies that have been conducted up to this point in the clinical setting including results from the REDDA study, the STAR study, the SPARK study, the Recife study. These are studies that involve phase three clinical evaluation of both acute and chronic bleeding -- chronic transfusion patients including acute cardiac surgery and chronic

transfusions in the case of thalassemia patients.

The results from those studies that have been completed so far have indicated they have met primary endpoint. The modified protocol which has been utilized in creating these products importantly has indicated that there are no autoantibodies that have been observed and no neoantigens present which was an issue with the first iteration as Dr. Benjamin outlined for the product configuration. The company has spent a great deal of time and effort demonstrating that this issue has not been problematic in the second generation of the product development that has taken place and has assays and methods in place to be able to detect the antibody which was primarily against the acridine moadin that is present in these preparations.

The -- Dr. Razatos described -- followed with the presentation describing the state of PRT for whole blood by Terumo BCT which is a method that uses riboflavin and light; described it as the same process that's being used for platelet

and plasma. There is a significant increased energy dose and treatment time that is associated with that product. There were several details that were provided on studies that have been done by the organization. That AIM study, the JICA study in collaboration with the Japanese Development -- or Japanese Corporation, and also the MERIT study which is a pending study that will be initiated under the leadership of Dr. Erin Tobin at Johns Hopkins University.

The primary focus has been on whole blood although the -- there have been activities related to red cells. She described it -- an investigator-initiated study by Dr. Trachlin in pediatric patients in Moscow at the Federal Institute of Hematology and Oncology for pediatric patients. She also described a -- the PRAISE study which is a phase three study on thalassemia patients which is being conducted in the United States. She indicated during that presentation that that study has been suspended as result of issues due to the logistical aspects of supply and

that that is currently under evaluation by the company as to how to proceed with that program.

Dr. Cancelas followed with a description of his experience in working with both of these technologies both in it from a preclinical evaluation phase as well as from radiolabel recovery and survival studies. He described the data both on in vitro and in-vivo results with the two PRT methods. With the amustaline process he indicated that the procedure does require a removal step. It is a centrigation [sic] and then resuspension of the cells in an additive solution. The product that was tested by this approach meets the recovery standard established by the FDA. There is a slightly reduced survival. This is at day 35 of storage of those products. It is -- there is no significant increase that was reported with the potassium and hemolysis of those products over the storage period that they have been evaluated.

In the case of the riboflavin and light approach there is no removal step that's involved,

Dr. Cancelas indicated. That product, however, does indicate that there is more potassium leak and hemolysis when in the treated products. The storage time for that product is reduced to 21 days as a result of those changes that occur during processing. It does meet the recovery requirement at day 21 as stipulated by the FDA, but there is a -- also a reduced survival that's observed in the products that are treated by that process.

And that was the summary, essentially, from that session.

DR. WAGNER: We heard from session four which was emerging innovations relevant to pathogen reduction technologies and alternatives. The first talk was given by Dr. Maclean who described the use of light alone with no added sensitizer. The light that was used in the system was blue light, 405 nanometers.

She first showed us data indicating bacterial kill in plasma at 92 percent to 100 percent or 99.9993 logs of inactivation. They

then went through dose ranging studies to determine what the effects might be on plasma proteins. They use SDS-PAGE gels as well as Western Blots and they were able to frame the conditions under which protein gel patterns were maintained and similar to controls.

They looked at two different light fluence rates; a high fluence rate and lower fluence rate and were able to identify that the lower fluence rate seemed to provide better retention of the protein qualities. They have also done some work -- some preliminary work with platelets and have been involved in developing a system for delivering the light that -- so that entire bags can be treated.

The next talk was given by myself. I described a photosensitizer, Thiazine Orange, which can be used for inactivation of viruses, bacteria, and parasites in red blood cells. The distinguishing feature of this system is that the photosensitizer is flexible and only becomes active when it is rigidly bound in a plane in

interacting nucleic acid.

The red cell studies involved looking at hemolysis, ATP, and potassium leakage. There was some enhanced hemolysis although levels were less than one percent. In addition, there was a more rapid potassium release and the clinical aspects of this system are unknown at this time.

The third talk was given by Colonel Cap and he reminded us that many hospitals in the United States don't have access to platelets and that platelet storage at room temperature made platelet availability problematic, not only within the United States but also for our troops overseas in need of platelets.

She [sic] described studies in cold platelets which showed better maintenance of aggregation up to 21 days than room temperature stored platelets. He described improved maintenance of mitochondrial function of platelets stored in cold temperature compared to room temperature stored platelets and better clot characteristics of platelets that were stored in

cold temperature compared to room temperature.

He outlined a study that was performed in Norway on cardiac surgery patients who received platelets and noted that the volume of the chest drainage was less and certainly not worse than patients who received platelets stored at room temperature. He also described some studies that were conducted by Dr. Goetz which showed that platelets stored in the cold do have a tendency with storage to aggregate to -- and reduce platelet number, but this can be ameliorated by storage in additive solutions.

He went on to discuss whole blood -- the storage of cold whole blood for patients who need both red cells and platelets who are bleeding and described some studies that they had been involved with which showed that cold whole blood -- the platelets in cold whole blood maintained hemostatic efficacy. In addition, he discussed the problematic issues of bacteria present in platelets because of their room temperature storage and showed data that cold temperature

storage of platelets does not enable the growth of many bacterial species in platelets.

So, thank you very much.

DR. ATREYA: Huh, which form is that?

DR. NESS: Yeah. Well thank you for those of you who I have not met or you don't know me. I'm Paul Ness from Johns Hopkins and I have been given the difficult task, I think, of offering some concluding remarks with insights for future research and development. I think I need to echo some of the comments of previous speakers to say that I really thank the FDA for pulling this together. I think it is a tribute to their wisdom that they had gotten in one room for two days almost all of the stakeholders in this issue in terms of people who are interested in regulating it, the companies who are willing to produce it, the blood centers willing to make these products, the hospitals willing to use it, the funding agencies, the various users, and I think that's really a tribute to their wisdom. I've learned a lot through the meeting and I hope

-- I believe you all have too.

So, in terms of thinking about disclosures, I've been around in this business for quite a bit of time and I had been a consultant to a company called New Health Sciences which is also known as Hemanext. They are working on an anaerobic red cell storage which we haven't heard about much today but, perhaps, it may have an adjunct to some of what we've talked about in terms of tweaking the various processes that are going forward. I've also been a consultant for Terumo BCT in their processes. Actually, my longevity with them is through four name changes so that I've been involved a lot with the discussions on Mirasol system and a lot of what's been used. And it's -- you know, it [sic] really a terrific opportunity to come here and talk to you today which I have truly enjoyed or hopefully I will truly enjoy; although in some ways it is a little different challenge. Formally, I would come to meetings and ask to be -- present some of our original research, some of the results of the

clinical trials we've done while at Hopkins or in conjunction with the Red Cross when I worked there.

Today's lecture is, sort of, a different type of lecture. One that as an earlier investigator when I was young I always feared that, you know, somebody was going to get up at the end of a talk and give this type of summary talk; didn't necessarily want to listen to everything that he or she had to say, but I guess when I look today in the mirror while shaving and got my aching body out of bed and reminded myself for dealing with my granddaughters who are very happy to remind me that I'm getting old and not any smarter, but I've finally have earned the title and the obligation to give this type of presentation.

So anyway, what I'm going to try to do and I certainly don't think this is the be all and end all of the talks that have been given, is to raise some things for continuing efforts going forward that I know that others have alluded to

and probably other thus far, and some of them may be somewhat independent and some of them probably are speaking for the broad consensus as the people are here.

But in terms of the ideas of blood safety and pathogen kill we've seen a lot of information about the various levels of the kill with the various systems that have been done; some that have been tried, some have been true. And the question that I think really remains is what does this do and how does this correlate with clinical efficacy? And we learned for multiple descriptions that you kill more things, you also have cell damage, so we're going to have to figure out how we can balance those two things.

So what is the appropriate balance between pathogen kill and blood component function? And we know that we're buying into -- buy all of these technologies at 20, 25 percent hit on cell function. Are there ways to tweak that such that we can still maintain acceptable cell function without having an acceptable cell

kill by compromising the existing systems or, perhaps, going forward with new ones?

So one of the examples is this that I wanted to point out is this is an old slide that -- from the Marisol system and it shows the various log kill measurements of viruses, bacteria, parasites. And if you look over on the far and the red column where there's parasites, they -- it was predicted based on their in vitro work that they would get a three to five log kill for parasites such as malaria. We've seen this slide in a number of ways although the point was not made or raised particularly. This is from the AIM Study that was conducted in Ghana. And as you see -- as you look at the differences between the untreated and the treated cells that the parasite loads were much greater than the ten to three or ten to the five predicted kill rates that the Marisol process would have had.

So this gives some example, perhaps, that what we are measuring, in terms of in vitro, may not necessarily correlate with how effective

these things will be in vivo. And what we're obviously going to hope to get is more in vivo evidence as to kill. Now this is obviously going to be very difficult to do because with the virus lodes our studies that -- the risks are too low to really measure them. And that's why I think if we talk a little bit about Erin Tobins' study that I'm working with him in Uganda, funded by the DOD, this hopefully will give us some real evidence as to what we really are killing in -- based on recipient studies and that's the kind of study I think we would want going forward.

The other thing I would raise as you look at the third point, this issue of how much cell kill do we need and how does it correlate with clinical efficacy? I know some people pointed out this paper to you, but Jeff McCullogh, Harvey Alter, and I recently put together a large review of this topic called the Interpretation of Viral Lode in Relationship to Infectivity and Pathogen Reduction Efficacy. It has been accepted for publication and transfusion. I'm no longer

the editor so somebody else accepted it, but hopefully this will add to the discussion of this, I think, very important topic so that we will know how we can balance, perhaps, cell kill with clinical efficacy.

So in terms of platelets, this is a -- obviously important topic. We have a licensed platelet system out there which is INTERCEPT and we had a number of discussions, sort of, about do the platelets really stop acute hemorrhage? And we know that most of the studies that have been done have been done in hemon patients where the use of platelets is prophylactic and we haven't seen necessarily any enhanced bleeding in these, sort of, noninferiority-based studies, but we really don't have a lot of reassuring information that in the acutely bleeding patient, pathogen-reduced platelets has current being performed whether by the INTERCEPT system, whether by the MIPLATE system, or the Mirasol system, really will stop acute hemorrhage. And I think this is something that we're going to have to look

at. We're given some information, for example, by hemovigilance studies that imply that, for example, red cell usage in countries that are using these have not seen enhanced uses of red cells. This, I think, unfortunately ignores the fact that we're also in a ten-year patient blood management program where all around the world people are now learning to use less red cells for clinical events. So we're really going to need better evidence, I think, for the acutely bleeding patients that these platelets do have some good function. I think what Dana Devine implied, based on the activation status of some of these platelets, may be reassuring but I think we need more clinical information.

Then again, I think the second topic really is important that comes out and was repeated multiply at times is can PRT damage to platelets be mitigated so that we can enhance the recovery, survival, and function? So do we have to accept the 20 percent or so hit? Can we do this in conjunction with other things? I think

we've talked about some intriguing possibilities and, perhaps, going back to cold storage with pathogen reduction to deal with some of these issues. There may be other ways with other anticoagulants, other solutions that we can do this, but I think this is an important goal because we want to deal with it.

We didn't spend much time at this session talking about how effective PRT platelets are in reducing alloimmunization. And just want to remind you, if we look back at this study called the TRAP study which was done many years ago at seven hospitals in acutely ischemic patients. You can see that various interactions using either leukoreduction or UVB light reduced the risk of alloimmune refractoriness in patients from around an existing level of about 13 percent down to about 5 percent. And this led to really the standard of care that we now do when we reduce leukoreduced platelets routinely for almost all of our patients, particularly for those with hematologic malignancies. It, sort of, doesn't

pay attention though to the fact that this study shows, with leukoreduction, we still have a five percent remaining problem. And if you're working at a large referral cancer center you still see patients coming in with alloimmune refractoriness either because of previous pregnancies, previous treatments with platelets, previous transplants. So this really does remain an important goal. I know that the Mirasol system is attempting to look at that; the initial results from the prepare studies. We're not as confirmatory as an initial study they did in France called the Miracle Study. We haven't heard a lot of information on the INTERCEPT system as to what it does in alloimmunization, but I think this is an important potential direction and that -- an advantage, if it's proven, that pathogen-reduction platelets would give us.

And then the other question I think we really need to totally verify is how effective are PRT platelets in reducing other platelet reactions? So this is a slide that I give when I

talk about platelet reactions and I think it's -- we've seen pretty convincing evidence that bacterial sepsis and transfusion associated graft versus host disease due to platelet transfusions really are eliminated by pathogen- reductions. In terms of bacterial sepsis or other means you could probably handle this and we've shown that we can do this with culturing or other tests, but these are two proven advantages of the pathogen-reduction.

Alloimmunization we just talked about, and it's not -- we -- the results are maybe a little discouraging but not yet clear. But in terms of the other types of potential reactions that patients suffer, we heard from Dr. Cap about the high incidence of fevers in oncology patients getting platelets. So will the pathogen reduction systems reduce the (inaudible) due to white cells or cytokines that are produced during storage? And also this other transfusion reaction that can occur; transfusion related acute lung injury which has to do with either antibodies or lipids; will

these reactions be reduced by pathogen-reduction? I think these are important things to add to the value of pathogen reduction going forth to, perhaps, make it a more comprehensive and easier thing to sell to the skeptics in our hospitals.

In terms of plasma we heard a number of talks about what is the effect of PRT on procoagulant and prothrombotic constituents and their balance in patients? And we know from earlier episodes that, for instance, Protein S, low levels in some of the plasmas led to thrombotic complications. So this will be an issue that we will obviously need to go forward.

The second question, I think, that we heard about is will pooled PRT platelets or products reliably reduce transfusion reactions? And as, sort of, a corollary to that, can PRT be added to plasma pools to improve current products?

So this is a list of, I think, issues that are problems with the current plasma product we're dealing with, FFP. And, you know, I heard Dr. AuBuchon say that there's no compelling reason

why hospitals want something better. Well most of us who deal with fresh frozen plasma in a hospital setting think it's the world's worst product. It has a whole host of issues that make it very difficult to deal with. Obviously, it has ABO antibodies meaning we have to have four different types. If we want to use it for universal patients, we have to use AB or sometimes A plasma. This is very cumbersome. This could be handled. It has variable content of coagulation factors and it has to be thawed and after it's thawed it has a limited shelf life. It has infectious risks. It also causes allergic reactions very commonly in patients who get plasma during routine transfusion episodes or, perhaps, during apheresis as we pointed out earlier. And there's a potential for volume overload in patients and the bottles -- in some cases it comes in glass bottles, in some cases it comes in bags and they break so they're not easy to use.

So there's been a lot of energy going on with improving plasma. A lot of you have been

involved in the idea of having frozen or lyophilized plasma that could be used, for instance, for military use, but I don't think we will ever get to the maximum of these potential things to improve these products without pathogen-reduction. So if you really want to get rid of the allergic reactions which plague some of our patients, you're going to need bigger pools than currently are going to be provided. And if you really want to have -- eliminate the variability of coagulation factors you're going to need bigger products. And if you have a manufactured product it may potentially, for example, be reconstituted in less volume so that you could use it for stable patients who have liver failure as opposed to just using it in trauma.

So I really do believe there is a real role for pathogen-reduction in plasma products. I think we underestimate the inappropriateness and the failure of FFP to really meet the clinical needs our patient's have and so I hope this is

something going forward.

So -- and then the other thing that has never really been mentioned is this final point on this slide. We talked a little bit about making whole blood to be treated making components from it. We know that right now when we make components; the blood centers make components, that there is plasma left over. Some of it goes into FFP. A fair amount of it goes as a recovered plasma in de-fractionation. So is it going to be acceptable to use whole blood PRT treated plasma for the fractionation process? Will this be a problem for manufacturers going forward? Will it require different types of regulations because if we can't use all of the plasma coming out of these products it will be a financial disincentive to those people who use it.

So in terms of, sort of, summarizing with the red cell issues, can damage to red cells with current systems be limited by new processes or additional manufacturing steps? And we heard that some of them are meeting the criteria fairly

well; some of them, the Marisol system seems to fall short. Are there ways that we can treat the -- tweak the system with different anticoagulants, with different ways to deliver the light source to deal with these issues? I think this is very important.

A second issue that we talked a little bit about is do PRT processes effect immunohematologic procedures? We know that the first-generation red cells were affected by antibody production. We saw some reassuring data that the second generation of -- on the intercell process doesn't seem to have these problems, although Dr. Benjamin admitted that there are some problems that will still exist. We don't think they're clinically significant but if we have products that are going to be made difficult to administer with our current immunohematologic processes, this is something we're going to have to deal with and have to understand.

And then I think the other thing we haven't really talked about is does the addition

of PRT in red cells reopen the age of blood controversy? So in a previous meeting I attended and spoke at we talked about whether the age of blood controversy is resolved. And we pointed out that there are a number of randomized clinical trials in adult and pediatric patients that showed no real difference between fresh and blood stored for longer periods of time looking for adverse effects and function issues.

But not everybody has totally bought into this. Populations at high risk have not been comprehensively studied. For example, trauma patients, sickle cell patients, that we have some data that we published and I presented on retrospective studies in patients who received older blood which is actually -- they didn't do quite as well as patients who got more routine blood. The animal studies from the NIH have very old blood in very sick animals suggests that there could be problems here. Dr. Hod and Spitalnik at Columbia have shown that older blood and -- beyond 35 days with current anticoagulants has some

problems. So I think this lays unanswered, what is the effect of PRT on this whole issue? Are we going to have to do another recess study? We're going to have to get -- Nancy had a lot of retirement to do another in-forum study. The -- I don't know how we will deal with this. Are we going to just use the traditional markers of red cell recovery, hemolysis, et cetera to say this is all fine or are we going to need more clinical data to resolve all of this issue and I think that's something we have to think about.

So other unanswered questions. Do we really need blood storage for 42 days or could the blood system in the U.S. handle shorter storage periods? We heard from Dr. AuBuchon and other people that the 21 day, you know, time would be a -- problematic but maybe we could get better. And we do know that for longer -- from distant hospitals and certainly for the military use, longer periods of storage are required. We understand that if we shorten the storage period, perhaps, due to the adoption of PRT for red cells,

outdating might increase, cost might increase, and -- but perhaps, some of these products' problems could be mitigated by the adoption of newer technology and advanced transport systems are a -- commonly used in other industries but don't necessarily get applied to transfusion.

So then, sort of, the final, sort of, summary is will FDA or can FDA adopt guidelines for industry that will allow the enhancements in blood storage or pathogen-reductions solutions to be licensed and implemented such that they will be cost sensitive? I think this is a big issue. I think the FDA here by having this meeting is saying they want to work with all of us to advance these goals. So I think this is a terrific start and we haven't heard much of a discussion of what they will do with these issues going forward, but hopefully we'll -- we will hear in printed word or word even here about other steps that they want to take.

But one of the, you know, questions will come up and come up to me, like, how much of the

new knowledge about red cell storage would need to be applied to applications and for licensure of PRT red cells? I mean -- we -- Simone Glynn at NIH funded a whole series of studies in vitro and clinical trials on red cell storage. We know a whole lot more about the mechanisms of red cell storage. Are these all going to have to be incorporated into regulatory review or will we just do the same review in the past? Will FDA continue to work with blood centers to increase efficiencies with modified procedure and elimination of redundant testing? We've seen that as an important issue to try to lower the cost. And then will these cooperative efforts result in PRT components that hospitals view as cost effective and worthy of the increased expense? So this is a real issue and I know one we'll deal with because I'm concerned that if guidelines and requirements become too burdensome, the clinical advantage is of -- some of the better solutions we're talking about that would help patients may never be realized. So I think these are obviously

the goal of us all in this room today working towards.

I think we've heard some wonderful presentations of the state of the art. We've heard some encouraging things about what might go forward. It would be nice to combine some of these events. For instance, I hadn't thought in the past of combining pathogen-reduction with cold storage even though I'm very interested in both. So I think this really puts together a wonderful opportunity for citizens of these ideas and hopefully bringing forward to our patients.

I thank you for the attention you have given me and my -- the permission to -- here to be, sort of, the final rambler. Thank you.

(Applause)

DR. VERDUN: So wow, I just want to say thank you to everyone. This has been, I think, a wonderful workshop and quite successful because of all of you. I, in particular, wanted to just thank Jennifer Scharpf and CD Atreya who did a lot of the -- not only the logistics but also some

behind the scenes things that really made this happen and come together today, so I wanted to especially thank them. In addition, I would like to thank our federal partners that have worked with us and our presenters today, and also all of the participants both in phone -- on the phone and in person.

And, you know, stopping short of summarizing the summaries, I'm not going to do that actually, but I just wanted to really say that FDA really is truly committed to moving pathogen reduction technologies forward. And really moving the needle forward is going to require collaboration among everyone here and we really do appreciate all of you being here. That definitely means a lot. And I really -- we really do look forward to working with everyone to advance the field. We take all of the considerations that have been brought up today quite seriously, including the concluding remarks from Paul Ness. I appreciate those and the questions -- the compelling questions that he had

for FDA. But I think this really truly is a partnership and we do look forward to working with all of you. So come to us early, come to us often. We're here for you and, again, thank you everyone for coming and for participating. Thank you. (Applause)

(Whereupon, at 12:42 p.m., the PROCEEDINGS were adjourned.)

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CERTIFICATE OF NOTARY PUBLIC

DISTRICT OF COLUMBIA

I, Carleton J. Anderson, III, notary public in and for the District of Columbia, do hereby certify that the forgoing PROCEEDING was duly recorded and thereafter reduced to print under my direction; that the witnesses were sworn to tell the truth under penalty of perjury; that said transcript is a true record of the testimony given by witnesses; that I am neither counsel for, related to, nor employed by any of the parties to the action in which this proceeding was called; and, furthermore, that I am not a relative or employee of any attorney or counsel employed by the parties hereto, nor financially or otherwise interested in the outcome of this action.

(Signature and Seal on File)

Notary Public, in and for the District of Columbia

My Commission Expires: March 31, 2021