

Thanks Jim. It's a pleasure to be here and I am certainly from the fringe, not only am I from a pharmaceutical company, but I'm not gonna talk about radiation at all, so I'm gonna leave it to your imagination to translate what I'm gonna tell you about two applications in radiation. More, what I wanna do is give you a sort of nuts and bolts approach to DC MRI, how you use it, how to get useful information out of it and then a couple of examples from my domain, the development of drug world showing just the fact the if everything is done appropriately, you can get useful information out of this. So, the first thing is really there's sorta two approaches that people can take with MR using tracers for tumor vasculature to get a handle on the tumor blood supply. One of those is using tracers that remain in the blood supply during the first pass. Those are

called vascular tracers, the second are diffusible tracers and those are tracers that start to get into the interstitium during the first pass and eventually distribute throughout the extra vascular extra cellular space (EES). Either case, what we have to do is infer the tracer concentration from single amplitude changes that are caused by the presence of the tracer. So, we're not having to change Hounsfield units like you might like to see in CT, so actually doing this inference of tracer concentration is not quite as straight forward but if you pay attention to the details you can reasonable numbers. So, for vascular tracer contrast agents the single changes take place because a contrast agent is in the vasculature and it's primarily because the susceptibility differences. When the contrast agent is in the blood there's a susceptibility difference between the blood and the tissue. This leads to a

loss in signal and the, consequently the effects are really only seen when the contrast agent is in very high concentration in the blood and that's why you really only see it during the first pass, maybe the first couple passes in the bolus injection unless you have a tracer that stays in the blood predominantly and even then as it starts to spread out you don't have enough of a concentration difference. So, here's an equation that is commonly used to relate what we see a concentration in the tissue, the volume of interest you're looking at to some parameters of interest. Here the concentration is a function of time and a volume of interest is given by this parameter.  $F$  is the capillary blood flow, often times called perfusion. The  $CA$  as a function of time, so this is in the blood, the arterial concentration as a function of time and this  $RFT$  is a vascular residue function.

That depends on the actual vascular structure. So, just to get a handle on how these come into effect, what impact can they have on the curve you're looking at, it gives you some indication of the sensitivities to these different parameters. So, here are three different cases of flow, roughly equal to what you might see in a tumor. For a given input function, you can see actually these tracer curves that tracer signals that you would see different are quite a bit different, so it's very sensitive to flow. The mean transit time which give you, gets into that  $R$  factor that has to do with the distribution of the tumor vasculature, so the mean transit time changes we see there's not quite as much of a change in the signal but it is somewhat sensitive to it and finally, if we look at different time courses for the arterial input function you can see that time course in the tissue can

be quite a bit different. Hence, it's important to know what the input function is in a

given subject. So, for vascular agents, and I'm really not gonna spend any more time on this because the dynamic contrast enhanced MRI mainly deals with diffusible agents. For these agents, 'cause of the time course is that we just go back for a second, there are over the first sixty seconds, all this is happening really in that, primarily in that first pass of the bolus, so it is very rapid. We need very high temporal resolution if you want to be able to measure these things so that makes it hard to get the temporal resolution you need and coverage of the whole tumor which you'd like to have. They are very sensitive to flow, sensitive to the vascular structure. This input function's critical and this has been used, the use of it is limited in the clinic. It's been used more in animal models, but in the clinic it's used mostly in the brain. There's been some work in breast and so forth. Often

times you'll see this called dynamic susceptibility imaging instead of dynamic contrast enhanced imaging 'cause in fact the signal's not enhanced, it goes down and it is due to susceptibility. Really, the main tool that's used very widely in oncology is diffusible contrast agents. Here the signal change arises primarily from an inter-reaction between the water protons and the unpaired electrons of the para-magnetic contrast agent and typically it's just gallium DTPA, the one that's used all the time in the clinic and this is present in both the vasculature and the extra cellular, extra vascular space. These don't get in the cells but they distribute through everything else. So these effects are evident until the contrast agent clears from this extra cellular space, which is a period really of several minutes to often times really hours. So, the concentration you see in the voxel

again now, because we have some in the blood and some in the tissue, it's gonna be dependant on both the concentration in the blood and the concentration in the tissue where the  $V_B$  here is a fractional volume of blood. Here is again our arterial concentration, the tissue concentration. If we look at an expression that's commonly used to represent the tissue concentration is a function of time. There are a number of different ways to do this, the most commonly used one uses a two compartment model with the term  $K_{trans}$  which is the volume transfer constant between the vascular space and the extra cellular space, extra cellular vascular space. This  $K_{ep}$  is the rate constant between the vascular and extra cellular space and the fractional volume of extra cellular space is simply  $K_{trans}$  over  $K_{ep}$ . So, this really depends on these two parameters

probably predominantly  $K_{trans}$  and the fractional volume of extra cellular space. So, if we look at diffusible agents do the same sort of thing that we did for the vascular agents. So, here we have an input function, first of all, note, now I have the time after the bolus arrival spread out instead of over a minute now, it's over ten minutes because again they stick around in that extra cellular, extra vascular space for a much longer time. So, here if  $K_{trans}$  varies and we'll get it in a second as to exactly what this  $K_{trans}$  represent, but as  $K_{trans}$  varies, again over a range that you might expect to see in tumors, you can see a considerable difference in these time courses in the tissue. If the fractional volume of extra cellular space, so that's how big the sink for this contrast agent to get into varies, you can actually see that can have quite a bit of difference on the time course as well and

of course just as for the vascular contrast agents, if the arterial input function changes its

shape, dramatically shown here, there is a shift on, in the tracer concentration time course as well, so really you need to, you would prefer to have this input function to get accurate measurements of K-trans and the extra vascular space. So, one of the questions that comes up often times, and you hear it talked about by many people in our community and the MR community doing these measurements and also in oncology where people are trying to use these is just exactly what are we measuring with gathering DTPA, is it permeability or is it flow? Well, K-trans, that equation that I showed you is actually very strikingly similar to an equation that Seymour Ketty introduced back in 1949, so it's been around for quite some time and used by the physiology community for over 50 years now and this K-trans is really the same as in K's equation, EF or the extraction fraction times

the flow. The extraction fraction is given by this equation, one minus E to minus PS over F, where PS is a permeability surface area product and F is flow. So, when you ask that question, well if the ratio of the permeability surface area over the flow is much, much greater than one, then E equals one and K-trans really does give us flow. Those are what are called freely diffusible tracers. If the PS over F is much, much less than one then E equals PS over F and K-trans is really giving us the measure of the permeability surface area product. If we tag the gallium DTPH or a large macromolecule like albumin, then you get into this situation and in fact you can use this approach to measure permeability surface area. However, in most tissues you're not in that situation for either, in fact, in the tumor, where this might be the range of blood flows you expect to see unless the

permeability surface area is greater than eight, so you know, a good ten fold greater than our flow, you're not gonna be measuring flow and \_\_\_\_\_ that's much, much less than .01, you're not gonna be measuring permeability surface areas, so really in tumors I think the general statement is we don't know what we're measuring, it can, it's really a combination of permeability and flow. So, for diffusible agents the same sorta summary as for your vascular agents, they require high temporal resolution that doesn't have to be very high. The higher you can get, the better, the consensus is that if you can have ten second temporal resolution and cover the whole tumor, you're in reasonable shape. It is very sensitive to flow and permeability, so we're really not able to distinguish those two. It's sensitive to the distribution volume of the tracer, the extra vascular space, you want a

have that input function to get reliable measurements and it really is widely used. I have this statement here and I think it's true, when people talk about dynamic contrast enhanced MRI, this is generally what they're talking about and especially in clinical studies, it's been very widely used. So, here, this is the rest of the talk now. I want a go through this line-by-line. Well, at least I got some chuckles. This is a poster that was presented at last year's AACR meeting. The cancer research in the United Kingdom, the CRUK has this committee that, Pharmacodynamics therapy advisory committee that met in October of 2002 to look at really what are the recommendations for, what are the primary requirements that you want in a DC MRI study to be able to reliably use it in clinical trials and that's what this really is. This is going to be coming out in the

manuscript but right now I really only have it in the poster form and I'd be happy to share it with anybody who wants it. It'll be in your handout but I don't know that you'll be able to read it. One of the recommendations from this consensus panel were that the primary endpoints one should use are K-trans, which we've talked about already, and IUC, the initial area under the concentration contrast agent concentration time curve, the IUC. We haven't heard about the IUC yet; so let me explain what that is. Ok, so if we have our single pixel time course, so here's a contrast agent as a function of time. So, we've assumed we've been able to translate our change in signal to contrast agent concentration without a lot of issues. Well, one thing obviously we can fit to the model

and get a K-trans value out, the other one is actually pretty obvious and this is an approach that we borrowed from the PET community is just to take the initial area of the curve. Typically somewhere from sixty to ninety seconds, so how does that relate to K-trans and why did they recommend that we might consider using that. Well, if we look at the IUC as a function of EF, so this is if we know the input function for both of these, you see that there is a nearly relationship over the range that one might see in the tumor but that does depend on the extra cellular space. Ok, so it's not a simple relationship, but it is there and really in tumors the extra cellular volumes are up in this range is pretty much what we would typically expect to see. In other tissues like muscle then you start to get to these much lower extra vascular extra cellular spaces. So, the one question that

immediately popped to my mind was how does, how do these relate to physiology? We've done some work with freely diffusible tracers and in the PET community they've used oxygen fifteen and ammonia, oxygen fifteen labeled water and eleven labeled ammonia and you really can get flow out, but here we're using something that we know doesn't give us flow or permeability. What if we take the situation where we have an agent that we know affects the flow. So, this is a drug Combretastatin A4P. It's what's called a vascular targeting agent. What it does is cause an immediate dose dependent decrease in the blood flow. We know that, so, Ross Maxwell, who's over the gray lab in England, did a study where he used DC MRI to look at these effects and analyze data both by the fitting and IUC approaches and then they measured the flow directly with

iodoantipyrine, which is a freely diffusible tracer. PS over F is much, much greater than one. This is a radiotracer. So, now you have a real measure of flow and your MR measures of K-trans and IUC. So, here are results for two different doses. So, at the lower dose this is the actual tumor blood flow measured by IAP. Here is the tumor blood flow as a function of time, so one hour where it comes down very quickly again. With a lower dose, it goes back up, so that it's almost back to normal out of twenty-four hours. At the higher dose it goes down and essentially stays down, non-measurable. If you look at the MR results, here is K-trans and here is IUC. The first thing you notice is they both have a very similar appearance. The second thing is to see that in fact we see something very similar to what happened to the flow. Not exactly the same but very similar where

we have the K-trans and the IUC coming down at one hour in the low dose, going back up to mirror pre-treatment by twenty four hours for both of them and going down and staying down in the high dose treatment. So, we do have some appreciation that what

we're measuring does actually relate to the physiology. So, when you do these measurements, one of the questions that comes up is, how do you choose the flip angle, and it's time for me to fess up to an error I made in a review I wrote in 1999 and there I suggested that what you want to do is maximize the signal change for the tissue and if you do that you find out that say for these, this particular case at five millisecond repetition time at eight hundred and fifty millisecond T1 before the contrast shows up. If you look at the change in signal as a function of the contrast agent over the range that you might

expect to see in tissues, this is getting pretty high. It turns out that fifteen degrees gives you the maximum increase in the signal and I have this other one out here at forty just because we're going to think about that in a second. The forty is kinda nice 'cause it's nice and linear, you can see there's some curvature up at the fifteen degree, but clearly down at these low concentrations you're getting more bang for the buck with a lower flip angle but if you do that there's a price you pay. Remember I said we want to know the input function and so that means you have to be able to measure the concentrations in the blood as well where the contrast agent concentration is much higher. So, if we expand that and here it was our plot before we were looking at just zero to one millimeter. If we now go out to ten millimeters, you see that this plot, the fifteen flip degree angle just

flattens out, so if you were to look in the blood vessels for an input function there, you would be insensitive to it at the high peaks 'cause you get very high concentrations in the blood so you pay the price at not having quite as much sensitivity to change in the tissue but you have something which will allow you to measure the contrast agent concentration in the blood. So, the flip angle's critical and I fessed up to making that mistake early on. The second recommendation from the PTAC is that for both K-trans and IUC measurements, you have to measure the tumor T1 immediately prior to contrast uptake and the reason for that is presumably that's a key piece that you need to have for you to be able to relate the change in the signal to the contrast agent concentration, okay, and that's true if the signal is affected differently for different initial T1's. Well, if we look at

the change in the signal, so just the signal with a GAD minus the baseline for this is now using our forty degree flip angle with a five millisecond TR, look for a range of seven hundred fifty, eight fifty, nine hundred and fifty millisecond T1's, you see there's almost no spread at all. So, in fact, if you use that change in the signal there's no problem at all, however the problem with doing that is you have RF core and homogeneity. So, the actual change in the signal may depend on change in the contrast agent but it also may depend on the actual B1, which is spatially dependent. So, normally what we want to do is divide by S0 which is what I had published back in '99 and when you do that then you see that in fact you do run into problems and it starts to spread out quite a bit. So that's why one should measure the T1 at the start of the study. However, one could ask the

question, well, does the RF in homogeneity or the T1 variation introduce more error 'cause you certainly have homogeneity in B1, you're certainly gonna have errors and measure your T1 which introduces the most. We don't have an answer for that right now. Hopefully, someone sitting out in the audience is gonna do that study and let everybody know how we should be doing this. Look at reproducibility, this is one of the questions

when you have a tool and you want to use it in a clinical setting and you're looking for changes, you need to know when you have a real change in an individual patient. So, you have to look at reproducibility and one of the things here where I said before, we want to measure that input function. This will, is a clear indication of why one would want to measure that input function. This is a study from the Dutch group. I'm not gonna try to

say his name, published in 2001 showing the reproducibility. Here, where they assumed an input function, so they used the same common input function for every subject and each one of these is a subject and you can see before and after. Some of them are kinda close, some of them are varied quite a bit. The root mean squared co-efficient variation for these subjects is about twenty-five percent. If they, this is an example from a subject where they had neck cancer, so here's the tumor and they identified regions that, where the signal took off very quickly at a very high extent and considered those to be vascular input functions and they recognized it's not arterial, but it's at least a concentration in the blood and it should give them a handle on variations from subject to subject. So, if they use that vascular input function, then what they get is, you can see the numbers are much

across the board, much closer to each other and in fact the root mean squared co-efficient our variation is down to seven percent. So, it really does have a big impact. You really do want to use that input function and just to summarize the data that were in the literature up to this year, really they're the only group who've reported reproducibility using the input function. There are ways to do that with the IUC. You just divide the IUC and the tumor by the IUC in the blood over that same time period and it does help out quite a bit. Neither of the groups who have done that did that, but I do make the point that the reproducibility for the IUC. If you use a common input function is actually better than it is for K-trans and that seems to be a common finding reported at meetings as well. So, then the question comes down to, does it work, so we have some idea of the things, the

details you need to pay attention to. Well, if you sweat those details, does it really work? So, here's an example that in fact it does and this is anti-angiogenic therapy. So, this is again targeting the tumor blood supply, but here the idea is you inhibit growth factor support at the native vasculature and that can have some or all these effects, reduce the permeability, reduce the perfusion, reduce the blood volume. Well, we know that the DC MRI should be sensitive to all those. So, here's the study from the Novartis that was published in the Journal of Clinical Oncology last year. This is the drug with a nice long name, nothin' simple to remember, but basically it locks single transduction by VEGF receptor tyrosine kinase. So, this is sort of a standard approach for these anti-angiogenic agents and what they did was DC MRI of course. They took a single slice, these were all

patient's with liver metastases, so they took a single slice oriented coronally so that they could reproduce. So, they positioned it in minimized motion. They used a common input function. They did the measurements at baseline. Day two after they started therapy and then day twenty-eight of cycle one. They also looked at plasma pharmacokinetics so that you have some measure of how much drug actually is the tumor exposed to and they calculated K-trans and IUC as indicators of vascular response. They didn't actually report the IUC and the JCO, but Bruno has reported it elsewhere. So, I'm only goin' to

show the JCO results here. The first thing you look for in these kinda studies is, do you have a biological relationship between the amount of drug that the tumor saw and the vascular response. So, this is the AUC the area under the curves, so this is the pharmacal

kinetic measure of how much drug was the tumor exposed to. So, the higher this is, the more drug it was exposed to and this is percent of the baseline K-trans. Ok, so a hundred percent means no effect at all and as it goes down you see you have a bigger effect. So, there is overall a reasonable relationship between the two and there's actually, you can see there's open symbols, closed symbols and I think those are at, that that information is better summarized on the next slide where they show not only do you have this sort of exposure response relationship, but you also see that the vascular response is associated with a clinical response. Whereas here what they're looking at is a size change at the end of cycle two. Ok, so this is one they figure out whether the treatment is working or not. So, if it decreases by fifty percent that's a partial response. They didn't have any partial

responders, but they did see that they tumors that decreased in size had a lower, it had a bigger effect on the, this is, this axis is the percent of baseline of the K-trans, so the smallness is, this is this way the bigger the vascular effect. So, the ones that had a decrease in size had a bigger vascular effect. The ones that continue to grow had less of a vascular effect. So, that actually gives you some sense that what you're looking at is not only related to the vascular response but also that this is somehow associated with a clinical response and when you're trying to develop a drug, that's a very important piece of information to have. The second and last study I'll present quickly is work that we just presented at ISM or Ammon and Asco this year. This is for a drug developed out in La Jolla. This is again in a phase I trial. So, this is in this case, these are all different kinds

of patients. These are the phase I patients. These are very sick patients, patient's that this is their last chance at anything working for them. So, you have tumors of a variety of types that have failed to other therapy. This is again block single transduction, it sounds just like the Novartis case. It seems like all pharmaceutical companies are after the same type of drugs. Here, we looked at a larger eight to twelve slices within individual input function in each subject, so these were performed at baseline in day two of cycle one and all the subjects in some time points at four weeks and eight weeks and some of the other time points. I know at least one of the members of the audience said Jackson participated in this study and Andy Anderson. So, again, plasma PK performed on day one and K-trans and IUC calculated as indicators of vascular response. So, first of all, I think one

thing that's worth looking at is really, what was the performance? So how robust was this method and where we stand right now it's not as robust as we'd like it to be. Ok, so, there were thirty-six total subjects. We didn't try to do DC MRI on the first cohort. So, we tried to DC MRI on twenty-six subjects and we found that we were able to get reliable data in only two thirds of those subjects. So, there are some problems with the robustness of the technique that we need to continue working on, but we do get reliable results in two thirds of the subjects and when we look at those subjects, here's an example. This is a subject that had a metastasis in the nasal cavity. So, this was a dose, one of the higher

doses. Fifteen milligrams four times a day and this color overlay is the IUC where here it shows the scale. So, red is higher and blue is lower and you can see that actually it starts

out with a large poorly perfused regions sorta of necrotic, but the region that is well perfused gets knocked down at day two, stays down at day four and at day eight and in fact the rest of the tumor just starts to disappear. So, this is actually a partial responder. This has more than a fifty percent decrease in tumor size and if we look at both K-trans and IUC, you see a similar sort of pattern in terms of the overall tumor vascular effect. The big reduction stays down at day four. It actually goes back up at week eight, but that's because now we're only looking at this very small part of the tumor and all this part of the tumor with the low volumes not added in. Again, overall, you want to see an exposure response relationship and this is now a semi-logged plot. We see a nice clear relationship between the exposure to the drug and the vascular effect. We had used a cut

off of great than fifty percent to indicate that in fact it was significant vascular response in that individual patient based on the reproducibility using...not using an individualized input function and these two subjects that have the red circles around them are subjects where in fact we have an objective partial response. So, we haven't, this is a different sorta phase I study than the Novartis study, so we don't really have the data that is in the Novartis plot, but we do see that we have a couple partial responders and in those partial responders, we did have a substantial vascular effect. Having the vascular effect doesn't give you a partial response, but if you don't have that vascular effect you don't get a partial response. So, in summary then, I hope I've shown you that DC MRI provides a sensitive but not a specific indicator of physiological, physiology. If we do it carefully

we can get quantitative information and we can do that treatment effects in hours for the vascular targeting agents the days, dose exposure response is evident and it may in some cases indicate eventual clinical response as early as two days after treatment. Thank you!