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CONCURRENT: Hyperthermia: The State of the Art

SPEAKERS: JOAN BULL, M.D.; ELIZABETH REPASKY, Ph.D.

MODERATOR: MICHAEL LUMPKIN, PH.D.

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P R O C E E D I N G S

MR. LUMPKIN: I'd like to welcome everyone to this session entitled Hyperthermia: State of the Art. First of all, I'm the moderator. My name's Michael Lumpkin. Our two speakers are Joan Bull and Elizabeth Repasky. Dr. Bull will be presenting first, as she has a time constraint, as we move on into the session. So please welcome Dr. Bull.

DR. BULL: ----- is a likeness of Hippocrates, and I won't begin to read the Greek, which is actually Latin here, but the translation I can manage. "Those diseases which medicines do not cure surgery may cure. Those which surgery cannot cure fire may cure. Those diseases which fire cannot cure are incurable."

Well, that is back a lot of centuries. And I think they were talking about cautery, not what we're going to discuss today, but I still love that quotation.

I am going to be talking about hyperthermia as an investigational component of multidisciplinary cancer treatment. You are all familiar with the traditional cancer modalities of surgery, radiotherapy, and chemotherapy.

We have newer therapies that are here now and more on the horizon that include liposomal drugs that Dr. Repasky will talk about, and I will mention also, cytokine therapy, immunotherapy, that is, Dr. Repasky's specialty, immunotoxins, gene therapy, and radioimmunotherapy, and we feel that hyperthermia can aid and add to, if used correctly in the right sequence, the right timing, to all these.

Historically over the last 20 or 30 years, 20 probably, clinical hyperthermia has been used largely as local or regional hyperthermia combined with radiation and it was induced and is induced by microwaves, ultrasound, and interstitial technologies. It was also believed, and Dr. Yu smiled at me because he remembers, that tumor temperatures of 43 to 45 degrees were the only things that they thought were possible to treat tumors and therefore those of us that did whole body hyperthermia were laughed at way back then.

It's not possible to achieve uniform tumor temperatures with local regional heat greater than 40 to at the most 42 degrees and yet with that limitation even at those lower temperatures, which were laughable 10, 15 years ago, there have been five positive clinical trials combining local regional hyperthermia and radiation in head and neck melanoma, breast, pelvic tumors, and glioblastoma. The latter is the only one on this slide that was done in our country.

However, I'm a medical oncologist and people seek my help, and I take care of just general oncology usually because they have or fear cancer metastasis. Treating or preventing metastatic disease needs a systemic treatment. Whole body hyperthermia is a systemic treatment and will address the whole body, get to the whole body like chemotherapy cytokines, vaccines, and monoclonal antibodies and others.

Whole body hyperthermia is a systemic treatment and can treat metastatic disease. Since the 1970s whole body hyperthermia has been used, and this is what I used to do, at maximally tolerated temperature. I told you that they thought we had to treat as high as possible, and the maximally tolerated temperature for a human being, whole body, is 41.8 to 42 degrees Centigrade or in Fahrenheit 107.6.

While this is well tolerated by most healthy people and even most people with cancer it has to be done very carefully and administered only by experienced teams of doctors and nurses and it has quite a lot of danger.

We began looking at a new, safer, a more physiologic or fever range whole body hypothermia. As you know, many infectious diseases, influenza, erysipelas, smallpox, tuberculosis, malaria -- I think we're most familiar with influenza -- each induced 40-degree temperatures lasting hours to several days.

We are trying to use the body's own, Mother Nature's, resources. So physiological or fever-range whole body hyperthermia, like a viral or flu-like temperature, its maximum is 40 degrees or 104 degrees. Now, probably most of you have children who have gone up to almost 104 degrees very easily. And the other difference is we administer it for six hours. And Dr. Repasky has demonstrated that it takes four to six hours to get the effects that one wants with the lower or fever range temperature.

Everything I'm going to tell you clinically comes out of the laboratory, where this data is backed up by using a mammary adenocarcinoma, the MTLN3, that metastasizes spontaneously 100 percent of the time to the axillary and inguinal lymph nodes, to the lung and the liver late. It grows rapidly, it shows a lot of necrosis, and it's relatively chemo-resistant. It behaves quite like clinically aggressive breast cancer and like other metastatic tumors.

The reason I use this model is because it is a metastatic model. Our hypothesis is that I alluded to very briefly earlier that the timing and sequencing of your modalities is very important, that when you combine drug with drug or drug with hyperthermia there are a better and a worse timing and sequencing to use.

We chose to look at gemcitabine, an antimetabolite, that shows synergy or superadditive cytotoxicity when you combine it with a number of other chemotherapy drugs and with radiation. And it is an observation that most everything that interacts with radiation tends to interact with hyperthermia, also.

Both gemcitabine and whole body hyperthermia interrupt DNA synthesis and repair, and a lot of studies suggest that gem is a heat-sensitive drug. We thought with proper scheduling we could do this.

So we set out to optimize the combination of fever-range whole body hyperthermia with gemcitabine to maximize the antitumor efficacy and minimize normal tissue toxicity. So we looked at three different schedules, starting with giving them simultaneously, separating them 24 hours and 48 hours, and we found that actually our best effect was combining them simultaneously.

We actually antagonized both the effect of the heat and the effect of the drug when we gave them 24 hours apart and 48 hours apart was about the same so we decided to use them directly together.

We also, because we were aiming to treat pancreatic cancer and lung cancer eventually, wanted to include the platinum drugs so we looked at the sequencing timing of cisplatin with gemcitabine, again for the same purposes, to maximize our tumor kill, minimize toxicity. So we looked at a simultaneous administration, gemcitabine given 24 and 48 hours prior to the platinum, gemcitabine given 24 to 48 hours after the platinum, and we found that in fact the best response was the platinum given 24 or 48 hours prior to the gemcitabine, and -- this was pure luck, not reasoning -- we also found this to be the least toxic combination, which is very fortunate.

Prior data that I'm not going to go through from our laboratory showed that if you give cisplatin and whole body hyperthermia at the same time you have a terrific anti-tumor effect; however, you get intolerable renal toxicity. But if you administer it 48 hours up to 30 minutes prior to whole body hyperthermia you can get a superadditive anti-tumor effect and tolerable renal toxicity, really not that much more than platinum itself if at all.

So using these preclinical data we designed a Phase I clinical trial combining platinum, gemcitabine, a low physiologic dose of interferon alpha, on which I'll comment in a minute, and whole body hyperthermia. Of all those parts I have least data about interferon but we included it in low dose to induce tumor apoptosis because interferon is by itself anti-proliferative and because, very importantly these last two, it is an anti-angiogenesis modality and it enhances the immune effector cells against the tumor. That is not from any data of my own.

Our Phase I clinical trial method is we use the Heckel radiant heat device, which I'll show you in a minute. We go to 40 degrees core temperature, maintain that for six hours, and it takes us anywhere from less than an hour to an hour to 180 minutes to get to that temperature.

This is a picture of the Heckel hyperthermia box when we are inducing the temperature. When we're getting the temperature up to plateau target we have the patient on a regular bed with the radiant heat device right up here and they're shut in this very loose, it's not at all claustrophobic, and as soon as they achieve temperature we take down the sides and fold them over much like a sleeping bag to insulate the patient. Usually don't have to heat again.

I'll just give these details. It's a Phase I regimen. Day 1, cisplatin. This is what we were escalating, 50 to 100 milligrams per meter squared, as it was written. Low dose interferon, a million international units per day. Whole body hyperthermia, 40 degrees for 6 hours on Day 3 plus gemcitabine, 600 milligrams per meter squared over 60 minutes. On Day 10, gemcitabine by itself and begin the whole cycle at Day 28.

Just showing you again, Interferon goes on for the whole duration of the protocol, whether it lasts two months or twelve months, cisplatin, 36 hours later gemcitabine and heat and then eight days later gemcitabine and then go on to the next cycle.

The toxicities of this physiologic range, fever range, whole body hyperthermia, relate only to the toxicities of the chemotherapy drugs, cisplatin, gemcitabine, interferon, and the drugs used in conscious sedation because we do this using conscious sedation. The purpose of the conscious sedation is to make this a comfortable regimen. I when we first started was heated in this apparatus to 40 degrees and stayed in it an hour. And then it was time to go to clinic and so I jumped down and I was glad.

The first couple of patients we sedated a little bit but not as fully as we wanted to and it's just very uncomfortable. It doesn't hurt but you get very restless and very irritable and very anxious just like you would if you had a high flu fever.

It's not comfortable so we do comfortable therapy with conscious sedation and this lets people sleep, even though I can talk to them if I want to, for the whole seven or eight hours of the treatment, which, if you look at induction and the six hours, that's a long treatment.

We've done 60 treatment sections for 22 patients. We always get a critical care anesthesia consult and they and I review the current medical history. You look at an EKG within 24 hours of heat, a cardiac ejection fraction within two weeks of the first heat, pulmonary function studies within two weeks, serum electrolytes, liver, renal functions within 24 hours, blood counts within 24 hours, a chest X-ray within 24 hours, and we insist on looking at new CAT scans and MRI to stage the patient within three weeks of the first treatment and every two months and, of course, a physical examination.

We like good cardiac function, a reasonable, with an ejection fraction greater than 40 percent, good exercise tolerance, an FE1 greater than 75 percent, and a good diffusion capacity. We look at history of snoring and short neck, not to eliminate people but it takes special technology if somebody has a sleep apnea type of physique. We look for plural effusions and ascites and, of course, we get the height and weight to calculate body surface area for our chemotherapy.

During the treatment, we monitor and record continuously the EKG, the cardiac rate, systolic/diastolic blood pressure, CVP, respiratory rate, O₂ saturation, urine output, and skin, rectal, bladder, and axillary temperatures continuously. And we do a lot of our fluid replacement monitoring based on specific gravity using a special refractometer, which is actually very easy to use.

And our conscious sedation includes Versed, Ativan, F-----, and Sublimaze.

We have treated 22 patients. They all had metastatic or very advanced disease. Their median age was 60, as high as 78, low as 25, nine females, thirteen males, sixteen Caucasian, five African-Americans, one Hispanic, and only one had zero. Most had had two to three, that was the median, and as many as five prior chemotherapy regimens.

The toxicities that we saw were largely at 70 per meter squared where we had neuropathy that was fairly severe but ototoxicity that was quite extreme and this is clearly not a level that we could go to.

We had two pressure burns that really have nothing to do with the chemotherapy and we have learned to avoid by padding the heels that rest on the bed. And we had one patient with a Grade 3 burn who was a very large patient who was close to the lights. Actually, we treated her after that without any burns. Leukopenia and thrombocytopenia was a problem only at the higher dose range.

We had no complete responses, nine partial responses, four objective responses which are measurable but less than 50 percent, and three patients with stable disease, altogether in this small group of patients, if you add these together, 72 percent.

We saw responses in pancreas particularly, not in colon, in gastric, lung, and in fact one of our longest responses from that woman that had the burn, who is now still going at 16-plus months who had widespread resistant small-cell lung cancer, renal cell carcinoma, bladder carcinoma, sarcoma, soft tissue sarcoma, head and neck cancer, and breast cancer.

We concluded that the maximal tolerated dose of cisplatin in this regimen is 60 milligrams per meter squared. Heat does not appear to significantly enhance the biochemotherapy toxicity and this particular regimen shows clinical activity and I plan to take this to a Phase II study beginning with pancreas.

We were doing another thermochemotherapy regimen which is quite similar to what Dr. Repasky will talk to you about, using Doxil, continuous infusion 5FU, interferon again, and whole body hyperthermia. You will hear a lot more about this from Dr. Repasky but our reasoning is that while normal tissue blood vessels, say, in the lung or the gut or the heart, have quite tight junctions, tumor tissue has holes, very faulty vasculature, and we can increase the size of those holes to let in large molecules such as a stealth liposome, which is what doxil is, stealth liposome holding doxorubicin, and increase delivery, going to a higher temperature of 42 degrees.

This is work of Mark Dewhurst (?) showing the extravasation of 100-nanometer liposome like doxil in tumor compared to normal tissue. You see that there's no increase in normal tissue and quite an increase in the tumor tissue and Dr. Repasky will go on and show you the same thing happens at 40 degrees. Particle size, increase in the tumor occurs at the higher temperatures.

Dr. Repasky's data, our data, and Dr. Dewhurst's data suggest that heat administered prior to doxil, it appears, the administration of doxil, induces and enhances the antitumor activity. And from some prior work of our own we know that a prolonged intravenous infusion of 5FU for five days ending 24 hours prior to the heat increases heat-induced anti-tumor activity.

So, again, we used the Heckel radiant heat device, 40 degrees, plus or minus three-tenths for our tumor temperature for six hours and, again, the same time to get to temperature, which is a long period of time.

5FU, 400 milligrams per meters squared, continuous infusion every day for five days ending 24 hours prior to the induction of heat, Interferon-alpha every day beginning with the beginning of 5FU, and doxil administered over two hours beginning two hours after the end of heat and our dose escalation of Doxil was 40 to 50 milligrams per meter squared, repeating thermo- chemotherapy cycles every 28 days. The number of treatments remain two and a half.

Showing you again low-dose interferon throughout the whole protocol, prolonged infusion 5FU Day 1 through Day 5 stopping 24 hours prior to the heat, heat given for six hours and two hours at the end of it given doxil and beginning again at Day 28.

Our post-treatment support uses leukine specifically beginning Day 10 to 12 for any granulocyte count less than one and a half million. We also use erythropoietin weekly for hemoglobins that are less than 10 and if patients begin particularly with low weight, as do many of the patients with GI tumors, we give them Megace and Nandrolone and Androgen to improve their ability to increase nutrition.

We've treated 11 patients with resistant metastatic malignancies, breast, ovarian, endometrial, cervical, adeno- and primary, melanoma, pancreatic, and gastric, median age 43 with a range of 32 to 78. Number of prior chemotherapy is median two, with a range of one to four.

And the greatest toxicity of doxil is not cardiac toxicity but is hand-foot syndrome, palmer plantar erythrodysesthesia, and we saw actually three Grade 2/Grade 3 toxicities at 50 per meters squared as well as severe mucositis.

We didn't have as much difficulty with leukopenia or thrombocytopenia but the hand-foot syndrome is pretty hard to tolerate.

We saw responses in breast, ovarian, cervical, mullerian duct, and melanoma, five partial responses, three stable disease, and four patients with progressive disease.

We decided that 40 milligrams per meter squared, which is the standard dosing for Doxil, is the recommended Phase II dose based on our data with the palmer plantar erythrodysesthesia and we also conclude that if you time this in sequence optimally with 5FU and interferon it's well tolerated and induces a clinical benefit in patients with chemotherapy-resistant tumors, many of whom had already seen adriamycin.

Whole body hyperthermia did not appear to significantly enhance either the myelotoxicity or the PPE, the palmer plantar erythrodysesthesia.

If I can summarize briefly the two clinical protocols, fever-range whole body hyperthermia appears to enhance selected chemotherapy-induced tumor responses, cysplatin, gemcitabine, interferon, or 5FU interferon Doxil. It doesn't appear to significantly enhance the chemotherapy toxicity. It may enhance the immune response and you're going to hear a lot about this in the second talk by Dr. Repasky.

In order to optimize your antitumor response, minimize toxicity, it's critical to learn the optimal combination sequence of each drug, drug to drug, drug to heat. And these two preliminary Phase I studies indicate that this combined chemo-bio-therapy fever-range whole body hyperthermia does have clinical activity.

I want to thank particularly Rick Strabel (?), who's a strong man in the lab, Wanda Roe (?), Dr. Sumiyoshi (?), who comes to us from Kyushu University, and the many people who have helped clinically, including my colleague, Dr. Garcia Menero (?), Steve Koch from Critical Care, who spends a lot of time, particularly Glenna Scott (?) from our clinical research center and the other nurses who support her, our pathologists, radiologists and, of course, the oncology clinic that does a tremendous amount of the background work for this whole protocol.

Remember, whole body hyperthermia is one of our armamentarium. Thank you.

MR. LUMPKIN: Thank you for that excellent talk, Dr. Bull. At the present time, I'd like to invite Dr. Dal Yu, who will serve as our commentator to express some views on this subject. Dr. Yu?

DR. YU: I'm Dr. Yu, medical oncologist here at the Washington Cancer Institute, Washington Hospital Center, mainly involved in instead of systemic hyperthermia local/regional hyperthermia utilizing intra-peritoneal chemotherapy with the GI malignancies and working with Dr. Paul ----- and his team and greatly appreciated the detailed systemic.

I think this is probably one of the most promising areas for systemic metastatic treatment area. Clearly, as you saw, the kind of patients that they're refractory to all other conventional chemotherapies and other systemic treatment. And I cannot emphasize that we need the systemic treatment. That requires whole body hyperthermia. There's no question. The question is, as Dr. Bull repeatedly emphasized, how to integrate that. We still do not know the optimum sequence and the dose ranges and a whole array of the data, which probably will be investigated further.

The only one small question that I have is that frequently when we present the hyperthermia data, particularly in Asian countries, that one of the most commonly asked questions is whether the size of the body, particularly the adipose tissue amount, has any bearing.

Asian, particularly Japanese, they're thin and the hyperthermia, particularly if you're using a local/regional and perhaps a whole body as opposed to a Western counterpart that presumably has a little more adipose --

DR. BULL: Not just presumably. I'm sure it makes a difference, the timing of the heating, because the rate of heating is a combination of metabolism, which your body size wouldn't directly impinge upon, but also the bulk that you're heating. So I'm sure I have not, as you saw from my data, an opportunity to treat an Oriental person but I think they're probably a little faster to heat.

Now, the effect should not be different that I know about. Yes?

QUESTION: A quick question on heating. I know if you work out doing an athletic type of endeavor your core body temperature really gets hot.

DR. BULL: Yes, it does.

QUESTION: And have you explored this as a way to heat people as opposed to -- it would seem like a more natural ----- and we would involve a lot of other synergistic processes to have the body actually heat itself as opposed to heating the body?

DR. BULL: I have not done that. I think it would be an interesting way to somehow if you could insulate at the same time because even though your body core gets hot it also tries to dissipate that heat so you've got to insulate that.

I think it's a real interesting idea.

QUESTION: I think there are some football players ----- replicate that protocol -----.

DR. BULL: And they're not with us any more. I know.

QUESTION: The football team might just need the football -----.

DR. YU: If I may respond, that might be a good idea for whole body but in terms of the local regional, say, intrapleural use of chemotherapy in conjunction with the hyperthermal intraperitoneal or intravesical, et cetera, et cetera.

Now, there you're targeting close to 41-42 degree, for example, intra- peritoneal cavity roughly up to a 41-42 degree right in the operating room right after the use of some form of the cyto-reduction surgery.

And while the fresh surfaces are opened up, and that is where you put the chemotherapy agent, with the heat and literally manually you're mixing the variety of ----- the whole thing right at the operating room table and for 90 minutes, at least, our protocol is that.

So that might be difficult to achieve that and localize in such a fashion but the whole body idea I think is very useful.

QUESTION: ----- interesting ----- using either a whole body ----- approach or local approaches or other types of ----- approaches in the context of -----?

DR. BULL: Absolutely. In fact our data is not ready. He is asking whether using heat could combine with anti- angiogenesis therapy. Dr. Folkman (?) and I think Dr. Repasky will mention that, also, has drawn our attention to a unique opportunity to kill tumors by specifically selecting those tumor vessels that I tried to draw your attention to.

And we have some data and other people do, also, that heat does increase the effect of some anti-angiogenesis agents but I'm not ready to show you that data yet because we need to do a lot more in the way of experimental work there.

But there is some indication that there is an interaction.

QUESTION: ----- see, I appreciate the response ----- but I don't see where the potential for harm is. That's what I really don't understand. And I don't understand why it's so necessary to ----- the drugs against the drugs plus the hyperthermia, when we know that at the very least, that you're getting partial response from the studies we've already done and the drugs are entering the cells at a greater rate.

There are other physiological benefits as well, and it's tolerable, and it appears to be safe. So I didn't really quite understand -----.

DR. BULL: Well, I work in the world in which we live and to do this we have to go a step at a time and I think that is based on trying not to do harm. I certainly object to any studies that go from test tube directly into patients.

And I feel that same care needs to be going from early stages to more advanced stages before you go to treating people who have other standard therapies that, say, would work.

Now, would I treat if you had a hypothetical tumor where there was no good treatment? Yes, that would be very reasonable, very reasonable.

DR. YU: If I may add one other comment I think it's at the moment at least through the peer review journals, where you have to publish your data and so forth, as Dr. Bull repeatedly implied, that if the treatment works in this refractory setting in the metastatic disease setting obviously it's going to be moved up rather rapidly and I think we are still in the process of trying to show safety and efficacy in a scientific manner.

Having said that, we do run into some cases, if I may, since pancreatic cancer with IRB approval. This one anecdote we had a pancreatic cancer with clear-cut peritoneal seeding, widespread, peritoneal carcinomatosis. By any standard at the moment peritoneal carcinomatosis from any primary is almost equal to death sentence. And Dr. ----- and his group in town have the one single case with the cyto-reduction, optimal cyto-reduction, and intraperitoneal ----- with the heat. And, I mean, although we ----- -- everything the patient made it okay and doing fine now, about a year and a half out.

But it was a very tough treatment. It required three months of hospital stay, prolonged -----, prolonged chemical peritonitis, prolonged liver toxicity, and so on and so on and so forth. I could go on and on.

And so I think your points are extremely well taken but there are cases that there are some problems that we still have not completely solved. Now, you can say the different tumor and the different way you have done your different studies but I think we still have to establish the safety and efficacy data based more on the advanced disease before we can move it up to the front.

QUESTION: Well, you know, I agree with you on a certain level and I'm well aware of this method, the reasoning -- the approach to reasoning about this.

But I also think that, you know, having also been a patient myself and being a physician, too, actually you, Dr. Yu, were one of my physicians ----- it concerns me a lot that we can't cut through some of this and really make maybe a little extra effort to see if we can't get from refractory patients the first responders and in a more expeditious way because I agree we get all sorts of paradoxical effects. And I don't know all the reasons why this case didn't respond, and, of course, ----- vanguard of all of this for a very long time in many areas.

But it still concerns me that, you know, we're not getting to this a little bit sooner and we're not using the principles in trying to ----- in a safe way, in a tolerable way with our study designs so that we don't have to wait, you know, three or four years even, when there's been so much, as ----- will tell you, German experience of these hyperthermia ----- and I don't mean to -----.

DR. YU: I think your points are extremely well taken and I'm grateful. Yes, definitely, oncologists are data-driven people and I was just mentioning from the audience from the previous meeting there's one prospective randomized intracavity bladder ----- bladder cancer that was just presented last month at the International Hyperthermia Society meeting in Rome that the Milan group urologists showed that chemotherapy and chemotherapy with a hyperthermia intravesical ----- transitional cell carcinoma the combined modality was a lot better in the abstract form.

But this is kind of study will be more published when the safety and efficacy starts showing. I think what you are commenting is very, very legitimate.

SPEAKER: It could be fast-tracked in a lot of ways.

DR. YU: Absolutely.

MR. LUMPKIN: Are there any other questions of Dr. Bull or Dr. Yu? If not then thank you very much.

Next speaker will be Dr. Elizabeth Repasky. Today her topic is Immunological Effects of Fever-Range Whole Body Hyperthermia and Its Implications for Cancer Therapy. Dr. Repasky.

DR. REPASKY: Thank you. Actually, listening to this discussion I listened here from a different perspective. I'm not an M.D., I'm a Ph.D. scientist at Roswell Park. I don't see patients. We do a lot of work with mice; however, we collaborate with clinicians at Roswell Park to bring our therapies to clinical trials and, having agonized over how to heat people and having people look at me for years and say what do you mean, you're going to heat people up for six hours and having to worry so much about the safety of that it's actually ironic to me that now when you hear data like this that it seems so logical that you could just move this right into early stage disease.

And I feel the same way but I also know how many people actually are quite worried about a heat treatment as natural as it might seem. And I know that from my own experience in trying to explain to even my family what kind of research we're doing.

But the work that Dr. Bull just presented actually provides a perfect backdrop into the research we're doing in our laboratory which is that a hyperthermia treatment, if we keep the treatments in the range of about 39.5 to 40 degrees or about 102 through 104 degrees is awfully similar to the natural hyperthermias that are found in fevers in response to infectious disease.

And interestingly as a society we treat fever as a disease. We take Tylenol, we take aspirin, when actually it's a symptom of something else. And what is that symptom? It's a very ancient, very conserved response to infection and yet the purpose or benefit of a fever has really never been understood.

Our hypothesis that has driven a lot of our research for about the past 10 or 15 years is that hyperthermia can enhance therapy of cancer. In other words it can be used as an adjuvant to improve cancer therapy. And in particular we're very interested in using hyperthermia to enhance immunotherapy, which is also what we're very involved with up in Buffalo at Roswell Park.

Our central theme in our laboratory and in our clinical programs is to try and integrate what we can learn about hyperthermia treatments and a set of proteins which we know to be induced by heat. And this set of proteins is induced not only in humans but is one of the most conserved protein responses in nature.

It's known as the heat shock or stress response way back to bacteria. And that understanding the basis of heat shock protein production and hyperthermia and tumor immunology can help us in the most scientific way to improve cancer therapy.

Now, what I'd like to do is to briefly give you a bit of the background and basic science behind this theme and why we're so enthusiastic about it and some of the clinical programs that are emerging from this laboratory data up at Roswell Park which are similar to some of the data that you've just heard and some ideas and plans for the future which we might address in questions as well.

As Joan has already mentioned, in the literature there are two forms of whole body hyperthermia. Of course, local and regional hyperthermia are much better studied and have achieved a lot more understanding in terms of what's going on, especially in combination with radiation.

But systemic use of hyperthermia has really had two forms, as has already been mentioned, a high temperature where the goal here is thermal kill, and if you could achieve a hot enough temperature at the area of the tumor that you might cause tumor cells to die. But, of course, at this high temperature you cannot use a whole body procedure for very long so these procedures have been as short as 15 minutes and as long as two hours.

And then something we had developed in our laboratory which we had identified as a fever-like or a fever-range whole body hyperthermia, which was much lower in temperature, about 102 to 103 degrees Fahrenheit, for two to twelve hours which we recognized was almost identical to the clinical protocol that Joan Bull was developing and using down in Texas.

We are interested in immunotherapy and the combination of hyperthermia with it for several reasons but one historical reason involves William Coley. I'm sure many of you have heard of this individual. He's considered one of the fathers of immunotherapy because he achieved some spectacular successes with a variety of different types of cancer by injecting individuals with bacteria.

And what was recognized about a century later was that -- and in fact one of these individuals is his granddaughter -- he didn't really appreciate the fact that the patients who did the best in terms of their long-term survival were actually those who had also the highest fevers.

He was giving bacterial infection. Of course, these individuals got really high fevers and so his best survival were those that got between 102 and 104 degrees. So this is interesting information from a small population of people.

Now, we are not interested at this point in my laboratory in using bacteria or bacterial products to induce the fever because we can't control that. Everybody responds differently to infections and viral infections in terms of what temperature is actually achieved in terms of the fever. Children respond differently from adults.

Actually, to address a question that came up a little bit earlier people's response to exercise hyperthermia is also different. It depends upon your body mass, your size, your being in shape or not. A variety of individual physiological factors come into play in terms of the ultimate temperature which is actually achieved.

And so it's not a most reproducible hyperthermia exposures and while it is a naturally occurring hyperthermia so is a febrile response or the ability to achieve a fever. I have something else to add to that but we can come back to that and that's shown right here.

Only mammals and birds actually change their body temperature themselves in response to infection and I always thought that that was the major difference between cold-blooded and warm-blooded animals, that we achieved a fever and, of course, a constant body temperature, and they did not.

And many years ago, actually as a result of my own children's experiences with pets in our house, I learned something that was fundamental and I attribute that day to one of the events which made me realize that the research we were doing in the laboratory had some relevance to real life.

And that was the day I learned that lizards when they become sick need to find a warm place and they behaviorally need to raise their body temperature. I was stunned when I learned that and I couldn't believe it.

I went back and started doing literature searches and found journal articles in our best journals, Science and Nature, about the behaviorally-induced fevers of cold-blooded animals.

And here we were all these years taking mice and putting them in a warm box, which may or may not be the most physiological way of achieving a hyperthermic state, but when I realized that most vertebrates do it exactly that way, by moving to a warm spot, and if you prevent them from moving to a warm spot their survival following infection is greatly impaired.

And that was what was shown here in this graph. You can see way down here at the bottom the lizards that were placed in the coolest environment had the poorest survival versus those that were allowed to go to the temperature that they would naturally seek out if they were infected. And this response goes back as far as earthworms and perhaps even earlier so it's a widely conserved response to infection, this need to raise body temperature.

And this is just one more paper. This is a Nature paper in 1977 which very dramatically showed that the fish that you allowed to choose which pond or which little box of water they swam into actually had 100 percent survival following infection from this particular bacteria and slightly less are those you force to go into exactly that same temperature, that they would prefer to move themselves, according to survival data. But nevertheless those you place in the coolest environment did the poorest.

Now, this is a textbook, William Paul's Fundamental Immunology, that we use in our graduate program and the training of Ph.D.s in immunology.

It's a very big book. It's the bible of immunology. Its most recent edition came out in 1999. And I just want to show you two quotes from that textbook which indicate that fever remains the most poorly understood of acute inflammatory responses and that we have no idea of why we need an increase in body temperature.

But I just want to emphasize again, this is a widely conserved response in nature, this moving to a warm environment or, as mammals have evolved, having a hypothalamus that allows us to automatically change a set point to raise body temperature.

This is how we do it. We have a warm box, an environmental chamber, as we call it, where we place our mice. We have temperature transponders now. We've actually gotten very sophisticated over the years. We used to use rectal thermometers. This was very tedious work.

Now, we use those same little magnetic strip devices that are in those thermometers that you can just put in someone's ear and automatically, a temperature comes up.

So we can take a whole cage of mice, wave a wand over the mice, and up on the computer screen comes the mouse's ID, and its internal body temperature. So we know that it takes about 20 to 30 minutes to bring the mouse to the core temperature that we're aiming for, which is about 39.5 to 40 degrees Centigrade.

And we maintain it experimentally for six to eight hours. We inject the mice with saline ahead of time. There's almost no toxicities that we've been able to note.

We do note that female mice do better with this treatment than male mice. Male mice tend to become more irritable with each other in the cage, so we usually reduce the number of mice per cage that we heat. I'm not quite sure what the basis for that is.

This is that one that I mentioned. This is a cage of mice. This is our warm box. You could see two cages of mice in here. We can heat four or five cages at a time so we've been able to really greatly increase the number of mice that we can use in experiments.

Now what I'm going to show you is a variety of effects which are visually easy to see from where you're sitting that hyperthermia all by itself affects parameters of the immune response and the hyperthermia in the range that I've described. What I'm showing you here is a lymphocyte. This is a cell that, of course, as you know, is very important in our defense against viral and bacterial infections and hopefully tumors as well.

And when they form these little pseudopods or uropods this is a sign that a cell can move or is about to move and has become migratory. And in fact one of the papers that we published that is in the handout that I have for this talk shows that a fever range hyperthermia all by itself, this is without a tumor, this is without an infection, has the ability to make lymphocytes move more quickly.

A larger number of lymphocytes start to move, either in a petri plate or if you're looking at whole animals out of the blood and into the tissues. We have several ideas for what's going on here. We think that as we raise the body temperature of the mouse we're fooling it into thinking I have a fever, there's an infection somewhere, go look for it.

And to our surprise motility and migration were the most dramatic changes that we see in lymphocytes in response to fever-range hyperthermia. This is another example of that phenomenon of uropod formation now in an antigen-specific cytolytic T-cell.

These lymphocytes are the lymphocytes that save you from viral infections. These are the cells that go out and kill flu-infected cells or a variety of other types of infections. And here again you find a significant increase in the number of cells that develop this ability to move a, uropod formation, in response to hyperthermia. Now, remember, there's no infection here. There's no bacteria, there's no virus, there's no tumor.

Going along with that, research from our colleague at Roswell Park, Sharon Evans, indicated that a fever range hyperthermia all by itself has the ability to make lymphocytes more sticky in a receptor-dependent manner, a receptor known as L-selectin, which allows lymphocytes to bind to the vascular endothelium and to several types of matrices that they find themselves in the body is greatly increased by 2- to 6-hour treatment by fever-range whole body hyperthermia.

And finally heat shock proteins by our colleague John Subject at Roswell Park is induced, of course, by high- temperature treatments and there is a large group of heat shock proteins. He is doing most of his work on the high molecular weight heat shock protein 110. And what we were able to find, that even low temperature fever-range whole body hyperthermia can induce heat shock proteins.

But something we're very excited about is the induction of proteins, as you can see here. In the control you don't see any of this particular heat shock proteins but following six hours of treatment there's great induction. And this is true in the lymph node as well with this heat shock protein.

The induction of heat shock protein is really in organs of the immune system at these low physiologically relevant temperatures, whereas in the liver it's the same in control and following heat treatment. If you went to hotter temperatures you would find induction of heat shock proteins at the liver but again it suggests that the immune response is aware of body temperature and in several different ways that I've shown.

Heat shock proteins themselves are now known to greatly stimulate the immune response and now we're seeing a connection where you have a febrile state or a hyperthermic state that causes genetic changes that induce proteins that are known as heat shock proteins to be produced and that these

proteins themselves are very potent stimulators of the immune response. And so quite a few numbers of people are very interested in using heat shock proteins from the outside injecting them into individuals in a variety of different constructs as vaccines to stimulate antitumor immunity.

Now some anti-tumor data that we started to look at about five years ago, when we started these studies. We knew that hyperthermia could affect the immune response but could it help control the growth of tumors?

And what you're looking at now is controlled tumor growth in a murine model and tumor growth following two separate treatments with just hyperthermia alone. And all by itself there is control of tumor growth but we've never noticed a cure. We've never been able to use this very mild treatment and have tumors go away.

We've prevented tumors from getting formed after we've added the tumors at the time of hyperthermia but in real life patients come to the clinic with cancers and you want to know how to get rid of them at that point.

But we do find a reproducible control of tumor growth each time we add this treatment and we're very interested in the biology behind this or the immunology and, of course, what we're most interested in is utilizing whatever is going on here to add to other standard or other types of cancer therapy to make it better.

This is what those tumors look like at Day 2 two days after the first heat treatment. What we're particularly pleased to see is a very large influx of lymphocytes. We know that they get up and move out of the blood, we know that their adhesion properties are changed, uropods are forming, so it makes sense that suddenly lymphocytes are maybe increasing in the center of the tumor, and this plays a part, at least, in how this tumor's growth is being controlled.

This is just another model. This is a colon-26 tumor now grown in a BALB-C mouse. The previous data, by the way, was a patient's tumor growing in SCID mice. We have a lot of basic studies going on in our lab right now, and we're very interested in changes in gene and protein expression. We're doing microarray and profiling of tumors and the immune system in response to hyperthermia. And we're looking at membrane polarity and dendritic cell activity and T- and B-lymphocyte activity following hyperthermia.

Now, the tumors do seem to be controlled a bit by this mild, very mild treatment. How is that happening? And one of the answers is that when you look at these green spots what these are is this an assay for cell death or cells that are undergoing apoptosis or programmed cell death.

And what you can see that eight hours following a whole body hyperthermia treatment you see a lot of cells that are suddenly undergoing apoptosis. And when you count these and quantify it you see this change in the number of apoptotic cells following hyperthermia.

Now, is this true in other organs of the body being treated by heat? And the answer is no, that in this case the thymus is the organ you want to look at if you're worried that your treatment is inducing apoptosis because there's such a high degree of apoptosis in the thymus naturally.

So if something were going to increase cell death you would look at the thymus and we were very happy to find no increase in apoptosis under these same conditions in the thymus.

Other end points that are probably quite relevant to what's going on in the tumor involve a model known as contact hypersensitivity, which I'm sure half of you here in this room are familiar with. And this

is a swelling model that's due, it's known from other types of experiments, to lymphocyte recruitment and associated edema from the recruitment of lymphocytes to the site of the response.

Hyperthermia greatly increased the speed of the elicitation phase of a contact hyperthermia response, again suggesting, especially since we see in large blood vessels here, that there is a change in lymphocyte recruitment and edema that's stimulated by heat treatment.

We have other data which I don't have time to go into now on the effects of hyperthermia not only on lymphocytes but also on a very important cell population known as dendritic cells. And one place where there is a lot of dendritic cells is in your skin. And just as we found for lymphocytes we find that dendritic cells when you're exposed to a fever-like state leave the skin in larger numbers, again perhaps going and searching for the cause of this increased temperature, that there's an infection somewhere.

And that's shown here. This is controlled dendritic cells in the skin. This is two days following whole body hyperthermia, and this is the decrease that you see in dendritic cells and a concurrent increase in dendritic cell migration from the skin to draining lymph nodes.

Now, I'm going to come to the end of this talk by going in a slightly different direction. I've been talking now about end points of the immune response that appear to be affected by heat.

Something which we did not expect to see was at the level of the tumor itself. What you're looking at here is the number of blood vessels in a tumor in which you can get dye that's fluorescent into. And the way these experiments are done is animals are injected in their tails with a fluorescent dye and then we wait at various time periods and take the tumor out and see how much dye is in a tumor.

And one of the paradoxical issues about cancers is that they do require a vascular supply, an induced one, but in fact that vascular supply is very poor. Most of the blood vessels in a tumor are compressed, they're not functional, they're leaky, and there's a variety of physical issues that constrict access of blood to a tumor which is why many regions of a tumor are actually hypoxic or necrotic and present one of the biggest problems clinicians face in giving chemotherapy.

The first tissues which take up chemotherapy are not the tumor. The first tissues which take up chemotherapy are the normal organs, which have a great blood supply. So you have to use very large doses of chemotherapy in order to get an appropriate dose of drug into a tumor and this accounts for a large amount of the toxic side effects and severe toxicities associated with chemotherapy.

Now, something we didn't expect to see was this effect two hours following our mild whole body hyperthermia treatment. Again, we're not curing any tumors. We're just controlling the growth with this treatment all by itself.

We find that there are no more blood vessels here than here but almost all of the blood vessels here now we were able to get some dye into. And that presented us with a variety of different things to think about, that no matter what drug we're adding, whether it's a lymphocyte, a vaccine, or a chemotherapy, if you use hyperthermia appropriately you might be able to greatly reduce the amount of drug needed to get into the tumor.

And this just shows the kinetics of this response. We know that this phase of enhanced access -- I don't want to say perfusion because I'm not really sure the blood's moving through the tumor; in fact we have evidence that it's not, at least at longer time points. It might right in the very beginning.

But for about twelve hours there is an increase in perfusion potential or access inside of the tumor following whole body hyperthermia. So right away we started looking at chemotherapy because we thought that this would be the easiest thing to do in the clinic at this current time. And we found to our

great disappointment that using most free drugs, and we tried several, we found no enhancement of tumor cell killing.

Then we decided to look at liposomally encapsulated adriamycin or Doxorubicin so the name Doxil.

This is a larger particle and now we thought that if we're increasing the space or the volume that's accessible in the tumor by heat first that now maybe using something which could become trapped and stay there that we could pack a lot more of this drug into the tumor.

And that's exactly what happened. We were able in this particular experiment to double but we've actually been able to get some very dramatic cases of increasing of Doxorubicin concentration in a tumor following whole body hyperthermia.

So then you might ask but you're heating up the whole animal. Why wouldn't you increase drug in the liver, the kidney, or the heart? The reason is that when we remove the heat normal organs and their blood vessels very rapidly constrict and come right back to their normal dimensions because in a normal organ the blood vessels still possess smooth muscle and possess all of the physiological control on vascular delivery.

It's like if you heat up your arm with hot water you see it turn red. But as soon as the heat's removed it turns back to its normal color very, very quickly. This does not happen in a tumor for another two days. So we have this window of opportunity, we believe, to increase delivery to tumors but not to normal tissues and that is in fact shown here with kidney and heart, two organs we're very concerned about in terms of increasing toxicity.

I just want you to notice the Y- axis here, 6,000 versus 400. This experiment does not mean that more drug gets into the tumor than in the normal tissues. Of course not. These are organs that have a tremendous blood supply in a normal individual. This is where all your blood's going every time your heart's beating. It's through the heart, through the kidney, through the liver, through the spleen.

So there's a tremendous amount of blood moving through there and, of course, a lot of drug. But the important part is that there's no increase in the amount of drug following whole body hyperthermia.

So based on this and other data we were able to go ahead and quickly do some antitumor effects and, just as we had hoped, the best control now in this same model that I showed you earlier is down here at the bottom.

This is the whole body hyperthermia plus Doxil where we get great tumor control as compared to controlled tumor growth or hyperthermia alone, giving us our typical sum control. Here's Doxil, which is a great controller of tumor growth for a while, but adriamycin and most chemotherapeutic drugs induce drug resistance after a period of time and that's shown here. This is another tumor model.

Here's a control. Here's hyperthermia alone and we had to sacrifice these animals because the tumors got too big. Here's Doxil at Day 12 looking really great but by Day 30 those tumors take off and there's nothing you can do at that point to really control tumor growth once the tumor starts to grow and has become drug-resistant.

But something that made us really happy was that we actually had two out of the five animals in this group never regrow a tumor after the tumor had regressed. So based on this particular data and a lot of other experiments we went ahead and have begun our second protocol at Roswell Park.

Now, the protocols I'll show you now are very simple compared to those you've just heard from Joan where she's using multiple chemotherapeutic regimens. The first one that we did, and this is already completed, was nine patients. And this is why I was very interested in your comments about why aren't we moving forward into earlier stage patients.

I cannot tell you the difficulty. We had patients who would agree to be heated for six hours even to 102 to 103 degrees Fahrenheit. It's a foreign concept still to most patients and it took us over two years to recruit nine advanced cancer patients for a trial that our institute made us do.

We talk about this quite often, and both of us are very interested in getting more institutes involved with these studies. But our institute's IRB board made us heat people alone first because of their great fear that this long temperature range and duration were going to hurt someone.

And yet from our mouse data we know that we don't get the effects on the immune response at all at two hours, barely at four, and it really comes into play by six to eight hours. And so we had to do a long Phase I study and we were so happy. And when we finally finished it, using hyperthermia alone, we find almost no toxicity.

And that paper, the abstract is in the handout for my talk. This has been accepted for publication at the International Journal of Hyperthermia. And it's now being followed with our second study that has now been approved. The IRB and the scientific review board approved now the use of the same protocols defined here in combination with one chemotherapeutic drug and that's Doxil. And we're accepting patients now for any advanced cancer, again where treatment options are limited. We're waiting on funding so we can pay for each patient going on this trial from an NCI grant which is now pending.

And I believe this is an announcement that came through the Center for Complementary and Alternative Medicine, where they're looking for trials using complementary therapy, and that's where our grant is pending now.

And we are very interested in conducting multicenter trials. And in development at Roswell Park - in fact we hope to have this trial in place in the early spring -- is a vaccine plus hyperthermia. And that's a vaccine using a melanoma antigen and to be followed by a breast cancer antigen vaccine using her ----

This is how, again, Joan already showed you the device that we're using to heat patients with at Roswell Park with its sides up, and learning from her we do exactly the same thing. Once a patient's core temperature comes to the range we want it we lower the sides and partially keep the patient covered.

We use a very mild, one sedative, and we haven't had any problems. I just want to show you one piece of data from our first trial. Our first trial was completed successfully but something that made us very happy.

In a Phase I study, as you know, safety and feasibility are the major end points. But we couldn't resist taking blood samples and looking at what was going on with their lymphocytes.

Are they moving out of the blood? Was there any evidence that the immune response was being affected? And the answer is yes, yes, yes, and this is one example. This is one patient's blood levels of lymphocytes at the control and one day following heat, and then it comes right back to normal levels by Day 2 or Day 3.

It's identical to the same thing we find in SCID mice. So what can we say from this? Not too much. We can say that hyperthermia affects lymphocyte mobility and homing and distribution but, of

course, until we get in there and biopsy the tumor we don't know whether those lymphocytes are leaving the blood and now getting to the tumor where they should have been to begin with.

I just want to tell you a little bit about this trial if there's anybody in the room that's interested. Again, we'll be evaluating Doxil only and we're very interested in comparing blood levels of Doxil when it's used alone and following whole body hyperthermia.

So pharmacokinetics is going to be a big part of this trial. We are also going to be now doing as many immune system end points as we possibly can and the eligibility at this point we're leaving it open basically to any patient whose tumor should show or could show effects with adriamycin or Doxil.

But we are allowing and hoping actually for measurable disease. Many studies such as this one usually don't want measurable disease but we're hoping actually to be able to biopsy some of these tumors.

This is the treatment schematic. This is Cycle 2. Cycle 1 is just Doxil itself. And then the hyperthermia treatments will be given at three different weeks, each week. The analysis again will be immune function and toxicity and hopefully, a response.

To summarize, I've told you that hyperthermia treatment may be a safe and effective new adjuvant because it affects the immune system and an adjuvant because it can help another therapy work better. But since I've been at this conference I've learned about the word "complementary therapy" for really the first time and I think I'll switch to that rather than "adjuvant."

I just want to remind you that at this time there are only two FDA-approved adjuvants. And so we're hoping to make the science that I've told you about with hyperthermia, their relationship to heat shock proteins, which I haven't had time to go into, and the immune response and integrated approach to our understanding of really what's going on. And we feel that before we can really move forward we're going to keep the trials really simple and the end points related to the immune response and by evaluating these different parameters of this connection, which is based, as Joan said, completely on nature that we think we can really hope to identify more rational strategies using hyperthermia in cancer.

I want to conclude by indicating my own e-mail address if anybody is interested in any questions or further discussion but also I'd like to identify the three other members of our Roswell Park what we call the HSP hyperthermia program. That's John Subjeck and Sharon Evans and, very importantly, Bill Craybill (?), who took bravely, I think, our attempts to heat people up and now is going ahead with the Doxil trial and now is going ahead with our vaccine trial for malignant melanoma next year.

That's just a summary of many of the components that we're working on at Roswell that I have not really described in detail here.

I'd be happy to answer any questions. Again, I'm not an M.D. I'm a scientist. And I'll try to answer clinical questions but I would rather that our clinical colleague sitting right here in the front row would handle most of those. Thank you.

QUESTION: It's a mouse question.

DR. REPASKY: It's a mouse question. I can handle that.

QUESTION: ----- I was struck by the fact that one of your -----.

DR. REPASKY: SCID mice. When they don't have an immune response? Is that what you're --

QUESTION: Well, -----.

DR. REPASKY: Exactly. I should have mentioned that but I wasn't sure anybody in the room would pick that up but you did. I'm always wrong.

QUESTION: I think that's particularly interesting because 20 years ago ----- cancer therapy. And we have subsequently realized that most spontaneously occurring ----- tumors are not -----.

DR. REPASKY: Well, they are and another way to look at that is they're highly immunogenic and by the time you see a tumor that's what's escaped the immune response. There's some selection that goes on.

What would happen if you had a very immunogenic cancer cell? The immune response would recognize it and kill it. So what does a tumor do next is a variety of mechanisms, ----- loss, antigen loss variants, to allow survival.

So it's possible that by the time you have a tumor that's what the immune response, you're right, can no longer recognize.

QUESTION: Suffice it to say that ----- usually does not recognize spontaneous ----- or it doesn't do so to the degree that --

DR. REPASKY: A transplantable tumor model?

QUESTION: ----- curious clinical result and what has been interesting about the natural ----- response was -----.

DR. REPASKY: Upon antigen?

QUESTION: On this -----.

DR. REPASKY: You're absolutely right. That SCID mouse data is very important because it shows --

QUESTION: ----- response to the injection -----.

DR. REPASKY: Right. Actually, we're doing a lot of antibody work. It might be here. It's not on here.

In conjunction with this phenomenon that we can improve access to tumors we're already using Herceptin, we're using Rituxan, and we're using R24 and we're very pleased by the increase in tumor efficacy killing that we're getting with herceptin, for example, in our SCID model, where there's no T- and B-cell.

But the SCID mouse data also indicate that the innate immune response is affected by hyperthermia and so you're absolutely correct in pointing that out, that there may be far more potential to the innate immune response than we've ever as immunologists given them credit for.

I remember just even a few years ago, when somebody said neutrophil or ----- I didn't want to hear anything about it and I was wrong again.

QUESTION: Do you have -----?

DR. REPASKY: Well, we have a tough time. What we do in my lab is we collect all the pieces of tumor from surgery that are left over that the pathologist has enough to make a diagnosis and I get the rest. And so we implant all those patients' surgical specimens of all solid tumor types at Roswell Park into SCID mice so we have a 3,000-plus bank now of mice and not actually actively grow the tumor for a while.

We usually take the tumor out and freeze it back after a couple of passages. But in each case sometimes the tumors grow and sometimes the tumors don't grow. And one of the reasons for that is some patients' tumors are recognized and killed right away by the mouse's ----- cells even though it doesn't have class 1, it's a xeno-graft so the murine ----- cell still sees something it likes to kill even though they have class 1. The paradigm for ----- cell killing in humans, with human ----- cells, is that as class 1 is lost, as tumors learn to avoid the immune response, that should turn on ----- cells because they go after class 1 loss. It's hard to tell what's happening in our model because with murine ----- cells in a human tumor yet hyperthermia enhances whatever is going on.

I have the nice experience of a large part of our research is not just on hyperthermia. We're interested in the existent immune response that patients have, whether they have an immune response to their tumor or they don't.

So if we grow a patient's tumor the physician who did the surgery is the only person allowed to call them on the phone. I'm not. But once the patient says yes, this person can call me, then I'm allowed to call them, tell them who I am, and did they remember on their surgery day that they signed a consent form that excess tissue could be used for research and somebody saying yes on the other end, slowly.

And then I tell them that it came to my lab and I put it into a mouse and grew it. And usually there's complete silence on the other end of the phone. And if it's a husband he calls his wife over and he says you won't believe this, you've got to hear this, and he puts his wife on the phone, and I tell them both that indeed we are growing their tumor. It's alive and well in Buffalo, New York, and they almost always want to see the mouse.

And it's a good way, though, for me to first of all tell them what we're doing and then get blood from them. We need their blood because after I have their tumor growing we have a large amount of it. Now what we do is we ask them for a lot of their white blood cells. And then we put them together and ask in the experiment does that patient have an immune response to their own tumor?

We do it in the mouse but mostly we do it *in vitro*. And there are lots of different assays we use to answer that question. And almost always they do, which is interesting because that suggests that in their bodies the potential for a strong immune response is present but it's just not working.

Now, one reason for that is that somehow there's a lot of escape mechanisms in place in the human or the lymphocytes can't get into the tumor, the access is poor. And these are great discussions to have over a beer but we don't really know what's going on. And so a lot of these things are there's so much to do.

I wish, again, just going back to your discussion earlier, I would like nothing better than everybody who first gets diagnosed with cancer go right away to hyperthermia to stimulate the immune system. But many people would object to that on this basis. Some people find that the immune response may or may not help hinder tumor growth. There are a lot of people out there that think a strong inflammatory response to cancer actually promotes tumor growth.

There are just so many basic science questions that need to be addressed before you can feel comfortable about taking something to earlier stage patients. But for as long as we've been doing this research I stop taking aspirins when I get sick. That's the one thing that I stopped doing.

QUESTION: I thought your slides on showing the poor vascularization of tumors was very --

DR. REPASKY: I know it seems paradoxical but it's not.

QUESTION: The question then is would the anti-angiogenic substances be contraindicated to be used when you try and get substances to --

DR. REPASKY: No, and I wrote a grant last year and it wasn't funded and I was really disappointed on this idea, that part of the value of anti-angiogenesis therapies or let's say anti-endothelial, tumor vascular therapies, is you've got to get the drug to the tumor's endothelium. If most of the blood vessels are constricted you're not going to get the anti-vascular drug to the right targets or very few of them.

Our idea was to use hyperthermia first, open up all of the blood vessels that we could, then add something which would kill the lining of every blood vessel. And we have data that demonstrates this is correct. The drug we're using is a cytokine called IL12, which is a very potent killer of vascular supply to tumors. It's through an indirect mechanism.

And what we're using now are more conventional anti-angiogenesis drugs in combination with hyperthermia and we're very excited about this data. But what the data suggests is that you don't need much to make a tumor happy in terms of blood vessels. Yet the anti-angiogenesis or anti-vascular drug world would say that if we constrict even that little bit we can kill a tumor.

But tumors have evolved to survive on very little in the way of blood supply that's normal. They're already hypoxic, they're already half-dead, and they're quite happy. So our thought is that it might seem to be reversed but to actually increase blood flow in order to really deliver the most appropriate killing targets to tumors.

So I do go to bed at night worrying about one other thing. If we're increasing blood flow into the tumor how do we know that metastasis isn't going to increase? We're breaking off pieces of tumor cells and now suddenly we're spreading them to all over the body.

I can tell you another reason I'm worried about going into earlier stage patients with hyperthermia is because of the potential. We have no evidence for it but these things have to be brought out.

There's a potential theoretically if you increase blood flow to tumors you're also going to increase the pieces of tumor that break off and spread through the blood. Do we have any evidence for that? No. In our clinical trial, our Phase I study, we have a couple of patients who are still alive. We, our clinical team, did not notice an increase in metastasis but these were patients who already had metastases.

We have one animal model system of experimental metastases that we're concerned about. It's not physiologically relevant. It's a model where we inject tumor cells in the tail vein and let them spread all through the body.

Hyperthermia increases that. Now, that's not the same as what happens to people. We don't inject our tail veins with tumor cells and so that also brings up this idea of duration.

We want the duration of increased blood flow to be followed very quickly by potent tumor-killing drugs or, what I'm more personally interested in, is the immune response. I want to do immunotherapy very badly with regard to hyperthermia. We want long-term systemic, chronic control of tumors. I don't care.

I heard this morning in a talk something that I agreed with very, very strongly. It probably is not necessary to eliminate a tumor. You just want to control its growth.

What better way to do that than with long-term T-cell immunity? So I'm talking too much. Go ahead.

QUESTION: ----- it's my understanding that tumor cells do not operate ----- then why ----- can you explain that?

DR. YU: I know that we have not well-defined answer for question. She's referring to pseudomyxoma. It's an appendiceal tumor. It just produces this tons of mucoid material, jelly-like material, and filling up the abdomen and literally chokes off bowel and everything and causing a problem.

And as far as we could see in the traditional pathology slide it's not a hypervascular so your question is a very valid one but, I mean, in it there are some blood vessels. We are talking about the density-wise. We expected that it's not like a hypervascular but it's still obviously capillary meshwork to go through.

Our theory --

QUESTION: ----- hypervascular according to --

DR. REPASKY: Hypovascular.

DR. YU: Hypovascular.

QUESTION: None of them are hypo or none of them are hyper?

DR. REPASKY: None of them are hyper.

QUESTION: Right, so you're agreeing with him -----.

DR. REPASKY: But some are even more undervascular --

DR. YU: It's the degree of the expression. For example, as you know, the hypercellular carcinoma some of them are very hypervascular relative to --

DR. REPASKY: Relative to other tumors.

DR. YU: Not compared to normal, among the tumor hierarchy.

DR. REPASKY: That's a good way of putting it.

DR. YU: So why is it that the relatively hypo lower end of them are responding to the heat or hyperthermia is not very well understood. Our theory is whatever the small amount of the blood vessel that is and if we can manipulate them probably that can enhance what Dr. Repasky just described, open up the gate, and then get the appropriate chemotherapy.

DR. REPASKY: Then close it.

DR. YU: As you are aware, that we do it 90 minutes up to 42 degree right in the operating room with the so-called ----- technique, where the belly is opened up and giving right there in the solution and

the manually manipulating entire abdominal cavity so that every corner of the space can have an adequate exposure of the cytotoxic chemotherapy. That's the only way I can explain. There maybe other plausible explanations.

QUESTION: I first wanted to say thank you for your research because you showed us some details why hyperthermia works. And ----- short time ----- then it goes back to normal.

DR. REPASKY: Right.

QUESTION: And I think you may be reaching the target, the tumor tissue, because of your ----- vascular constriction, and -----.

DR. REPASKY: You said it better than I did. So I appreciate the way you say it. Thank you.

QUESTION: ----- we come to the question what we do, anti-angiogenesis or do we do hyperthermia ----- and the thing that's interesting ----- human tissue selectively reacts the way you described it by normal tissue that ----- that's not.

And this way we have access to -- we have tumor access ----- like Doxil. But the question really is do we treat this way or do we use anti-angiogenesis? And the question that you made clear we do have anaerobic tumors that do not need blood supply to grow.

So basically they are completely unaffected by --

DR. REPASKY: Anti-angiogenesis.

QUESTION: ----- have to realize that it goes back ----- do need a certain oxygenation in your blood, otherwise you paralyze your immune system.

We do know that there is actually ----- when you have reduced oxygen supply. Angiogenesis is a two-sided sword ----- for instance, studies ----- and actually doing ----- and we showed how the immune system went ----- because ----- angiogenesis was completely ----- tumor but it affected the entire system.

And this is something that you have to do when you treat this anti-angiogenesis. You have to make sure ----- are you really reaching the target? Are you helping the patient or are you maybe doing the opposite?

And one more question, since you mentioned you are getting so many different tumor tissue ----- -- everything that's coming -----.

DR. REPASKY: That's big enough.

QUESTION: What is the difference in your vascular reaction and therefore your enhanced tumor access -----?

DR. REPASKY: I wish I could answer that. We've noticed such a variation among patients, though, even with the same cancer. I'm really unable to compare, standing here, anyway.

Colon cancer versus breast cancer, if you took 10 patients' tumors growing in SCID mice and looked at all 10 of them they would be so variable in terms of how much vasculature that's still present in the tumor that's human.

That's lost after a passage or two because now that tumor that's cut out by the surgeon we put it in the mouse. There are some human blood vessels that last in there probably for 8 to 10 weeks.

But at the same time that tumor's calling upon murine vascular elements to vascularize it so after a while there's a mixture of human and mouse vascular supply. That's the down side of the model. We don't claim that our model is as good as using a patient and we all wish we could do experiments on human tumors in humans but we can't.

This is what we consider to be the next best thing. The next thing we can do is use a cell line, which is a single cell implanted into a mouse which you allow to grow up as a tumor, and then a vascular supply is also developed while the tumor is growing.

But I was very interested in what you were talking about earlier in terms of anti-angiogenesis. Is there any data on immune response following anti-angiogenesis therapy?

QUESTION: -----.

DR. REPASKY: But I think that's a very important question. I had not ever thought of that, what's actually going on with the oxidation status and the effects on the immune response. I think you make really a valid point.

QUESTION: ----- and also ----- fever I can see -----.

DR. REPASKY: Yes. There had been so much anecdotal interest and also some very good studies of hyperthermia. Now we're adding another component. We're adding this low temperature whole body hyperthermia.

It's an intriguing idea but one that needs a great deal of more science behind it. And hopefully that's what we're starting to do is to really try to understand what heat does. And I'm a firm believer that it does something or else it would not have been conserved through these millions of years since earth worms.

I'm very impressed by that data on the survival of cold-blooded animals in response to hyperthermia. And also personally it makes me feel better about taking a mouse, putting it in a warm box, because I'm not giving it a fever. It's not exercising on a treadmill. I'm very defensive about our model not being physiological but then when I look at most animals in nature, though, that's exactly how they get their fever is they move to a warm spot. So I think it's okay for a study.

QUESTION: If you go to the scene of an accident -----.

DR. REPASKY: There was a study published in the New England Journal of Medicine on colon cancer patient survivors. Again, this was in Europe.

In the United States and in most places you have your abdomen opened. Your body temperature falls to 34 degrees for most plain old abdominal surgery and colon cancer surgery.

You're undressed, you're on a cold metal slab, and they did a study where they simply kept the patient at 37 degrees. They didn't get hyperthermic but they kept some kind of warm air. Maybe you've heard of this.

And they shortened hospital stay by an unbelievable number of days and they measured it by cost of in-house stay. Simply by not allowing patients to get cold they actually improve the time needed to recover from intra-abdominal surgery. I was impressed by that.

QUESTION: On the basis of your research, which I found very fascinating, do you feel that there is justification to do clinical trials ----- before the hyperthermia or do you think you've established the fact that -----?

DR. REPASKY: What you're bringing up is very important because, as you notice from Joan's study, she had found that the optimal time but she's using free drug. And she's using 5FU, cisplatin, and gemcitabine.

We're using liposomally encapsulated Doxil or doxorubicin or adriamycin so unfortunately we can't compare our studies. But we found no enhancement by using free adriamycin with hyperthermia whether you added it before or after.

And I said that but very quickly so I'm glad you brought this up again. This is a very important point. It was the particle size that mattered for our studies. We think hyperthermia allows us to trap a lot more particulate drug inside that tumor. Free drug, at least with adriamycin, comes in and goes out.

Just having more blood there doesn't really help a free drug because it comes in and goes out probably even more quickly. Tumor blood vessels are very leaky and, again, this is why patients get so sick. It's not because the drugs are bad. It's because they work incredibly well. But you can't get enough into the tumor at the same time that so much more is getting into normal heart, normal muscle, and all these normal tissues are affected because you have to add so much in order to maintain some level of efficacy inside of a tumor.

These drugs work incredibly well *in vitro*. That's why they were all approved. They make patients sick because we have to use so much of it in order to have any effect *in vivo*.

QUESTION: -----?

DR. REPASKY: That's what mattered here.

QUESTION: -----?

DR. REPASKY: No, we couldn't get any in without the hyperthermia. No, the hyperthermia matters. We only get the effect that I showed you if we do hyperthermia first, then Doxil.

If we did the reverse, which I didn't show you, we get no enhancement with hyperthermia and Doxil.

QUESTION: -----?

DR. REPASKY: You can liposomally encapsulate any drug. There are companies out there, though, that we have to depend upon to do this and they do not liposomally encapsulate most. For example, 5FU is a very standard post-colon cancer surgery drug. For some reason, adriamycin was in a stealth liposome and it's used a lot in ovarian cancer and I don't know why it's not used in breast cancer.

QUESTION: -----?

DR. REPASKY: Yes, it's grants, grants, grants always.

QUESTION: -----?

DR. REPASKY: It traps it.

QUESTION: It traps it in the context of increased -----?

DR. REPASKY: That's right. Remember, Doxil is not FDA-approved. It's used because the side effects are apparently less but it's no better than free adriamycin, apparently, in control of tumor growth in patients.

We feel that it's better if you use hyperthermia right before it, though. But that's just with preclinical data we have. I hope that we're going to show that in our trial at Roswell, though. I hope we're going to show even in a Phase I study but we'll see.

MR. LUMPKIN: I just wanted to give everyone a chance to continue asking their questions but for those of you that need to leave or you have appointments please feel free to go. And we'll consider this the formal termination.

And for those of you that have additional questions why don't you just come on up and talk to Dr. Repasky?

DR. REPASKY: Thank you. Thank you very much.

(Whereupon, the PROCEEDINGS were adjourned.)

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