

I want to thank Lynn and Bruce for asking me to address this audience, unfortunately I forgot my slides at home, and I have to scramble to put something together. Any how I changed the title of the talk a little bit, perhaps we can have the lights down a little bit, to improve visualization and characterization and radiation treatment of the continuum of cancer stages. You say well what are you talking about, what is this continuum of cancer stages. About 10 years ago Ellen York, a colleague of mine, and others wrote a paper describing the tumor growth and metastasis formation. Basically the tumor grows with a formula called a compression growth initially very quickly then slowing down and during the growth some of the cells could acquire the ability to form metastasis and some of them do actually metastases, and grow into metastasis. Now if after treatment you fail to cure the cancer the tumor can re-grow and also

form metastasis and a mathematical formulation has been developed by Ellen and her colleagues. Now, actually now we understand that there can be heterogeneity within the tumor instead of our previous concept cancers clonal. There can be multiple phenotype and these are indicated by this yellow and blue, and so when I say the continuum of cancer stages it means that it could be the frank tumor that you diagnose or micrometastasis form and other metastasis you could see, and aside from the continuum of cancer stages there's also biological heterogeneity. So now as it turns out that we actually can use radiation to treat the continuum of cancer stages, for example even if you don't see it prophylactic treatment could be delivered right now using chemotherapy, but radiation immunotherapy is one of them, and metastasis right now people are using radiostereotaxic radiation treatment or they are proposal to use so called multi-step approach of

RIT which is delivering the antibody Co and then use a radioactive conjugate to treat them, so they are radiation approach to metastasis, and obviously for frank tumors we have this high dose, high position intensely modularly radiation therapy even differentially deliver dose to different part of the tumor, and lastly we could modify the radiation response with radiation modifiers. So that's radiation treatment and so this is just an illustration for the people in the imaging field that how conformal we can shape the dose region, the red region, is the osteosarcoma, you need to avoid the spinal chord and so the radiation oncology physics has been developed to a fairly refined state if you will. So how will imaging help us to treat the continuum of cancer stages? Well, I think that improved imaging is, could occur in at least two areas. One is improve visualization, just that you can see it better. So that would help us to find earlier diagnosis of

frank tumor and more accurate delineation of the tumor extend and it could help us see smaller and smaller metastasis and perhaps even one day micrometastasis. The other aspect is not just seeing, but characterizing to understand the biology of it, and this biological characterization perhaps is the larger opportunity of the two, and this might apply to all treatment modality as I will illustrate subsequently. Now I think that David Townsend is gonna talk about PET, and Ted is gonna talk about MI, I won't talk about this. So there physical approaches to improve visualization and characterization. Biological approach as I said is probably the frontier that we haven't even entered into, in terms of clinical application. It's defined fact is a fact of radiation response and this could apply to several modality; chemotherapy, targeted therapy, radiation therapy, and then designing methods and probes to interrogate these factors that effect the

treatment response if you will. This is only possible because now what is called, what I call biological imaging is, where as previously we are more likely to want to know about the structural and anatomical information. Now we have multiple parameter that we can understand that include functional, the metabolic as exemplified by FDG PET, physiology blood flow and even gene expression in self trafficking, and I use this descriptive biological to characterize this large body information that can be gathered about the biology of the tumor. Now just as an example of what biological images could be so and how they might help radiation treatment. For example, in terms of radioimmunotherapy, you might be interested in, to see the expression of the antigen, how abundant they are to calculate how many antibodies you need. Now in terms of

IMRT and stereotaxic dose painting, you want to know the dose intensity and that your graphic distribution of the dose intensity to give the most application dose distribution and then lastly in order to apply, modify to radio ratio response it would be nice to know the factors, the phenotype and the genotype that effect radiation response and I coined this term radiobiologic phenotyping, basically the characteristic of the tumor in terms of the response to radiation. Now in thinking about this I quickly realized that there are many factors and this realization is not mine alone. There are many factors that effect radiation therapy and this is a slide that I borrowed from Bert Vandego who we are meeting in Mumbai and basically showing you the diversity of the things that you can acquire in the fruit store in India, which is likened to the variety of factors that effect radiation response. Proliferation, how quick the tumor grows. Tumor hypoxia, the level of

oxygen in the tumor. The repair ability of cancer cells and genes that effect tumor growth and repair and also invasion and metastasis that affect the tumor characteristic. So anyhow, so realizing that one cannot study all aspect of it, we have focused on one aspect of the things that affect radiation response and that is tumor hypoxia. Now this phenomenon has been known for 50 years, Tomlinson and Gray examining some pathological samples in the 1950's came to the conclusion that after ten cell diameter away from the blood vessel, cells become hypoxic, and further away the cell begin to die in necrosis, and so the hypothesis was because hypoxic cells are three times more radio resistant, meaning it requires three times as much doses to kill a hypoxic cell, visa via the oxygenated cells. Hypoxia mediated radio resistant might be one reason why radiation therapy fails in some circumstances. And this is a modern slide showing

you the same thing using a technique called immunohistochemistry, basically using antibody that targets certain things, and here this would be the blood vessel, this would be oxygenated cells, here the brown that you can hardly see are the hypoxic cells and this is just tumor necrosis. So 50 years later we're coming back to the subject that has been advanced previously. So why are we, why is there renaissance in trying to study tumor hypoxia? In a sense it's because the clinical data now. So this is a paper published eight years ago, and it is cancer of the cervix and these authors actually use an oxygen probe and stick it in the cervical cancer and they, subsequently they follow the patient and this is, I can't quite read but this is some sort of survival data of patient that have a oxygenated tumor visa via hypoxic tumor as determined by probe measurement. So this is statistically significant that oxygenated tumor does much better than

hypoxic tumors, and there are many papers now that support this observation. So hypoxia is bad. Now not only is hypoxia bad for radiation they also reported patient that underwent only surgery the hypoxic tumor is bad as indicated by here. So these clinical studies raise some puzzle and

which was subsequently answered in laboratory study and actually laboratory scientists have found the tumor hypoxia not only leads to radio resistance, it also is hypoxic cell resistant to some chemotherapy agent and under this unfavorable environment cancer cells that survive acquire a more aggressive characteristic and are able to metastasize easier than cancer cells that die in the hypoxic environment. So it's an environment sorta like the ghetto. If you survive the ghetto environment you develop this characteristic, which is bad. Now in the meantime some clinical data, laboratory study, using animals and now the molecular biologist that defined the molecular pathway of

hypoxia. This slide just impressed you that I know something about molecular biology, not a lot just enough to get by. As it turns out now this cartoon published a number of years ago illustrates what is given in this schematic here. There is a switch; the switch is hypoxia inducible factor of HIF one alpha. This switch is the key ingredient that senses tumor hypoxia and leads to things that happen. So in an oxygenated condition it would go through an enzymatic action that leads to the degradation of this HIF one alpha, but in the absence of oxygenated this pathway is not available so HIF one alpha is stabilized meaning it generates more and more HIF one alpha and this is called promoter, it promotes something. When this is built up it leads to things, genes, that are turned on subsequently, and one very important one is this VEGF, vascular endothelial growth factor, which promotes blood vessel formation. That's how a tumor grows. It grows so

fast that it outgrows the blood vessels, it needs this pathway to promote tumor growth. So we understand the molecular aspect and the clinical implication and so on, so forth. So it's very important now for us to characterize a tumor in terms of tumor hypoxia and there are many ways, and I will focus on one, but just to let you know they are dynamic NMR techniques and obviously PET techniques to measure tumor, but for as a surrogate of tumor hypoxia. Bioenergetics because tumor hypoxia would lessen the energy supply of tumors, lactate which, lactate acid is a byproduct of anaerobic glycolysis, so lack of concentration of lactate is another surrogate, but I will focus on some discussion on use of hypoxic cells marker, which is exogenous thing that you apply to the patient and using positron emission tomography. So this is one such trace it's copper ATSM and this compound has been pioneered by Mallinckrodt Institute of

Radiology in St. Louis and they in a serious report they indicate that copper ATSM preferentially goes to the hypoxic cells and this is a publication the rare presumably is the high uptake of copper ATSM and therefore the hypoxic region and then if you give more dose to what perhaps you can circumvent radio, hypoxia induced radioresistance. Now several years ago perhaps three or four years ago, my colleagues in the laboratory started to examine three compounds. FMISO, which is pioneered by the University of Washington, IEZG compound which was Don Chapman's favorite initially developed in Canada and subsequently brought to Fox Chase, and Philadelphia and ATSM is the compound that I mentioned from St. Louis. Now this is one of the published data by Zanzonico earlier this year, where we compare head to head this IEZG compound and the FMISO compound to different traces. The major differences they are both a

class called trinitrate _____, don't worry about the name it's a chemical family, but the, but isotope label attached to it I-124 has a four day half-life where as F-18 has only a two hour half-life. So when we image this same animal, injecting FMISO first and image at one hour, three hours, and the tumor is here at the left limb, if you will, and at three hours you can see it quite clearly, but both at one and three hours the visceral organ uptake of this compound is quite high.

So it might compromise a signal to noise if the tumor is close to the visceral organ. Now in contrast because of the four day half-life of I-124 you can wait until the clearance of the compound from the visceral organ at 24, and 24-48 hours, you see the tumor very clearly with almost no background at all. That doesn't mean that in the clinic we can see this or that we are proposing clinical trials to test the efficacy of IEZG visa via MISO. Now we then went to

copper ATSM and comparing with FMISO. We also went to a larger animal, because mice have limitation, you can only grow the tumor to approximately 1 cm, which is inadequate to observe heterogeneity, meaning that nonuniform distribution in the tumor by PET imaging. So we went to a rat where we can grow the tumor to two or three centimeter and in this case we see there is a heterogeneity distribution of both FMISO and copper ATSM and there's some resemblance of the four hour post imaging image of FMISO and the 16 hour post injection image of copper ATSM. We went one step further because, just because something lights up on the screen, just because something is taken up more in the tumor and you think its tumor hypoxia doesn't mean its hypoxia. So we went to do probe measurement and try to correlate images with actual probe measurement and they dramatically aligned manner and this is not perfect. We're developing

better ways that I do not have time to report, but there is a rough correspondence between the high uptake region in the images and low PO₂ level as measured by probe. Now we are going one step further, this is from Jason Couches lab where we are using the same model and template system for stereotaxic image correlation and putting RF coil in there in order to measure lactate, measure dynamic contrast, imaging as a surrogate of tumor hypoxia and basically do multi modality imaging which I have to mention because of the theme of this is dual modality session. Now actually when we start studying this we found there are many problems, many difficulties and I only have time to illustrate one or two. One is a matter of scale. As we mentioned that the dimension of tumor hypoxia is very small, as illustrated here by the green slithers. These are a mock up pneumonitis or exogenous marker and a red blood vessel. So the scale of tumor

hypoxia in, at least in rodent tumors is approximately 100 or 200 micron y, which is ten times smaller than the 1 mm resolution that you can have with dynamic MMR. And this is approximately one sixth of that of the special resolution of clinical PET. So there is a matter, there is a question of scale. How do you relate this image to this fairly ribbon like tumor hypoxia? It's a issue that we contend with, but the physicist obviously would apply a convolution of this pneumonitis image, this fine distribution, and when you _____ it with the resolution of the two millimeter of micro PET you get this image, this what we would want to see in a micro PET, a rodent camera visa via immunohistochemistry, but when you go to the clinic it's another story. So the last thing that I want to talk to is about this support, or so-called reporting, reporter gene image technology. What I've indicated just now is exogenous, you give

something into the tumor and you look at the image. Now what we are trying to do, we are trying to relate to images to the initial event, the molecular switch that is turned on by hypoxia. So how do we do that? You remember that there is a, the factor that I say HIF one alpha, okay. So, we constructed a reporter gene that will response to HIF one alpha. When the HIF one alpha level goes up the cell will generate a signal that we could see. So it's a reporting system, trying to relate the molecular event to the exogenous marker imaging. So this is a fairly complicated slide, but I will walk you through it. Basically you deliver a gene to a cell. How do you deliver

a gene? Well actually you make the membrane of the cell porous okay, and then you throw this viral particle on this cell and because of the porosity of the membrane the cells, the virus start going in and get incorporated into the DNA, okay. Now what is the gene deliver? The genes

that we deliver have a element called hypoxia responsive element that the hypoxia induced effector reacts with. What does reacting mean? When it reacts with it, it turns on this gene, this gene, this herpes simplex virus one TK gene. It's just basically enzyme of a viral origin. Now what is the reason for this enzyme of the viral origin? Well, when this gene is expressed okay, this is called transgene expressing. When this gene is expressed, it will enzymatically reduce a substraight that you put in, you inject a substraight, and the reduction product of the substraight is trapped in the cell, okay. So this is a reporter gene, it's a report of substraight and this gene is only turned on during hypoxia. So it's a reporter system for a hypoxic system, for tumor hypoxia. Now, so this is a factor, this is just a factor with, this is the HIF one alpha enhancer, or the hypoxia response element it will turn on this TK gene. So the next slide is just a data from

the lab that relates the molecular event turned on by tumor hypoxia with exogenous marker with FMISO. Here what we did. We have actually three tumors here. One is a parental, meaning that cells that did not have this reporter gene put in. There is a positive control where the gene is under another promoter that's turned on another time. And a third tumor, which is responsive the gene is only responsive to tumor hypoxia and these are obviously controlled. A positive control, a negative control and this is the test. So into this tumor, into this animal we injected FMISO 2,000 millicurie and this marker substraight 200 millicurie. The reason for this ratio is that we want; we want this to overwhelm the signal initially. So here is the three hour image, and this, and you see there is all three tumors light up because all three tumors are hypoxic and they all take up this exogenous marker, okay. Now if you wait 24 hours, this F-18 has decayed

away, it's dominated by this and now the positive control lights up like crazy. The parental no reporter gene is, there's no signal and here the hypoxia response element gene shows uptake very similar to the exogenous. So this is a way to relate the molecular event with exogenous imaging, but with markers. So, now I've two more slides and our enthusiasm for this sorta work is heightened by a abstract that was submitted into ASTRO in 2004. Basically this group of investigators use FMISO to find out whether the tumor is hypoxic or not, and then they also used chemotherapy, or a chemotherapy agent, plus a hypoxic cell toxin, okay. So concentrate on the purple curve and the green curve. These are the hypoxic tumors. The purple and the green. The purple is hypoxic tumors that were treated with the hypoxic cell toxin and this is hypoxic tumors not treated with hypoxic and this is tremendous difference in survival. So this give a hint that

hypoxia imaging maybe very useful to chose the appropriate modality for treating a particular patient. And so I give you a glimpse of the work in terms of trying to have biological characterization of a tumor with example, concrete example as to one parameter that effect radio response and how we can subsequently use this information to improve cancer care. Thank you very much.