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Good morning, everybody. Thank you so much for joining us here this morning. We are super excited to kick off The first annual symposium of our Center for Cancer Systems Biology.

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Which is a NCI-funded center joined between MIT, Dana-Farber and a few other institutions that we will meet today during our morning talks.

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My name is Francisco Miguel. I'm a professor at the Dana-Farber Cancer Institute at Harvard.

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And have the great pleasure to co-lead the center with Forrest.

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Who will also be our first speaker and introduce us to the overarching goal of the center.

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As far as this research. Forrest holds a bachelor's degree in chemistry from Framingham State College and a PhD in analytical chemistry from Florida State.

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He then did a postdoc at the University of Virginia. And left for industry, but luckily for us, came back and has been at MIT since 2003, where he is now a professor of biological engineering.

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And the only downside is that he is not an Antonio because all others in the morning session are Antonio, so he is an honorary Antonio for this morning.

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And we very much look forward to your talk.

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All right. Yeah, so thank you all for coming this morning. I know it's kind of early.

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I'll get started with a quick overview of our center. So I won't go through the whole name because it's rather long. I think we just decided to throw everything together into a single name. But we have really two projects. And the center is really focused on understanding the interactions between GBM tumor cells and neurons.

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As well as GBM tumor cells and immune cells in the tumor microenvironment.

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And then ideally, sort of bringing all this information together to try and understand the interaction and the dynamic evolution of GBM tumor with its microenvironment, including the neurons and immune cells.

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The center is composed of Francisca and I as the co-PIs, and then we have some awesome, talented people, including multiple of the people you're going to hear from today as our co-investigators in our center, covering a range of different topics.

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I'd also like to just give a quick shout out to the National Cancer Institute. So Hannah, Monica, and Shannon are the program directors and deputy director.

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For the Division of Cancer Biology. And they've been fantastic supporters as well as giving us guidance for our program.

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Most importantly, I'd like to call out and give a quick shout out to Isadora, who's sitting in the back so she doesn't get embarrassed. I think in the other room maybe. But anyway, Isador put everything together today and tomorrow for our site visit for NCI and has just been phenomenal. Getting everything set up.

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Okay, so I won't belabor an introduction to GBM. Just quickly, it's one of the most common and most deadly form of adult brain cancer.

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Survival is relatively limited and treatment is really focused on resection, radiation, and chemotherapy. And unfortunately, as you'll hear probably a couple of more times today.

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That treatment paradigm really hasn't changed much over the last 20 or 30 years.

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And there's been just dozens of failed clinical trials in this disease.

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And so a couple of the challenges that I'm going to sort of hit on today, one of them is the invasive disseminated cells.

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So these are the cells from the GBM tumor that crawl into the normal brain and invade the normal brain.

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And not only the brain around the tumor, but also evade across the hemisphere to the other side of the brain.

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And these disseminated cells basically drive recurrence after surgery. And often lead to that recurrence being fatal.

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And so what we'd like to be able to do is to actually characterize and target these disseminated cells that lead to recurrence if we can.

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But it's been super challenging because identifying and isolating these disseminated cells when they're hidden behind billions of neurons is really, really challenging.

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The other very challenging aspect of GBM is that it's incredibly immunosuppressive. It's a cold tumor, meaning that there's not a whole lot of mutations present, and there's minimal T cell infiltration into the tumor.

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But a lot of this is driven by these glioma-associated macrophages and microglia, the GAMs. And these GAMs basically drive this immunosuppressive phenotype, secrete cytokines that drive away T cells, and really limit the efficacy of immunotherapy in GBM.

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So we'd like to be able to tackle these and talk about these sort of in order today.

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The first one is the invasive and disseminated cells. And so if we look at a couple of tumors, GBM6 and GBM12, these are PDX tumors, patient drives intograph tumors grown in mice If we look at the opposite hemisphere, you can see there are little tumor cells that have crawled over and have invaded that opposite hemisphere.

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And are hidden behind, like I said, the neurons and normal brain

tissue.

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And in GBM 6, if we look at this region here, we get about 5% of the cells that might be tumor cells in GBM 12, we have a very small amount of cells that are tumor cells that have invaded through.

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And the challenge really is isolating and characterizing these cells, ideally while preserving their physiological signaling state. So we can't just associate the tissue because that changes the cells as we dissociate the tissue. So what we need to do is find some way of sort of fixing that state of the cells in order to isolate them and identify them.

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And so, Yehen Ahn, one of my former postdocs in the lab, recently developed this technology that she's calling Insight.

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And it's a way of basically being able to isolate and characterize particular cell types from tissues.

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For downstream phosphoproteomic analysis. And so the way that this works is we take out the frozen brain. So we take out the brain, freeze it. We can then cut it into hemispheres if we want. We can section those hemispheres on a cryostad and then drop these sections into formalin to fix them.

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And this basically preserves the physiological state of those cells because they've been frozen or fixed the entire time.

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We can then dissociate them into single cells and then sort by cell types on a cell sorter to isolate out particular cell types that we're interested in. We could sort by CD45 positive cells to get the immune component.

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We can sort by EGFR status to get the tumors. We can then lyse those cells and then basically process them through our standard MassFact protocol to enrich for phosphopeptides and then quantify phosphopeptides from these various cell types.

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And then at the end, we get basically peptide identification, including the phosphorylation site on the given protein, and then quantification as to how much it changes across the different samples

that we might be interested in.

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All right, so Jen applied this to… GBM6 and GBM 12 in collaboration with Jan Sarkaria's lab, where they injected GBM6 or GBM12 into the right hemisphere, wait 24 to 28 days, and then basically take out the tumor and take out the brain and freeze it. And then we cut it into halves, either the left hemisphere or the right hemisphere, and then dissociate and enrich

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For EGFR positive cells from either the left hemisphere or the right hemisphere, EGFR being a marker of GBM for these particular patient-derived xenografts.

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What this leads to is 54 populations from GBM6 and 18 populations from GBM 12.

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And allows us to compare the The tumor cells to the non-tumor cells and also the disseminated tumor cells to the tumor cells from the primary or the core tumor.

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One of the big challenges here is that when we look at tumor cells from the distal hemisphere, we're getting on the order of tens of thousands or just thousands of cells of tumor cells from that distal hemisphere.

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And we really want to be able to characterize this small amount of cells from that opposing hemisphere. All right, as we started analyzing the data, what we see looking at either the proteome or the phosphoproteome of tumor versus non-tumor is that the tumor cells are basically lit up for EGFR signaling, as you might imagine.

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The non-tumor cells have a lot of sort of classic markers of brain biology, including a lot of oligodendrocyte precursor and oligodendrocyte markers.

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We can take the phosphoproteins that are enriched in the tumor and then load those into the kinase library and predict which kinases are active in the primary tumor. And one of the ones that comes out is EGFR, as you might expect, as well as a bunch of cyclin-dependent kinases and casein kinases in a variety of other Banks that are known to be involved in tumor progression.

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So it gets a lot more interesting when we look at the disseminated cells versus the primary tumor cells because sort of for the first time we can start actually characterizing the signaling networks in these disseminated cells.

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And so one of the things that you might notice looking at the protein or phosphoproteins is that the disseminated tumor cells aren't that much different from the primary tumor cells. They're still tumor cells.

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They've just crawled away from the tumor and are now surrounded by neurons.

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So even though they're fairly similar, there are some differences. And we can start looking at what those differences are by using gene set enrichment analysis, for instance.

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And what we see is actually upregulation of neuronal systems, neuronal signaling, transmission across chemical synapses. Basically, these tumor cells, as they crawl away from the primary tumor and go into the normal brain, upregulate processes so that they're able to communicate with the neurons.

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And this neuronal communication can also be seen here as we look at particular phosphorylation sites that are enriched.

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And what we see is, for instance, this Gria2, which is a glutamate receptor. You can see the protein level doesn't change, but the phosphorylation level goes up fivefold. It's much more active in the background of the neurons in the contralateral hemisphere than it is in the primary tumor. If we look at CAM kinase 2 or this sodium potassium transporter, again, we see that the protein level hasn't changed.

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But the phosphorylation level has gone up significantly, indicating that these proteins are actually much more active in these disseminated cells compared to the primary tumor.

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Interestingly, we can go in and characterize this as well by immunohistochemistry. So here we have an antibody that recognizes this tyrosine phosphorylation site on the Gria2.

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Glutamate receptor. And you can see by these little green dots here that we're getting these sort of punctate foci.

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On the tumor cells, if we quantify the punctate foci in the tumor core versus the tumor margin versus the left hemisphere, you can see an increase in the number of punctate foci as the tumors start crawling away.

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So one of the other sort of interesting findings that came out of this is this protein called Hornerin.

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And it's actually a pretty poorly characterized protein. There's only a couple of dozen papers on it. But Horner is upregulated about sevenfold in the disseminated cells compared to the primary tumor. And then it has multiple phosphorylation sites that are all upregulated.

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At least twofold up to fourfold in the disseminated cells compared to the primary tumor.

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And so one of the questions that we asked is, what is Hornarin doing in these disseminated cells? Because it was one of the strongest hits that came out of our analysis.

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And so to figure that out, we did a short hairpin knockdown of Hornerin in GBM6.

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And you can see we use two different short hairpins here. We're able to effectively knock down Hornerin levels.

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That also knocks down invasion of these cells in an in vitro assay.

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We can do the same thing on LN229, which is a classic GBM cell line. We can knock down Hornerin. And again, that leads to a decrease in invasion.

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What's actually really encouraging here is we then take these knockdown cells from GBM6, we inject them into the right hemisphere.

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And then we look at how the tumor grows over time. And you can see that our tumor volume has dropped by about 80% when we knock down Hornerin.

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And I think this is due to the fact that the tumors are not able to invade and push away that normal brain and so are really limited in their growth potential to be in that one spot.

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And so through this characterization of the disseminated cells, we can now start highlighting particular kinases that are active in the disseminated cells compared to the primary tumor. And we can start characterizing what makes these cells disseminate compared to the primary tumor Including, not surprisingly, things like more mesenchymal cells. They've upregulated neuronal precursor-like signals. And the primary tumor is really driven predominantly by cell cycle regulation and proliferation because these cells are rapidly growing where the cells that have invaded are more migratory and invasive.

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All right, so the cool thing about this approach is that not only can we look at these disseminated cells, but we can also start quantifying the treatment response in those cells.

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And so as most of you know, a lot of the drugs don't cross the blood-brain barrier very effectively.

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And so one of the challenges has been that we're typically looking at the tumor, but not necessarily characterizing these disseminated cells that lead to recurrence.

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With this approach, we can now quantify how these disseminated cells are responding to different therapies, including temozolomide.

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We can also look at treatment response across basically any tumor type. So looking at not just the tumor, but also how the various treatment response might affect tumor cells compared to immune cells or neurons.

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And then lastly, we're starting to apply this approach to breast cancer metastasis, where we can start isolating, and then quantifying and characterizing those metastatic cells in the lungs or liver

following breast cancer tumor formation.

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All right. So in the last couple of minutes here, I just wanted to talk about the other aspect of GBM that's been particularly challenging, which is the immunosuppressive nature of GBM driven by these glioma associated macrophages.

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These GAMs comprise about 30% of human tumors, and they drive tumor progression, therapeutic resistance, and immunosuppression.

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Ideally, what we'd like to be able to do is to target these innate immune cells and wipe out some of these GAMs, which would lead to Slower progression, better therapeutic response, and also immune response rather than immunosuppression.

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And so one of the ways that we've been doing this has been driven by Yufei Kui.

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And Yufei is a very talented grad student in the lab, and she's been looking at MHC peptide presentation on the tumor cells or on the macrophages.

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And then seeing how these MHC peptides, which are presented to the immune system and then signaled to the immune system to attack the tumor, for instance.

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How these MHC peptides change as the tumor and macrophages co-evolve together.

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And so we can take the tumor cells by themselves, or we can take the macrophages by themselves and analyze their MHC peptides, or we can put those tumor cells and macrophages together, let them co-evolve for a few days, and then analyze how they have changed on their surface expression.

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And ideally, what we're looking for, this is the worst acronym ever, by the way, Eufei, are co-culture-induced very significantly peptides or civis peptides. And so we're looking for these peptides that are upregulated by co-culture.

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And the first thing she's done is to sort of characterize that when the macrophages interact with the tumor cells, they change their state and they become more TAM-like or tumor-associated macrophage-like.

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When they're interacting with these tumor cells. And so they upregulate various signals associated with sort of an anti-inflammatory response. And then if we look at the immunopeptidome on these macrophages.

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One of the interesting things is that as the macrophages interact with the tumor cells, they change what is being presented to the immune system on the surface of these macrophages. And there's multiple peptides that increase in response to co-culture with either CT2A, which is a more aggressive send genetic cell line for GBM or GL261, a less aggressive syngeneic cell line for GBM.

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We get, in both cases, we get upregulation of MHC peptides on the macrophages that are associated with cytokine signaling in the immune system.

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And interferons and interleukin signaling, sorry. Euphay's then gone in and quantified these peptides using an approach that we call Shurquan MHC.

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This allows us to go in and get accurate quantification of these peptides and not only quantification, but actually copies per cell. So we can now start identifying whether or not these peptides might be targets for either bispecific T cell engagers where you're in the thousands of counts per cell or maybe TCR T cells or CAR T cells when you're in the tens of copies per cell.

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And so using this information, we can start identifying sort of the treatment modality that we might want to use to target some of these macrophages after they've interacted with tumor cells.

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And then she's also gone in to quantify basically how the tumor cells are changing when they interact with macrophages.

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And what's perhaps more surprising is that the tumor cells respond more strongly to this interaction with macrophages than the macrophages do with the tumor cells.

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And so what we see is actually a pretty strong upregulation of a variety of different peptides after these tumor cells have interacted with macrophages.

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And what's being driven here is this signaling by row GDPases. Rho GDPases basically drive migration and invasion.

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And what's interesting is as these cells are interacting with the macrophages, we're getting upregulation of these processes associated with migration and invasion.

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So we can again go in and quantify selected of these peptides by our short quant MHC method.

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We get up to a 20-fold upregulation of particular peptides after they've interacted with the macrophages. And these are actually the ones that we want to start targeting because these are the peptides in the tumor that have been upregulated by their interaction with macrophages. Okay, so can we start targeting those peptides? What Eufei did next was to identify a handful of these peptides that were presented on either the civis peptides, sorry.

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That were identified on either the tumor or the macrophages. And then in collaboration with Daryl Irvin's lab, generated an mRNA vaccine to try and target these, get the immune system to target these peptides.

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And so using an mRNA vaccine injected on day one or day one and day 15 or injected on day 8 and day 15. So we tried two different methods.

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Of trying to stimulate the immune system to attack these tumors. In both cases, what we see is a nice increase in LE spot formation. We get a nice infiltration of T cells into the tumor.

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And it looks like we're able to generate an immune response against these particular peptides.

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Unfortunately, this leads to a very slight improvement in or a slight decrease in tumor growth rates, but no overall improvement in survival.

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And so one of the things that UFA is working on now is trying to understand why these T cells, when they infiltrate into the tumor, are not necessarily reacting to try and kill the tumor. Are they becoming exhausted or what is the mechanism by which they're basically failing to attack the tumor?

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Perhaps the most exciting thing about some of this work is that we've now gone in to look at human tumors and in collaboration with Kenny Hu at Memorial Sloan Kettering, he's resected a couple of patients now and then taken that tumor and divided it up into CD45 positive macrophages.

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Or CD45 negative tumor cells. And what we see is that the CD45 positive macrophages actually have signaling by interleukins and cytokine signaling upregulated on their cell surface.

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Whereas the tumor cells have row GTPase and signaling by Rho GTPase.

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And in some cases, we actually see the same source proteins represented on our mouse model as we do on these human tumors.

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Which suggests that if we can figure out a way to actually tackle and target these particular MHC peptides.

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Then we actually might have a way of moving some of these forward into human tumors.

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So this would give us a way of trying to basically eliminate the macrophages and attack the tumors at the same time.

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Okay, so the work I talked about today was really done by two really talented people in the group, Yufei Kui and The rest of the white lab shown here provides awesome support and a lot of other really cool projects. Most of this work was done in collaboration with Jan Sarkaria and as

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It's been supported by a variety of funds, but especially the NCI Cancer Systems Biology Consortium.

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And lastly, I'll just give another shout out to Isadora, who helps with everything associated with our group and helped organize the symposium today.

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Cool. Any questions?

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If you have any questions? First of all, thanks.

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That's very cool. I have a question on the… Any plans to actually look at sort of imaging lattices, so use the mouse in the 7T or 14T and look at DTI, DSI imaging.

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And or angiogenesis because eventually you'll need that to put the models up, right?

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It's a good question. So sorry. So basically, imaging supported tissue lattices that you need for computational modeling later on if you want to go to discrete because that's where you need to actually go to these. Yeah, yeah.

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Yeah, it's a great question. And so we've started working with Natalie Agar using her cyclic immunofluorescence platform to try and quantify the spatial localization of different cell types.

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Not only in the tumor, but also in the tumor microenvironment and in surrounding these disseminated tumor cells to try and understand sort of how they're being supported, like you said, with endothelial cells or with vasculature. One of the interesting things that I didn't get a chance to talk about today is that when we see these disseminated cells.

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If we see them as either individual cells or as a small cluster of cells.

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Their phenotype and some of the characterization has changed between the single cells and the small clusters of cells.

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For instance, single cells have really down-regulated most of their EGFR, whereas as they start growing in a cluster, we start seeing EGFR levels come back up, which is kind of fascinating, but it makes sense

as they go into more mesenchymal state, they downregulate EGFR. But then as they start to proliferate.

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Egfr comes up. And so I think combining some of our work here to identify potential targets and then look at them by imaging is really a critical component.

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Oh, she's better. I'm the gaps.

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Great stuff. I have two questions. One that actually just came up from your last response, which is that you said that the clusters of cells had different phenotype than the single cells.

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Have you looked at associations with immune cells in those cells and are they different? And then related to that, I don't know if I misunderstood it or if the slide was labeled incorrectly, but on your slide where you're showing data, CD45 positive versus negative tumor cells.

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What you said was CD5… CD45 positive and negative was reversed. And either way, it's really interesting data, but I'm just curious. Yeah, no, great question. So we haven't gone deep enough into the imaging yet to really start characterizing the immune cells around these disseminated tumor cells. But I think that'll be fascinating to try and get a deeper understanding of what's happening there. On that slide, it was actually

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Reversed. So the CD45 positive cells have ROG DPase, and CD45 positive cells have tumor cell, CD45 positive cells are the macrophages.

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And those have upregulated interleukin signaling and cytokine signaling. And the CD45 negative tumor cells have row GTPAs.

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Yeah, yeah. Of course, great work.

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I wanted to go to the experiment with your small GL261, the vaccination experiment, where you did not actually see increase survivorship and you thought that maybe the T cells are exhausted, but you're looking at that.

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I guess the surprise is because that model is pretty immunogenic. You know, you can You can get survivorship fairly easily. So I'm just running. So I think that may give us an opportunity to really look at it.

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Immune evasion in a model that should be fairly immunogenic. And the question, do you think it's just T-cell exhaustion?

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Yeah, I think… Could it be something that's changing with the myeloid cell population? It's a really good question because… I thought we'd be able to do something with GL261. And unfortunately.

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We're not. And I think the big question that comes out of this is the antigen selection.

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Process. And so one of the things that we've been chatting with a variety of different immunologists about is once we start identifying these MHC peptides and we're thinking about making a vaccine against them.

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Should we be picking the peptides that are very abundantly expressed?

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Should we be picking peptides that are sort of in the middle or should we be aiming for some peptides that are very low abundance? And the difference being that as the T cells come into the tumor, if we're, in this case, we pick peptides that were pretty abundant.

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As the T cells come into the tumor, they see target everywhere. And that's a signal for them that this could be self and therefore they would get exhausted pretty quickly.

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On the other hand, if we just go for super low abundance targets, maybe we don't get a response. It's actually pretty challenging to know sort of what's the right range here. And unfortunately, this is a part of immunology The immunologists tell me they don't know. So it's not like this is a well-characterized thing. So I think one of the things going forward is trying to figure out like.

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As we're targeting these MHCs. Which range do we go for and then which particular peptides are the right peptides to target.

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And then we haven't been combining this with anti-PD-1 or some of the other checkpoint inhibitors, but we could try.

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Activating the T cells with a variety of different mechanisms here.

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Okay, so we have two comments online. Rakesh Jain. Hi, Rakesh. Has a comment, another mechanism GBM serves use to migrate to the contralateral hemisphere.

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Is a paper that says that there's a glial signature in Win7 signaling that regulates glioma vascular interactions in the tumor microenvironment, a cancer cell paper.

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Cool. Thank you. And then we have a question from Divya Sinya who is asking, sorry if I missed this, does the TAM and or TME downregulate MHC1, thereby inhibiting this cytolytic activity downstream.

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Yes, that's a great question. In our data so far, we have not been seeing significant down regulation of MHC1 in vivo.

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That doesn't mean it doesn't happen. It just means in our system so far, these cells don't particularly downregulate MHC1 in vivo.

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Excellent. And then, in fact, I can ask another question. So you showed great data co-culturing the tumor cells with macrophages.

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What about co-culturing more widely with different subsets and frequencies of different subpopulations? So not just macrophages and tumors.

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Yeah, it's an awesome question. I think moving forward, adding in dendritic cells, adding in T cells, trying to make more complex multicultural system, if you will.

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Will be important, but also very challenging. It's challenging because some of those cells are adherent, some of them are not adherent. It's interesting. Making a diverse multicultural system like that in vivo is sort of automatic, but doing it in

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Plates in vitro is actually much more challenging than I thought it would be. And also, one of the biggest challenges is Those cells tend to grow at different rates. And so even with the tumor cells and the macrophages, as we add those two together, the tumor cells continue to divide. The macrophages don't. And so over three days, as the tumor cells grow out and the macrophages don't, we basically shift our tumor to macrophage

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Ratio, and this would be similar if we had DCs or T cells or anything else. And so we have to figure out a little bit like what are we aiming for and where do we start in order to get to that point.

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And also the spatialization then changes, right? And who knows what that influences downstream.

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Do we have any other questions?

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Okay, cool. And thank you first.

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All right. Next, we have our first antonio. First but not last Antonio. We have Nino Kyoka. Maybe I'll go forward with the introduction while you set up.

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He has served as the neurosurgeon in chief and chairman of Department of Neurosurgery at Brigham and Women's.

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And as the Harvey Cushing Professor of Neurosurgery at Harvard Medical School since 2012.

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Prior to these roles, Dr. Kyoka held assistant professor and associate professor of faculty positions at MGH.

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And in 2004 became the first chairman of the newly developed Department of Neurological Surgery at Ohio State.

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He's a graduate of the University of Texas Medical School and completed his neurosurgery residency training at MGH.

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But I would be admissed to say, to not say that he is European and grew up in Parva.

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And with that, we're very much looking forward to your time.

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Thank you, Francisca. Great introduction. I appreciate it. Thank you, Forrest.

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For starting this great symposium I love the fact that you have a systems biology group here on glioblastoma, this awful disease. So I'm going to tell you the story of a true academic bench to bedside.

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Approach for a particular compound. So this is a glioblastoma clinical timeline.

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So Forrest gave us a great introduction to glioblastoma. I have the date there, 2025. That has not changed in several years.

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This is what happens fairly routinely. And I know the neurosurgeons in the audience know this, so please forgive me. But this is one of my patients, mid-40s.

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Cpa who had an episode of speech arrest A father of two beautiful young girls. I saw him in the office and he has this mask and as you can see here in his In his left temporal lobe, left frontal lobe close to the speech area. So what's usually done in the United States, in fact, in most countries in the world, is to take this out by surgery. So we're taking the surgery, take this out.

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And then he undergoes chemoradiation. And then it gets treated with adjuvant temozolomide. You can also give optoon, which is another device that one can give and it's FDA approved.

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But unfortunately, this is very typical. Within six to nine months, the tumor occurs. Most of these tumors recur locally. They don't metastasize. They usually recur in the surgical bed, like in this case, around the ear.

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And so what do you do now? Now this is when patients can undergo more surgery. Potentially they can undergo more radiation.

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They can do a clinical trial. In this case, we took them back to surgery because he was still performing really well.

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Take this out again and then started a clinical trial. But unfortunately, this tumor just recurred within a few months. And you can see now at this point it's become unresectable. It's across the corpus callosum.

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As Forrest shows, the tumor is invading. It's basically going throughout the brain.

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And of course, she passed away within a few months from this. So he lived, what's the median survival for these tumors, which is about 15, 16, 17 months.

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So… The median survival time for initial diagnosis is reported to be 15 to 20 months. This really varies based on genetic and demographic markers. For example, if you have a methylation of the MGMO, MGMT promoter, you can actually diminutive survival is up to two years. If you're older, you live less. If you're younger, you live longer.

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But we really have not made much of progress in terms of media survival.

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We know a lot about these tumors. These were one of the first tumors to be sequenced to the TCGA approach. So we have a lot of targets.

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But the clinical trials of more targeted therapies or even double targeted therapies have really failed There was a lot of hopeful anti-angiogenic therapies. Those that failed.

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And even the more recent clinical trials of immune therapy that have been so successful But a lot of the solid cancers, especially the immune checkpoint inhibitors, failed with this tumor.

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So that is what I want to discuss is the failure of these immunotherapies.

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So very simplistic. One of the problems has been that these tumors are the prototypically immunologically cold tumors. That means that they're scares of lymphocytes. We see very few T cells or tumor attacking cells within the tumor.

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And these tumors are full of myeloderized suppressor cells and immune evasion particles. And so even when you're trying to make a T cell less exhausted, the T cell is just not getting to the tumor to attack the tumor itself.

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And so we've approached this by trying to change the microenvironment of these tumors. And so there have been several clinical trials that we and others have done trying to change the microbiology of these tumors from a microenvironment that's full of immunomyelosuppressor

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Mild cells and very few reactive T cells. By intralesionally injecting either oncolytic viruses or gene therapies that express potent cytokines like IL-12 and others. Or some people have also now starting to put some anti-tumor gene proteins on scaffolds that will just stay within the tumor itself.

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And with this, we're hoping to change this microenvironment to one that's Last time you react in immunosuppressive myelose cells and lots of immune reacted T cells.

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So for the people in the audience, if you want to go to sleep, this is my talk right here. This is what we're trying to do.

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Go to sleep now. So… So the compound I'm going to tell you about, we started working on in my lab over 20 years ago.

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And it's based on an oncolytic virus. This is a tumor-selective virus.

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And the original mechanism of action of these oncolytic virus was thought to be due just to direct cytotoxicity.

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The virus infects a tumor cell, it kills it, and then propagates this infection, bi-distributes and kills the entire tumor.

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That's what we thought we were doing. But it turns out that because

there's a pathogen, there's also innate inflammatory responses that occur And so you have macrophages that get simulated, lots of cytokines, lots of interferons, and a lot of these have

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These inflammatory signals have anti-tumor effects. In fact, in some cases, they even have anti-angiogenic effects.

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And so now we know that a lot of the mechanisms more than cytotoxicity is this activation of innate inflammatory responses.

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But even recently, there's this thought that as you get this inflammation going into this immunosuppressive tumor.

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This tumor now becomes much more propitious for infiltration of T cells, but also recognition not just of viral antigens.

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But also two more antigens. And through this process of epitope spreading, which I think is the only greopful of immunotherapy, every immunotherapy you see up there, peptide vaccines.

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Viruses, gene therapy or CAR T cells. Is counting on epitope sweating to get more T cells educated against other tumor antigens.

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So we're hoping to get an adaptive response against tumor antigens with each tumor.

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So… This young coronavirus we designed in our lab. So this slide shows you about 25 years worth of work.

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And this is based on herpes simplex virus type 1, a common pathogen that's present in human population.

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And there have been several ERP simplex type 1 oncolytics. One has been approved for melanoma.

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A different one has been approved for glioblastoma in Japan. And we designed this, and it's a little different from the ones that have been approved.

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I'm not going to go into the specifics, but basically we're expressing one of the viral genes called ICP345.

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That's responsible for inflammation. This really does a lot of inflammation.

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But instead of having this inflammation occur throughout the brain because herpes is neurotropic.

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We restricted this inflammatory insult to the tumor because we put under control of a promoter for the intermediate filament called nastin.

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Which is highly expressed in gliomas, glioma stem cells, but the adult human brain is not expressed.

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So now we have this inflammatory gene, basically. That's expressed primarily in the glioma. So we want to call that gliomitis.

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But not expressing neurons in our brain, so we're not causing an encephalitis.

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And… this virus can be turned off for antirepatics.

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So we… did from a lab.

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Got IND enabling studies. Got an ID with the FDA, all done academically.

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And then started this. Phase one clinical trial in patients with current agricliomas.

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That's just GBM. We also had some patients where IDH mutant gliomas that were grade four as well as some recurrent anaplastic owls. But the majority were GBMs, glioblastomas.

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So we first did what's called a dose escalation trial, where we did a

single injection. We're not giving this IV, we're giving this by direct intratomoral injection.

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Of this virus called, we now call it CAN310. What you'll see in these slides, I'll call it herpes, oncolytic, CAN31-0, sometimes it says arcunestin. They're all synonymous. They're all the same thing, okay?

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And so we went from a dose of 10 to the 6, 1 million viral particles, all the way 10 to the tenth.

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We did 30 patients. We did not see toxicity. Actually, this was really well tolerated. Most patients went home the next day. I actually had some patients fly in and fly out.

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So really well tolerated. And we got all the way up to 10 to 10th platformer units.

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After this, we dose expanded. We treated another 12 patients at a dose of 10 to the 9th, a little bit less than 10 to the tenth, just because I did not have enough vials to do additional patients at this high dose.

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And we finished this phase in 2021. This was published in 2023, this clinical trial, and that's actually what I will discuss, the results of this trial that was published.

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And this we call the RMA. We had a second arm called RMB where we actually pre-administered cyclophosphamide to immunomodulate the patient I will really not discuss that in this paper is in preparation.

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And then through this consortium called Breakthrough Cancer. Michael Chima Forrest participated in this. They've really provided us the funding to do a serial injection and serial biopsies over four months for an additional 12 patients. So in these 12 patients, we're doing

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Time-dependent injections and at the same time get several CO biopsies.

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So we can see what is happening in these tumors as we treat the

patients.

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And so far, we've accrued eight out of total patients for this. And the analyses of these, we have over 400 biopsies. The analyses of these biopsies, CO biopsies are undergoing right now.

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So I'm going to show you a video because I told you patients tolerate this really well. I'm going to show you a video of a patient, and she's going to tell you her story with this trial. In the middle, it breaks and goes to another patient and it's actually your daughter that's speaking.

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Because that patient who is her mom did extremely well, but unfortunately passed away two years after treatment in a car wreck. She was actually not the driver. The husband was the driver. But her daughter will tell you the story about her mother's experience.

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This video was shown on a local TV channel and they named me, so it's my five minutes of fame.

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But really what's important is the patient and the patients.

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The audio was working before.

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We tested before and the audio worked.

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Is that a problem.

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Let's see if that works.

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No. All right, we can just skip it.

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I'm just going to skip it.

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Okay, so this was really well tolerated. The first 42 patients in that arm 30 plus 12.

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No, it does limit accessories. This was a phase one, so it was not a phase one.

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To look at safety, but Like every phase one trial, we were looking for any tidbit of information that we could get from these patients to see if there was anything any demographic marker.

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Any cellular mark or any genetic marker that could tell you which patients did best.

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So one of the things we were collecting prospectively for antibodies to HSV-1. As I said, this is an HSV-1 based engineered virus.

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And patients… But in the human population, there's about two-thirds of us have antibodies to HSV1.

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So interestingly, when you look at the seropostitial patients. We're actually surprised to see that the patients that were seropositive And that's the curve in blue were the ones that did best.

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The medial overall survival time was over 14 months. For recurrent GBM that's not resected, in other words, we were just injecting the GBMs.

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The medial overall survival time is more in the seven to eight month range.

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So we kind of doubled that. And when you compare it to the HSV seronegative patients, the patients that did not have antibodies, the universal viral time was seven months, which is exactly what the historical median survival time was.

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So we were surprised by that because the Oncolytic herpes virus that's been approved for melanoma in the US and the one approved For GBM in Japan did not show this.

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Actually, if you had antibodies, you did a little bit worse probably because people think that the antibodies got rid of the virus.

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But it was not statistically significant. So that was kind of an

interesting finding.

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So then I said, well. Maybe there's something different about these patients. Maybe these patients have something else.

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And so we did these parametric cost proportional hazard ratios to see all the potential prognostic factors that could differentiate this population to this population.

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And even when we did this, the serology to HSV-1 showed here.

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Was independently as an independently significant factor for survivorship compared between HCC1 positive and negative.

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Age was significant too. The size is too much significant to, but all the others were not.

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So this is interesting. So serology, if you're seropositive, you did better.

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It seems to be an independent prognostic factor for that.

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The next question I asked, well, maybe there's something else. Maybe it's not this. Maybe it's something else.

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So luckily, we were also collecting antibodies to a related virus, HSV2, as a control. I don't know why we were doing that. I just had put it in our protocol.

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So we collected this antibodies, so we checked to see If zero positivity to HSV2 had anything to do with this, it did not. Actually, the patient ratios are positive probably did a little bit worse, but this was not statistically significant.

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And then at the same time, Steve Francis, who's an epidemiologist, genetic epidemiologist at UCSF, published a paper in oncology We had a large data set of gliomas and glioma patients and was looking at Sheer positivity to a number of different viruses. Now, these are just a general population. They're not doing this trial. They're doing

something else.

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And they published this paper showing the HSV1 serology is not associated with GBM overall survivorship.

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And so this seems to be very, very specific to this particular compound, CAN310.

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So this is interesting. We'll have to prospectively validate that to show that this is really true in another trial But the second thing that was most interesting to me was the fact that we really tried to get back tissue. So this is a sort of a timeline of what happens to a patient. So these are all recurrent GBMs. Sometimes they're second, third, or fourth recurrence.

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So at time zero, we would inject the virus into these tumors.

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And then sometime in the future, these tumors seem to recur.

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That more enhancement. Maybe the patient was doing clinically worse. So really strive to get back tum either by surgery. In a few instances, the patients donated their brain, so we also had autopsy tissue.

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And we also could collect blood. At all these time points.

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And that could do all of these complex multi-omic analyses to see whether there's anything that correlated with outcome.

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And so at the beginning of this, I told you that when we put a virus in here, we change the microenvironment.

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Is that true? Most of what I told you is based on mice. Is that true in humans?

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So the first question we ask, is your evidence after injection that you see more CD8s, more CD4s, more B cells in these GBM infiltrates.

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Second, we're comparing before injection to sometime after injection. So you can do this visually.

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So this is a patient before treatment, patient 16 here. Before treatment, and then I think she got resected like four months later.

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And what you can see is that you see all these CD8s and CD4s.

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Sometimes these are three different patients, 16, the same one as here, 19 and 21.

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So what you can see is that you see perivascular accumulation of lymphocytes thinking of that these cells are coming to the bloodstream and then trying to expand into the tumor, infiltrating the tumor.

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Sometimes you see it in these parian aquatic areas. But because we had about 30 of these paired samples before and after. We could also do some quantitative analyses.

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So these are quantitative analysis, these paired samples before and after. And you can see there was a statistically significant increase in CD8s.

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After injection, CD4 after injections. B cells, we were not statistically significantly increased. Some patients had lots of increases. Some were much less so.

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You see the averages are here, but again, there was a little bit too much of a range to make this significant.

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What is this interesting Can you correlate that with anything?

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You see more CD8s, more CD4, you've injected a pathogen, great.

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So the next question we ask, is there any correlation between the infiltration of T cells and survivorship outcome.

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And so this is work by Alex Ling. Alex was a postdoc in my lab, and

now he's a junior instructor at the Brigham.

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But what Alex did is started putting On the x-axis, the change in CD8s in these specimens.

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After oncolytic virus injection. Or the change in CD4s, and then looked at survivorship as a continuous variable.

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So this survivorships of continuous variable, which is a more I say robust statistical test in just saying long-term survivor versus short-term survivor.

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So you join these dots as a patient. And what you can see is if you look at all the patients that we looked at, it was not significant.

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But if you looked at the seropositive patients, the ones that lived the longest, there was a significant correlation between these changes in CD8s The change in CD4s and survivorship.

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So again. What I showed you so far is that So your positivity seems to be involved with survivorship. You have more T cells in there.

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And in the COPUSA patients, the more T cells you have in there, the longer your survivorship.

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So I said, well, let's do another test here. Let's try something else.

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So we did what I call the $250,000 experiment. We made genomic DNA.

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From our specimens, sent it to adaptive biotechnologies, which can then sequence from paraffin samples, the T cell receptor, particularly the beta chain of T cell receptor. So now you have what are called T cell clones.

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And we correlated that T cell clones with survivorship. And so here we're looking at the tumor TCAR betas again.

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And we're looking at survivorship as this continuous variable versus a

change in the tumor T cell fraction that's just And in the case of the change in tumors. And again, what we can see is that there was a significant increase

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In survivorship, the more the T-cell clones were in there. And this was also related to T cell diversity. Diversity is a metric of T cell diversity, which is how well the T cells respond to different antigens, for example.

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And the more diverse they were. The better the survivorship.

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And this is also seen in the PBMCs. From the same patients. Again, there was a pretty significant increase in survivorship the more of these T-cell contacts you saw coming into the tumor.

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And again, this is also associated with survivorship. So what I've shown you so far is that there's evidence of increased CD8s and CD4s. We've really changed The microenvironment is these tumors.

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With more T cells. The more T cells that come in there either just counted grossly by immunostochemistry or looking by T cell clonal types, the better the outcome.

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And the last thing we ask, is there any changes in the tumor immune signatures by bulk RNA sequencing?

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Are these changes correlated with outcome? So this is a complex slide, but basically.

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Here we're looking at p-values And so this red bar is a p-value of 0.05. So everything's the left.

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Is significant. And the y-axis, we're looking at 13 different immune signatures by transcriptomic analysis.

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What I'm just trying to show you is that in the COP positive patients that we could study in blue here.

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All these signatures are positive after injection of the virus. So

there's definitely a change and most of these are immunoactivity signatures.

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In the seronectave patient, you don't see as many as shown here in red.

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So the way we put all this together. Is that really what we're doing here is an intralesional booster vaccine. In other words, in patients that had been previously exposed to herpes They have antibodies and probably T cells that recognize the virus, maybe an essential memory

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A pool when we inject a virus in the tumor. You start getting a profound pretty rapid antiviral immune response. Maybe it's antibody mediated, maybe it's T cell mediated, I think it's T-cell mediated personally.

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Which starts cleaning virally infected cells, particularly tumor cells But we also think by epithelial spreading also some tumor bystander cells.

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And clearly you can get a better response in that aspect.

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Or if you're seronegative and you inject the virus, these tumors are growing fast. There's just no way for the for this viral therapy to really catch up.

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So these are the conclusions. We can change this tumor marker environment with injection of this virus.

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We actually see these inflammatory infiltrates even several months We've seen as late as three years out.

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So we can truly turn these tumors into some durable cold to hot tumors. We have not achieved a total of, I think, 61 patients. We've not seen neurotoxicity We can see that survival patients treated with this oncolytic associates with positive HSV1 serology, although we do have to prospectively validate this.

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These patients are the ones that also have More increases in T cells and T cell chronotypes. We actually have some specific TCRs that seem

to be associated with survivorship.

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We see more new signatures. I didn't show you this, but the seropositive patients also clear the virus. We do not find virus.

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In the serial positive patients, even the ones that live long.

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In the serone active patient, we find virus even several months to years later.

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We're doing additional analysis, single cell RNA sequencing codecs with the SUVA lab, the George lab, and the Wookoff Finney lab.

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And we're now in the midst of doing this large sampling and injection of multi-institutional trial, which is founded by Breakthrough Cancer.

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Do I have time? So I'm just going to tell you. So these are all the individuals. Again, it takes, I don't say it takes a village, it takes a city.

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Over 20 years that I've worked in this, a lot of the funding has come through the NIH, ACGT, philanthropy.

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And there's multiple, multiple individuals that have been involved in this.

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Now… I just wanted to really give a shout out to Breakthrough Cancer. This is a collaboration between MIT, Dana Farbo Brigham, Memorial Sloan Karen, Johns Hopkins, MD Anderson.

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But we're doing this longitudinal assembly platform to try to understand what is happening in the tumor microenvironment.

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As we do a therapy. Initially, we want to do a systemic therapy, but we thought that putting patients through six or four multiple injections Without the treatment may not be approved by the IRB. So we added this intratomoral injection as a therapeutic so we could also do these

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Analyses. And so the idea here, again, is that When you have a therapeutic, you could do these sealed time point assays in these tumors and then analyze this by multi-omic approaches to see what is happening in the tumor itself.

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You can also collect CSF. You can also collect blood to see if you can associate the biofluid changes with what you find in tissue.

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So actually, we've done eight patients, patient number seven and eight are currently undergoing treatment.

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But these are the first six patients in the first court that have undergone this.

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And this is definitely feasible. In some cases, like this patient, we got up to 26 biopsies from four different locations over 120 days.

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In some cases, we stopped early because either There was a little bit too much, looked like too much progression, and so these patients either underwent surgery to resect the tumor.

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And in fact, a lot of times we actually found a lot of inflammatory progression, more inflammation than tumor.

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Or they decided to do something else. And one of the questions, what can you do with some serial samples? I was talking to Antonio before. And actually, you can do a lot.

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You know, you can do a lymphocyte immune analysis, you can do podium analysis, you can do RNA analysis, you can do DNA analysis.

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And this really involves a gigantic team, over 100 scientists over these five institutions that are doing these studies.

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And some of them are shown here. In this slide, each one of these scores goes to a bunch of different labs, including the ones at MIT and Nina-Faba, but also MD Anderson.

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Memorial and Johns Hopkins. So… Patients, we have more patients than slots on trial. That's my major as the IND sponsor, that's my biggest problem is always trying to find a spot for patients that want to undergo this.

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We have not had serious adverse events Odyslamic accessories.

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We can get multiple sites from tumors. We've been able to get at each Each site from 6 to 26 biopsies. Right now, we're eight patients, we have over 400.

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And each one of these is of sufficient quality and quantity to get great scientific data via OMIX approaches.

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So thank you and thanks to the entire Breakthrough Cancelaz. Some of them are here in the audience. I appreciate it.

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Fantastic. Thank you so much. Questions? So, Nino, you mentioned this briefly, but maybe you could go over it a little bit.

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The CAN 3110 immune response seems different than other H.

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Herpes virus therapies that you compared to. So what is it again that's different?

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What's different is the genetics of the virus, how the virus is constructed.

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So our virus still retains some of the immunovasive properties.

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If so, there's one gene called ICP47 that allows MHG to downregulate. So actually in infected cells, it's pretty immunovasive.

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That allows it to be more robustly replicating. Number one. And I think that that's why you get this importance within serology.

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The other ones are pretty feeble. They don't have that, so they get recognized pretty fast, I think, by innate inflammation and get

removed by the circulation.

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With the TVEP, which is a meloma trial, they can never find virus.

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They're very satisfied these tumors whether it's about or negative. So I don't think these virus hang around very long. So precisely the thing that might have scared you from a safety point of view. Yes. And that's why the FDA made me start, 10 to the 6th by Alpha log, using 9-inchra outputs of MRI to make sure that

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The injections were in the right spot. But it's actually been extremely safe.

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I mean, some of the other immunotherapy trials, patients are really sick.

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They're in the hospital for like several weeks. I'm not going to name the immunotherapy trials, but you can imagine.

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With this one, I've had patients fly across the country get their injection and fly back the next day.

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So, Nino, I imagine you didn't do any pre-selection of the patients initially. And I was wondering if you had any consideration of the status of the tumor before treatment.

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To understand the response. In other words, also. You seem to allude the most in your talk to the state, the cold state, right, of these tumors that then become immune health with more infiltration of immunoactive cells. But as you also mentioned many times.

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Some GBM especially can have really a large infiltration already in the beginning, a diagnosis.

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Of immunosuppressive myeloid the cells. What happens to those cells? Have you checked?

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There is a role of the myeloid cells in predicting the response.

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Yeah, so the In the first study, this RMA study that I showed you that was published.

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The only way we could really look at moderate cells well, because we tried to do by immunochemistry, but these stains are crazy.

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So you are to enumerate the very complicated and I gave up.

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But you can do it by transcriptomes. So we can clearly see, particularly in the seropositive patients, that there were many more, if you believe the M1 to M2, M1-like transcriptomic synergies after injection of the virus. So I think there's still mild cells in there. They're just much more immunoactive.

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This is a mixed bag of gliomas. There were current, recurrent, recurrent. Some of them had previous treatment.

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But again, all that I've shown you is the GBM. They're IDH wild type. Gbms are not the others.

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Yeah. And, you know, I'm sure you're here because one of the next steps is to somehow combine this with the previous talk, right, to look at Are you actually targeting more migratory cells or more proliferative cells with this and what's with the

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Signaling pathway because you actually have shown that there's some extraization and some higher density of whatever you do around the blood vessels, which makes a lot of sense.

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So that's a comment. And then the question is, I'm super surprised that there's no edema like 25 years ago. And, you know, is this because all these patients are It's a recurrent tumor and there's some cavity and there's not a lot of pressure that is being generated. What do you see on the MRI? There is edema. The virus does cause edema. But there's no headache? There's no headache or no seizure activity? So there were no DLTs. The worst SAE, serious events, were persistent seizures in two patients

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Were young and were IDH mutant gliomas. They were not actually GBMs. That's been the worst. We've had some… headaches. We've had some… So

we saw patients even a few months later that showed more enhancement. So we took them back to surgery.

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We take him back to surgery, there's a lot of inflammation. There's a lot of lymphocytes. It's mostly tumor confined.

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But there is edema. I mean, this is an inflammatory agent. It causes edema around the tumor.

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Yeah, so the way we inject this is right into the gallonium enhancing area. Sometimes maybe we get flare. I think the virus is just providing an initial How do I say it? It's the initial match.

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The startup process. The virus is not going, well, I shouldn't say this.

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In theory, the virus should not be going away from that.

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We do have a patient that was actually in a paper who had multicentric glioma.

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We injected one tumor. And that tumor became an aquatic on MRI scans over time, but a second tumor temporal that kept on growing.

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He donated his brain. Eight months later.

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And when we did the autopsy of his brain. That tumor that was going at you was full of virus and T cells. I have no idea how the virus got from the injection site to that.

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Side. There were three other spots that you cannot just see on the MRI scan, but there was some tumor there and three other spots in the contralateral hemisphere.

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There was no virus for immunosometry, but there was virus just in the tumor, not in the brain, by PCR.

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So I actually don't know how I got there. And there's a postdoc in my

lab that's got all these ideas and working on it.

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He's been at it for two years now. It's interesting that the two patients who had seizures were IDH1 mutant. Do you think there's any biological significance to that, that they would have more edema and therefore more seizures? Well, I think that there's some data, the IDH mutant

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Patients versus the younger do seem to have more seizures. So I think they had seizures even before.

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The seizures that got worse afterwards. And in both cases, we actually gave the patient aclovir because we thought it was Because of the virus.

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Finally, we got the seizures under control. Okay, excellent. We have four questions online. Given the success, Divya Senya, given the success of oncolytic virus in this case.

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Curious to see if you have seen or would expect to see similar anti-tumor activity in other mimicking non-viral models of cytosolic delivery, such as mRNA.

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Peptide vaccines. Wondering what the principal mechanism that makes this work and if that would be leveraged for customizing a more effective emission. Can you say that again?

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Maybe easier to read it, actually. That was a long question. Yes.

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Yeah, I don't know. I mean, I think that there's what they're asking for, asking is if you put a mRNA vaccine or a peptide vaccine that mimics viruses.

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Could you see a similar effect? I don't know. I think there may be some common mechanisms amongst all these inflammatory, whether it's a cytokine virus or maybe even a CAR T cell.

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And then there's maybe different mechanisms. I think the problem with the mRNAs and single peptide vaccines is that they're very targeted. This is pretty untargeted. You know, this is just… You start setting

up an inflammatory response in the tumor and then you see what the immune system does.

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So I think those would be the differences. Excellent. And then James Vega Luccarelli.

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Are there more tumor specific CD8 T cells in the virus-treated group?

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And did you look at the impact of treatment on glioblastoma stem cells?

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So there are more. Yeah, definitely. We saw just a tremendous influx of CD8s and CD4s.

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Correlated with outcome. You can also do this by genomics. And… What was your second question? T-stars and stem cells. Yeah.

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Yeah, that's difficult. You know, with glioblastomas, the stem cell hypothesis is that there are some stem cells in there that you can look by nastin expression. Again, this virus is driven by nestin promoters, in theory.

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It should definitely affect glioma stem cells. By humans.

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It's very hard to do that and figure out whether the stem cells are getting infected just grossly looking at that. And you almost have to like get him out of culture, put him in culture. In culture, if you believe in glioblastoma stem cells, the virus definitely infects them.

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I don't know in vivo. Excellent. Last question from Chelsea Chergina.

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Great talk. Do you believe that there would be any correlation between SNPs and the tumor cells and length of survival in patients?

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Yeah, we didn't look at that. I mean, there's some data about GWAS and SNPs potentially correlating with survivorship.

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I'm not sure if there's any… That's solid, but we did not look at

that.

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Thank you for your questions. Anything else? Excellent. Thank you so much.

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Onward to our second Antonio. Antonio Yamarone is a professor of neurological surgery

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He's also the deputy director of the Sylvester Comprehensive Cancer Center at the University of Miami Miller School of Medicine.

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Before that, he spent 20 years at Columbia. I wonder whether you miss it these days. Last is Professor… of neurology and pathology and cell biology.

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And the Institute for Cancer Genetics. He was also a research fellow at UCSF before that, Sloan Kettering.

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And the Albert Einstein College of Medicine. And his MD is from the University of Rome, even though you grew up in Napolei, right?

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You're from Napoli originally? Yes, sir. Non-napoli. It's another city. We don't want to mention. It's a very small called the Benav.

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Antonio from Peneventa. Okay, I hope I… I did everything right with the connection. Fantastic.

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So, Nino, thank you very much for the great talk. You gave that works also as a perfect introduction because obviously I will present to you the work that we do in the lab.

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But I will start with our frustrated presumption that we should try to have an impact in the clinic.

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We think that we have been thinking that since quite a few years, especially because we understand that when we study these tumors, as you heard from Forrest.

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We can understand really at very high detail the biology of these tumors. We understand that GBM is incredibly heterogeneous. At the genetic level, at transcriptomic level, proteomic level, really at the multiomic level, every single tumor looks different from another one, and we can

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Quite accurately these days classified these tumors and come up with some presumptive idea of what could be the best option for that patient.

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But all these type of thinking is a zero input in the clinical setting.

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As you heard from Nino, essentially all patients with an IDH wild-type GBM, despite these formidable.

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Heterogeneity, they all receive the same standard or create approach. This intervention completely bypasses the potential research efforts that could be used to identify truly precision therapy for these patients. And I will start telling you some low-hanging fruit idea that obviously

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The precision therapy opportunities in GBM in the clinic of glioma patients have so far failed, and they have really failed also because they have not incorporated some very basic concept.

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Such as where the genetic alteration is a tranquil alteration, which is simply present in the vast majority or as a tranquila actually in 100% of the tumor cells, or where there is, for example, has been the case for most of the EGFR gain of function alterations that have been targeted in the hundreds of field clinical trials in EGFR. Basically, these are most of the time

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Branch alterations that a best will result in removal of a branch without any satisfactory elimination of the root of the tumor origin for a particular GBM.

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The low-hanging fruit for us is being the attempt to identify alterations that represent the tranquil alterations and that could represent, there are not many in GBM, then they could represent relatively easily target. And one of those that represent actually

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Greater frustration for us, and I'll tell you why, is the GFR3AK3 that has emerged after our initial identification.

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In 3% of IDH wall-type GBMS, the single most frequent gene fusion across all human tumors because at this low rate, this particular fusion has been essentially discovered in many different types of solid tumors.

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And the reason why I was saying is being frustrated is the fact that we discovered the diffusion in 2012.

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And as of today, a proper clinical trial in GBM has not been done yet. And the reason why it has not been done yet is because the disease is present in only 3% of patients with the GBM, IDH1 type. And when the standard, I would say, clinical neuro-oncologists here about 3%, they get terrified. They really don't like this type of trials. And it has been extremely complicated.

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To select these patients for a precision therapy opportunity. So these fusions have ever been studied now, mostly in other tumor types, and in basket trials, there has been actually an approval by the FDA of erdafitinib, probably the single most effective FGFR inhibitor.

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For treatment of patients with locally advanced or metastatic ureteria. Carcinoma. And in the contest of these trials, several GBM patients, such as those indicated here, have shown very significant response. So since we arrived to the University of Miami, there was actually one of the reasons why we moved there, was actually to try to inspire the establishment of some

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Clinical studies where FGFR3 positive patients, there are also FGFR1, TAC1, but the vast majority of adults with GBM are FEG for treatach 3 can be preselected for the inclusion in treatment with irredafetinib. And you see the scheme of the trial that now is currently ongoing with Macarena de la Fuente, who leads our clinical neuro-oncology clinic.

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These trial has now recently moved to the dose expansion court. We have already rolled, I think, approximately 12 patients between the initial phase and the expansion court. And the results so far have been very encouraging. Obviously, these are interim results.

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But you can see here some very significant response actually over partial response, more than a partial response that have been detected in record.

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In a recurrent GBM. The format of the trial here is the standard phase two, so these are all patients that recur standard of care, of course.

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We believe from a preclinical studies, I'm not showing this to you, that the administration of FEGFR inhibitors upfront might result in even better clinical response. But this trial is proceeding in the context of ETCTN, which is NCI network and with them we have to be cautious, so this is the way to start but hopefully

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After we completed the trial, we will be able to move to a more advanced scheme.

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Now, the reason why these fusions are exciting genetic alterations is not only because they represent probably the easiest target today identified the NGBM patients, but also because they were associated with a very unique biology that allowed us actually to understand some fundamental biology of all

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Idh-1 type GBM. And the unique biology is that these fusions activate mitochondrial metabolism and oxidative phosphorylation as a primary mechanism of transformation of mechanism of action that actually can also be used in a contest of targeting.

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So by what we did in the lab, after we identified this mechanism of action of FEGFR3 tractor fusion was to think whether now we could think about classifying GBM based on the biology of these tumors, based on the biological functions, the functional activities that are activated, and as we said in a heterogeneous manner in these tumors. I'm sure you know that in many other tumor types.

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The transcriptomic classification of tumors. So a typical example is breast cancer, but really there are many other tumors.

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Where a transcriptomic activities of tumors can help to stratify patients based on clinical outcome. And also, as in the case of breast cancer, also direct patients to completely different type of therapeutic opportunities.

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This is not the case of our GBM. There has been a classical classification that includes These are three groups of proneural, mesenchymal, and classical, but really classifying disease tumors into these three groups resulting in very little information in terms of patient survival, inability to recapitulate a tumor heterogeneity, but most importantly.

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To inability to drive a stratification of or personalize the therapy. So actually with Luciano Garofino, he has been the primary leader of this work, we started an approach that aimed to classify single tumor cells using a single cell ERNAsic based on a pathway-based classification of individual tumor cells. And this was complemented by a bulk

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Classification that was also associated with survival. And this type of analysis led to a new classification of GBM, a pathway-based functional classification of GBM that resulted in the identification of two independent functional assays One that we call a metabolic axis that includes the mitochondrial GBM, exactly the subtype that was initially inspired by the FGFRTAC diffusion

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That is encountered by a very aggressive subtype that is a deglycolytic lower metabolic subtype, the subtype that is rich, the weight, the high number of myeloid infiltrates, but that is also characterized by the redundant activation of all type of metabolic pathways except

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The oxidative phosphorylation that is unique for the mitochondrial subtype. These axes are opposite to what we defined as a neurodevelopmental axis that is actually differentiation, lineage commitment access that moves from a proliferative progenitor or stem-like cell reach the subtype that we call PPR, that then is associated with a neuronal subtype that includes tumor cells that acquire

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Synaptic competency for the ability to establish connectivity with the normal.

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Neural cells. Now, these are classification, as I said, was initially generated from single cell transcriptome validated on a bulk transcriptome, bulk genetics, but then we added the possibility to have access to The multiomics data from the CPAC, and you can see that when we aggregate all the different platforms that are available in CPDAC, this work has been done by Simona Miyotze, who is a partner of

Luciano and who recently got a very beautiful baby. And this work, as you can see.

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Basically identifies that the four subtypes independently for each different platforms with actually a complementary but sometimes different features that could not be inferred from the transcriptomic alone.

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And in particular, I want to point to you because this will come up again, the identification from proteomics and phosphoproteomics specifically of the DNA damage responses that we found in proteomics and phosphor proteomics that we had not identified the Fortress atomics as a key hallmark of the PPR subtype. Now, all of these type of classifications, of course, can be recapitulated at the single cell level and at the bulk level, but where initially

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Identified from the whole tumor mass. When we go and try to dissect the different region of the tumor from a multi-regional analysis, from image-guided multi-regional analysis in which we have the core contrast financing and the REIM non-contrast financing, we could see, here you see from these seldo time single cell-based analysis.

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That as we move from the core to the rim, what typically happens is a key hallmark of the transition is the change of the PPR stem-like state of the tumor cells towards a neuronal highly differentiated tumor cell.

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And this point has been validated by multiple studies. This is a complementary study. The one I showed you before was a study done from a limited number of cases on the single cell level. This is not at the single cell level, but is at the multi-regional level done with actually the Mayo Clinic with Nantran, Mayo, Arizona.

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And you can see that the key cluster that we find associated with the non-enhancing room is a cluster that is massively reached neuronal tumor cells.

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This idea that the mechanism of invasion of GBM might be driven primarily towards a transition to a highly differentiated synaptically competent neuronal expressing tumor cell state is a concept that is now emerging from many different reports, including, as you will see later in a proteomic report.

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But I wanted to show you some data that actually are providing additional evidence for this concept. And this data that we have just recently started to generate are this data that we generate with the cosmics spatial molecular imager platform.

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It is a platform that uses spatial trust atomics at the single cell level with a highly accurate segmentation of individual cells where basically we can clearly assign the transketomic official or to individual cells, both to tumor cells and non-tumor cells. And as I said, this is a

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Work in progress, clearly we have now a number of results that we are trying to solidify, but this is a finding that I wanted to show to you because it's a finding that came from the complete reconstruction of both the bulk core front of the tumor and the invasive rim where you have a mostly the normal brain here, and you can see that the only tumor cells that are present actually into the invasive normal brain are actually those classified into the green neuronal tumor cell status.

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When we see these cells that are typically scattered, surrounded mostly by non-tumor cells into the normal brain, these cells are assigned to a neuronal state.

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This is different from what state happens in the core of the tumor, where you can see that the majority of the tumor cells are classified as proliferative progenitor.

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And those are the cells that clearly results in the expansion of the tumor mass.

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So these are classification I alluded before the motivation for us to go after this classification was to try to understand if we could generate some prediction opportunity for patients following the classification and obviously then based on those predictions.

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Also to try to trigger some therapeutic opportunities for these patients.

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And as you can see here, this is actually finding that recapitulates what we see with the effici of R3 target 3 mitochondrial functions of

the F3T3 patients.

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Is that the mitochondrial subtype is associated in the context of exclusively IDH1 type of GBM with a significantly better survival, something that you can see in all the possible comparisons, but more importantly, if we use oxford inhibitors, different type of inhibitors of mitochondrial metabolism and mitochondrial respiration.

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You can see that we can have a significant anti-tumor effects in PDX in patient-derived xenografts, intranial PDX, treated with deoxophores inhibitors. If we use a mitochondrial subtype PDX, but this is not the case.

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If we use other type of PDX, they cannot care less about mitochondrial inhibition. So Luciano developed a clinical grade probabilistic classifier. This has been published. It is publicly available. And the DZ classifier can be used using a typical RNA-seqa from about FAP or frozen results and can be used to retrospectively evaluate. So for example, in the case of Nino, if Nino wants to know if his responses are typically associated in the clinical trial that he described.

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To a particular subtype, we can use the RNA-seq from the original tumor, so to determine the association of the therapeutic response with this subtype. But of course, the most important use of this classify is a prospective use.

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To do a patient selection for prospective clinical trials. And I told you that clearly the most mature group in terms of clinical impact is the oxidative phosphorylation subtype, the mitochondrial subtype.

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However, in the clinical setting, there have been a lot of negative results in terms of testing mitochondrial inhibitors. There have been actually a number of papers that have described the failure of these compounds in the clinic, mostly because of the extensive toxicity that has been associated with these compounds.

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So we have worked with many of them, but we have identified a compound that is a derivative of metformin. Metaphorin is obviously a drug that is widely used, a very safe drug, is not a clean drug. It is a drug that in addition to inhibiting a complex one of mitochondrial expiration as many other functions.

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But then this compound, the lexumistat, has been identified as a derivative, a much more powerful and more specific of metformin and has shown a very safe.

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Profile in many phase one trials that now ever been reported.

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We basically tested the activity of Lexumistat in addition to the activity of the many other oxaphorce inhibitors that we had previously tested. And here you see the patient derived the cells from different tumors that have been classified as either mitochondria or is glycolytic capillary metabolic. And you can see that while in the glycolitic pleura metabolic this compound, just like the others, showed very little response, we have encouraging preclinical responses

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In the case of the elixomistat for mitochondrial GBM. So these are the summary of the preclinical data. This has shown activity against mitochondrial GBM.

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Both in vitro and in vivo, as shown in the ability to cross to the blood-brain barrier, although not with a concentration in the brain tumors, and is a compound that can be used in clinical trials. Now we have a discussion that hopefully is advanced. It is in advance of the stage with Jan Parne and his team at EMEO to do a window of opportunity and possibly also a phase two trials.

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To target the mitochondrial GBM in the context of the glyoblastoma therapeutic network supported by NCI. So this is where we stand the mitochondrial GBM, but these are type of a paradigm that was relatively easy. We identified the biological functions. We think about the drug.

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Did not work, simply did not work for the other three subtypes. Clearly the other three subtypes are much more complex. So if we, for example, think about inhibiting glycolysis, so we think about inhibiting lipid metabolism.

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For GPM subtype, this was not doing anything, probably because of the redundancy of these pathways in the case of these other subtypes.

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With the Luciano and Simona, we thought about the possibility of using these clinical proteomic data, primarily proteomic and phosphoproteomic data, to try to deliver at the proteomic level what

we had previously done when we were at Columbia, in particular the work we started with Andrea Califano.

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At Colombia a while ago in which we used the reverse engineering to identify the master regulator, transcription factor, so that are associated with a particular GBM signature.

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Here what we did is that we integrated the proteomic and the phosphoproteomic signature tissue specific of RGBM, and then we came up with the prediction of the kinase network of all the kinase networks that are active are potentially active in the GBM context. And Luciano developed an algorithm that we call Sphinx.

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For substrate of phosphocyte-based interference for network of kinases. And this algorithm was applied to GBM, of course, but then it was also applied to other tumor types in which we were also identifying, I forgot to mention this point, that diesel classifier, even though was initially developed for GBME, is actually also operational. It can also predict

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The same subtypes also in other solid tumor types, such as breast cancer in the lung squamous cell carcinoma. And you can see that we found some PPR, master kinases, namely kinases that behave as master kinases and the primary most active kinase in a particular subtype.

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Across multiple tumor types. And these are two other kinases that really scored at the top as a specific subtype, specific pandemaster kinases.

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Pique siderta is a master kinase over the GPM subtype and DNAPK.

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These master kinase for the DNA damage response for the PPR subtype.

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In terms of the neuronal subtype, the finding of the master kinase of the neuronal subtype was actually inspired from another study.

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That we did with our collaborators in Korea, John Be Park, because with them we actually collected the larger sector of matched peers, primary and recurrent GBM, that we used.

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To do multiomics, including proteomics and phosphoproteomics, something that had not been done in the context of CPTAC.

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And as you can see here, recapitulating what we see at the spatial level.

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We see exactly the same type of a primary transition, a primary evolution of IDH wild type GBM when we look on a temporal level. So when we look at the main difference between a primary and recurrence, match the GBM, What we see is a net expansion of the neuronal subtype of tumor cells compared with instead at the expense of a decrease of the PPR proliferative progenitor tumor cells. So the neuronal subtype

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Is what we see enriched in the rim, possibly remaining as a resistant tumor cell after standard of care in these type of tumor state is the one that we recover as the most reached eight recurrence, something that now we have

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I'll be at a different rate recapitulated also at the single cell level in the context of a care project with Mario Souva and the Taitiros.

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That we have all been doing together. And now having the opportunity to study the neuronal subtype in recurrent GBM is really a prominent state.

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We identified BRAF, wild type of BRAF. In this case, there is no mutation of the BRAF gene. It's a post-transational activation of BRAF is the top scoring master kinase for these recurrent neuronal GBM. And as you can see, the use of different type of inhibitors of BRAF results in the recurrent state. In this case, we were using a patient-derived the cells.

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From MEX, the primary record states. And you can see that in the recurrender state, we can clearly see the elimination of these neuritus tension, these differentiation related features.

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That are, of course, the hallmarks of the neuron RGBM. But more importantly, together with John Babark, we were able to show is that if we now combine we could significantly extend the survival all of these recurrent neuronal GBA. So these are classification can be, as I explained to you, recapitulated multiple omics level. I didn't

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Talk about the radiomic level. Again, some work that we have done.

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Very recently in which we have identified a number of radiomic features very specific cardiomic features that are associated with these different tumor subtypes, but we hope that especially if we are able to do a study now in which mitochondrial GBM

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Will be selected in a non-genetic manner. Again, these are non-genetic selections of these tumor subtypes. We hope that at least for the PPR, for the GPM, these type of subtypes can come next as other opportunities for for targeting. So I want to finish in the last couple of slides with just telling you about what is the most frequent tumor predisposition conditions in which actually brain tumors are identified and in particular glioma. Of course, everything we discuss today and in any

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Discussion with glioma is the context of sporadic glioma. But in the case of neurofibromatosis type 1, in which there is a germline mutation of the NF1 gene.

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We have a syndrome in which there is a very high incidence of brain tumors, in particular optic glioma in children, but also we have a five-fold increase of non-optic glioma at any age, including the possibility of the development of high-grade glioma.

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In these patients later in life. Now, it's been extremely challenging to study these tumors and that there are very few reports because the surgery is not the choice, the approach of choice.

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In this case, it's because of the multiple morbidities that these patients have, so there is a decision to try to have a conservative approaches rather than having a surgical resection in this tumor.

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So a few years ago, we created what we called the Landing Consortium that includes now 30 centers across different continents to basically aggregate a sizable number of both high-grade glioma and low-grade glioma from patients with an F1 These are both adults and children, and they can be, in both cases, low-grade and high grade.

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And we studied these tumors at the bulk level, both at the genetic

level, and at the transcriptomic level, and then at the single cell immunostaining level And we were able to identify two very distinct modules.

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Of genetic alterations that characterize a dehydrid glioma and the low-grade glioma in the context of NF1. You see the key features here. The high grade is associated with the typically higher mutational burden as expected, and there is these three groups of genetic alterations that include the p53 CDK2A,

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And ATRX, these tumors are almost always IDH1 type. We actually think.

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Found one ADH mutant, but beside that, all of them are at the edge of wild type, whereas in the low-grade glioma, these are type of aggressive genetic alterations are absent, and we instead identify primarily alterations over the MAP kinase pathways that are gain-of-function alteration of MAP kinases.

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And the other key finding that we reported was the fact that in the context of the low-grade, exclusively the low-grade, we found a group that we call the high moon.

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Because these tumors distinctly present a large number of CD8 T cells clusters of CD8 T cells that were not otherwise present that could be distinguished with what we defined as the low immune. So the low-grade group, the low-grade glioma group of NF1 patients

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Can be subclassified into this ion and low immune. And obviously, this was work at the Bulk level, here you see again the characterization of these tumors.

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In which, of course, I think I already told you that the mutational burden is higher typically in high-grade glioma, typically in adults. And there you see the more granular classification of the CD8 T cells in the high immune that are typically associated also with the cytotoxic phenotype that represents the indication that these cells might be able

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To mount a sufficient immune response. Now, again, Luciano, together with the Fulbiodangelo, who had also worked on the original studies, recently started to analyze partially overlapping set. Actually, most of these tumors were completely exhausted, so we had to start again

the request for a new collection of cases, but here you see the new cases, 46 tumors.

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That we have been able to analyze at the single cell level, at the single cell thrastectomic level, and here you see the typical classification of a tumor versus a known tumor and the microenvironment that is associated in these in this particular type of an F1 tumors.

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This is a still work in progress. We are in particular still trying to deconvolute the tumor cell states because we believe that there are some tumor stations that are unique for the NF1 glioma state, but what I wanted to present to you is really the more accurate, if you want, the classification of the microenvironment that now we generate.

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From these tumors because what we see is that the immune high not only has what we had already identified, the presence of these distinct CD8 T cells associated specifically with this tumor, but what we found is that the myeloid cells appear to be characterized different than the typical situation of the high-grade

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Gbm that you also heard from Nino is instead composed by a pro-inflammatory microglia and macrophages that we believe have a clear cooperation with the CD8 T cells. So these are tumors, so these LGG immunoh of an F1 glioma are definitely an optimal subtype

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For a subtype that might respond to immunotherapies. We also identified an immuno-exhaustive subtype, again using a single cells. We had not identified before, but this is a subtype is a significant number of CD8 T cells as a mixed myeloid microenvironment and is completely different from the immunocort, where essentially no T cells could be identified.

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The high-grade glioma are very diverse, but they are not substantially different, at least based on what we saw so far.

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From the sporadic high-grade glioma. So we think that For NF1, there is a really specific opportunity for the low-grade, and this is the final slide in which I am showing you the wonderful group of people. There is a Luciano here, but these are really representing of a

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A dream team who is in the lab and many of them moved from Colombia to

Miami. I think they are all happy to be in Miami and all of you who are really tired of Boston, you should write it to us because we have

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Fantastic opportunity to rescue you in Miami. Thank you.

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Thank you, Antonio. Do we have any questions? Enum.

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Antonio, as always, beautiful, beautiful talk. If I understand correctly, this neuronal subtype is more at the border.

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And it makes sense that it sets up to occurrences because that's why we cannot deal with surgically or even with radiation because She's pretty focal.

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You have good drugs against the other subtypes with those We have other stuff that we can take care of.

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What do you think is going to be… successful against these neural subtypes. It's susceptible occurrences. Is there something that you can think of? I think you may know that now there are probably even Too many, if you want, approach is trying to target the synaptic connectivity right of

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Ogbm with the explosion of the concept of brain neuroscience, I think Michelle, I don't know if she's here, but also Frank Winkler, Europe is doing a number of studies. I mean, we have the Perampanale study.

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That is a part of our study here. Of course, the neuronal subtype, we believe, is driving resistance, is ultimately driving resistance to standard of care, right?

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But it's not necessarily the subtype that is driving the growth of the tumor.

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The growth of the tumor in the context of the neurodevelopmental axis is coming from the peripative progenitor subtype, the stem-like cell subtype, right? That's the stem-like cell subtype. So most likely, the association of a neuronal targeting drug With the standard locator that is probably working well against the PPR is really the only subtype that is shown in our hands some activity in the context of the

standard care, possibly even

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With some inhibitors of DNA damage, right, that are those that we think can specifically target the PPR.

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You see, in that context, probably we can have some positive expectation.

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Also highlight that while in the context of these neurodevelopmental axis, we see the neuronal tumor cells infiltrating in the normal brain.

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By all means, these may not be the only mechanism, right? There could be what have been called the mesenchymal tumor cells, right? Before there is actually ostensible literature that mesenchymal state is what drives the invasion. And this may be true in the context of the metabolic axis. So if we cannot at least distinguish, right, the GBM tumors into these two very divergent states, right?

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Which are the metabolic and the neurodevelopment. I think we would really make a significant progress. Because this is also a point at which more or less even with different terminologies, I think the whole world more or less agrees.

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I can ask one more question. How do you put this finding?

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With Mario's paper showing that oxid drives Some of these anatomical Asian

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Absolutely. Hypoxia can be the contest of the metabolic state, right? Because hypoxia is the hallmark over the GPM subtype. So we have correlated this, right? And as I was saying, we can use a different terminology, but the biology is the same.

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Please. I was wondering if you think that the neuronal subtype is driving migration. You think that signaling is going to be important and anti-accidatory anti-glutamate would actually do something.

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But it also may impact any immune signaling that may be in the area.

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So there could be a plus and a minus. Have you looked at that? And then secondly, to Nino's point, if you actually debulk the main part of the tumor, wouldn't you expect that you have by definition more of the neuronal and the migratory world. Absolutely, absolutely.

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So on the first point, Lushan, you should really listen to this because we have always found a particular subgroup of microglia Which is different from the typical myeloid-derived suppressor cells.

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Reached in the GPM subtype that this state that this microglia subtype, not better defined because This is a great priority, right? Associated with the neuronal subtype, right? So I think that there is absolutely an opportunity there to understand what is the immune state and the crosstalk.

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Between it is a neuronal cells and especially the microglia, which is the primary immune cells associated with the neuron receptor.

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In the context of what you just mentioned, I guess the answer is yes, in the sense that if you do it, the bulking of the tumor, what you remove what Nino removes is primarily the core PPR, the highly, and now you are left with the neuron, right? And in this preclinical model that I showed you.

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Where we actually identified BRAF as the master kinase of the neuron.

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What we see is that unless these tumors start regrowing, the neuronal state is highly preserved.

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When did they start to regrow, they can go anywhere. At that point, they are… completely plastic, right? They can become a GPM, they can become PPR, they can become everything.

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But there, we have lost the butt.

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The force mentioned in sort of in passing is that there may be an element of collective cell migration.

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When you look at some of that, is there actually more self-sell talk

between the glioma cells in these, whatever it is, spheroids that show up below the imaging resolution threshold and or with the neuronal, in this case, mostly axon fiber.

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When the nuclei, there may be some neuronal cells. So is when there is higher density of these cells in the migratory area.

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A talk or glioma neuronal talk. I would argue that most of the crosstalk is a glioma neuronal and possibly other non-tumor cells. Let's not forget microglia.

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Oligodendrocytes, possibly astrocytes, right? They could all participate in this crosstalk.

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In the spatial analysis I was showing you. Is that if you look at the peripheral rim where we see exclusively neuronal tumor cells, right, in the invading front of the brain.

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The tumor cells are highly scattered, are completely scattered, right? So they are totally surrounded by normal T4 cells. You don't have any neighbor activity between the tumor to tumor.

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If you start having a contact between the tumor and tumor, these cells are starting to grow, of course, right? They are starting to proliferate.

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So now they start to change their state. They acquire a plasticity.

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Excellent. Unfortunately, we're out of time. So please find the speaker during the break, which we will have now. And I will also ask you to answer an online question, actually, during the break, if you don't mind.

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We will take a quick break. We will meet back here in 12 minutes at 11.15, please.

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Thank you.

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Yeah, they're going to look it up. All right. So we're going to get

started again.

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You guys can take your seats.

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All right. So welcome back from the break. It's my pleasure to introduce Francesca Mihor.

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Co-pi on our U54 center grant. Francisca is the… The Charles Dana Chair in Human Cancer Genetics, Professor of Computational Biology at the Dana-Farber Cancer Institute in Harvard. And she does some amazing computational work she's going to talk about today.

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Thank you, Forrest. Oh, the expectations are high. It's a great pleasure to do this with you, Forrest. And I'm so excited that everybody's here for our symposium.

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The way I set this up today is to show you a few things that we have done with the goal of inspiring and inviting collaborations across the different data sets and approaches that we have within the center.

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Here are my disclosures. We've been focused on trying to understand evolutionary dynamics of human cancers using computational approaches.

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And so we've been interested across the years in both initiation and progression, both spatial As well as genomic and epigenetic.

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As well as the selection pressure that the treatments administer to tumors.

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To create this bottleneck that unfortunately in many cases can be overcome when relapses occur.

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And so we've been interested in quantitatively characterizing these different stages of disease progression across different cancer types.

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Both with inpatients and across patients. And so what the lab does is to have a very data-driven approach where we start with either in vitro and vivo patient data sets to understand what the structures and distributions within these data sets are to then come up with

integrative analysis pipelines with the ultimate goal of creating predictive computational modeling frameworks.

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With which we can ultimately impact patient care. And so the example that I want to show today is something we started years ago.

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On GBM, but that has led to many follow-up investigations that are still ongoing and that are supportive our CSVC going forward.

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And so in this quest of investigating the causes and consequences of tumor evolution, we were interested in this particular example to come up with a qualitative description of how glioblastomas responds to treatment, different combinations of treatment.

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But particularly radiation therapy. With the ultimate goal of designing novel clinical trials that would be predicted to outperform existing fractionation and combination schedules.

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Particularly focused on existing agents, but of course also branching out into other approaches. And so we're particularly excited about the BTC work and what Nino has just shown us.

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And so GBMs don't need an introduction at this point anymore, but what we set out to investigate was the question whether different radiation fractionation sketchers might help maximize efficacy.

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Of a given amount of radiation. And so standard fractionation is two gray a day, five days a week.

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For six weeks. But many investigators have investigated alternative fractionation scatters, including hyper and hypofractionation.

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Over the decades that different fractionation sketches have been investigated, no significant differences were found in survivor depending on the fractionation schedule. All that seemed to matter was the total amount of those.

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As the first line, treatment is 60 gray over five weeks.

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We thought that maybe there was an opportunity to further improve and further investigate alternative fractionation scatters given that we now have a better understanding of intra and intertumor patient hierogeneity, thanks to the TCGA and Single cell.

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Rna-6 studies and other studies, such as the one that Antonio just showed.

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Which then enabled novel, more predictive mouse models to be built, which then would enable computational models based on quantitative descriptions and measurements obtained from these mouse models.

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And so one of the early introductions of TCGA, of course, showed different subtypes of the disease.

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That are partially overlapping with what we just heard from Antonio, but suggests that different subgroups Gioblastomas that are driven by a specific molecular And that enabled Eric Holland's lab, who we started collaborating with when I was still at Sloan Kettering, to build a mouse model which is based on the RCAS TVS system, which is an avian virus, the receptor to which is engineered

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Into specific subsets of cells within the mouse brain, including nestin positive cells.

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But also differentiators marked by Oleg2, which is a stereos marker staining differentiated tuberous somers.

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And so those cells that have been engineered to express the receptor can then be transfected with whatever molecular abnormality that this avian virus was engineered to deliver.

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And so you can build specific mouse models through this system that have stereotactic injection of the correct mutation of interest or overexpression of a syllable factor of interest at this specific time are so sequentially and within a cell type that you're interested in. So we started looking at the PDGF-driven subtype of glioma.

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Where we deliver PDGFA overexpression through RCAS TVA to nest in positive cells in the mouse brain.

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And those histopathologically plus genomically look very similar to human GBM. And we can then use this mouse model to very carefully characterize the microenvironment and the dynamics of radiation response.

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Of different subsets of cells within this. Mouse model. And so if you just stain for H&E, for instance, you can see that you have endothelial cells here in the middle of this leoblastoma, cells line, the endothelial cells are around. If you stain for nestin.

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Which is the stem cell marker. Which I'm so grateful that Nina already introduced this.

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It looks as if in this mouse model these stem-like cells that stay in positive and estin line the endothelial cells, so more closely localized at the center of the tumor lining the blood vessels.

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While the only two positive cells, the differentiated tumor cells, are more distal from the endothelial cells.

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And this seems to be supported by nitric oxide signaling that originates from endothelial cells and maintains these stem-like size, at least in this mouse model.

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In an undifferentiated, more radio resistant state. And so this is important because we can then quantify using this mouse model.

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Serially, over time and over different radiation fractionations. How sensitive versus radio resistant individual subtypes of cells are and how the time course of radio response for different localizations and cell types looks like, which of course we can't do with human samples.

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And so this is why we set out to investigate this based on this particular mouse model in collaboration with Eric Collins lab.

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And so this is a paper that was already published. A decade ago, but where we used the quantitative estimates of this mouse model treated with different fractionation schedulers and different total amounts of radiation administered to these mouse populations at different time points and then measured how many cells remain certain times after

administration.

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Of radiotherapy. And that enabled us to build a mathematical modeling framework that is very data-driven, so directly derived from the radiation response that we observed within this mouse model.

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In which we have two types of sires, the nestin positive stem-like cells and the olig-2 positive differentiate cells.

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If we administer a dose of radiation to these cell populations, then a fraction of the differentiate size. Smaller fraction of the tumor stem cells die because they're largely radio resistant.

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Parameterized based on the quantitative measurements of this mouse model. There's also a small proportion of these differentiators that can de-differentiate into these more radio resistant states.

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We then use this parameterized mathematical model to optimize over all possible dose administration schedules of 10 gray given during the span of one week, so just one week of the schedule, it would be administered to humans.

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But in mice, we can't give 60 grays. Optimized for Tengray. And we came up with this kind of non-standard schedule where we give one gray of radiation Monday at 8 a.m.

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2 p.m. And 5 p.m. Tuesday at 5 p.m. And so forth.

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Which we then implemented as a large mouse trial. Comparing this optimized radiation fractionation schedule to several other 10 gray schedulers given all at once at standard fractionation or an a sub-optimized randomized control schedule that was predicted to not be significantly different from standard of care.

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As well as a 20-grade schedule that was administered at the standard of care fractionation. So 20 grade total for two weeks.

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But too gray every day, just double as long. So double the total amount of radiation.

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And what we found was that this optimized schedule, even though it only administers 10 grade total, almost doubles the efficacy of each graph.

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Radiation administered because it's indistinguishable in terms of survival benefit in this mouse model.

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From a 20 gray standard fractionation. Suggesting that there is some scope, at least based on this mouse model, that we could think about optimizing how we administer existing agents in the best possible way to maximize.

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Efficacy. And so the mechanism seems to be that we enrich for nestin positive cells through this fractionation schedule, we give an additional dose before the potential stem-like cells either have been eradicated or replenished from the differentiation of the differentiated RDC2 positive size.

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And so this characteristic, very non-standard fractionation schedule seems to enrich for these nest in positive SARS.

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Which are even more radio resistant, but prolong survival because they have a lower turnover rate.

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So it seems that the mechanism of maximizing survival given this non-saturation fractionation schedule is to enrich for even more radio-resistant cells, which in principle doesn't seem like the greatest idea ever. But given that it's not curable anyways, at least it belongs to viable, at least in this

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Mouse model of the disease. And so there is supporting evidence.

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That this might also be a mechanism in humans, even though this is not definitive.

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But there was an Italian trial for the Antonios where patients were biopsied before and after radiation treatment, and those patients that had more than median enrichment in nesting positive cells had significantly longer survivor than those that had less than median enrichment.

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So it seems as if this could be a mechanism that could also be at play in humans. But of course, many more follow up.

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Investigations need to be implemented. We then decided to design a clinical trial based on this in mouse validated schedule.

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Where we are interested in comparing the feasibility, first of all, we're not at efficacy quite yet.

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Of this non-standard fractionation schedule. Against the historical control trial, which is RTOT505, that administered 35 gray in 10 fractions to recurrent GBM. And of course, we start with recurrent GBM, even though this is more of a mouse model that mimics

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Newly diagnosed GBM because it's easier to recruit and more feasible to implement.

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In those patient populations. The practicality constraints were a maximum of three fractures per day and not more than three days total that have more than one fraction a day.

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And so keep in mind that those are very sick patients that have recurrent GBM and so therefore can't be bothered to come in every day for many, many fractions.

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And also, therefore, to reduce the number of fractions as much as possible.

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So we redesigned this optimum schedule to something that's not the global optimum.

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But almost as good as this optimum that we had identified in the mouse model before, but simplified with the hopes of being more clinically implementable and more feasible.

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And so this led to this clinical trial here. Which is called Mars-Clio, which stands for Mathematical Model Adapted Radiation Skechers in Glioblastoma.

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The study objective was to employ a novel mathematical model adapted radiation fractionation schedule in patients for glioblastoma. We involved 14 patients.

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After translating this mouse model using a series of other clinical outcomes where clinical trials were designed based on preclinical evidence based on the same mouse model.

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To see how predictive the outcomes of this mouse trial preclinical evidence would be for human disease.

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And we then reparameterized using some data from human patients to come up with this Mars-Glio schedule, which looks like this.

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Two weeks total, first week, one fraction every day at 3.96 gray.

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24 hours apart. And then the second Saturday, Sunday off. The second week, the first two days, the same as the first week.

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And then the last three days, three fractions each at one gray, each with a 3.25.

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Our interval in between. And that by this validated mathematical model would have predicted to… if scaled for efficacy.

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Would lead to superior outcomes, but here we are only powering for feasibility.

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Which ended up being 14 patients with the assumption that within one hour of these three fractions a day.

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Time slots. Patients would be adhered into the schedule. And within three hours of the single fraction day, patients would be adhering to the sketcher.

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That's how we do the power calculation. For 14 patients. So you have patient characteristics for the patients that we enrolled, median age

53.

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Four women, ten men, the rest we can look at as well.

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Progression-free and overall survival look like this. It's not a randomized clinical trial, but it's compared to a historical control arm of the RTHG545, so we don't have randomized progression-free and overheard survivor.

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But in a Cox regression. The progression-free survival is significantly longer.

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So hazard ratio of death is smaller as opposed to this historical control trial.

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But of course, take it with a grain of salt because it wasn't randomized.

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We don't see the significant difference in overall survivor. Here's the patterns of response in progressive disease across our 14 patients. We had some that maintained a partial response for a pretty long time.

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Of course, also patients that progress very quickly. The most interesting finding were the patterns of failure within these patients where in the external control arm, the RTHG505 trial, we had a distant failure rate of 16%.

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So almost everybody who recurred recurred within the field of radiation. There was the planning target volume of radiation from the beginning versus in our trial.

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We had out-of-field recurrence in the majority of patients. And this is a very significant difference where we see in just two cases recurrence within the planning field, most of them actually recurred outside of this field of radiation.

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Which suggests to me that we are far from having efficacy readout because it was only 14 patients. But we do see that we have significantly steered the evolutionary dynamics of these tumor cell

populations in a different direction because it looks as if

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Within the time span of survival of these patients, we have sterilized.

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The radiation target planning fields and recurrence actually arises outside of that.

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Now the question, of course, is what to do about that given that we can't give that much radiation to the entire brain.

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But I think the next step is then to look at efficacy.

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Which we are now talking to Brigham and Women and Dana-Farber Radiation Oncologists that also ran our first clinical trial to actually expand this to 40 gray. There's another RTO3 trial that administered 40 gray to the recurrent setting.

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Because the hope would be that Of course, the higher we go, the more efficacy we will have and the lower the enrollment numbers are going to be in order to see statistically significant outcomes.

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And so the standard schedule here that we are going to compare to is 2.67 gray in 15 fractions.

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So this is now given over three weeks, five days a week, and then the same fractionation every day for the historical control, which gives us this constraint of BD equivalency of 93 grade that we can administer in this optimum schedule. And this is all work in progress.

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But Chris, who's here, where we're now looking at optimized schedules for this 40.05 gray.

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Schedule where we have already identified several candidate schedules that are predicted to significantly outperform this standard fractionation schedule.

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Some of these catchers look like those that are optimized, look like the de-escalating sketchers are best, where we start with high dose

and then later on have lower dose but more frequent fractionation schedulers. And if you look at the normalized volume change of dose catchers.

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As opposed to the standard. Again, this is going to be the historical control. Then we have a significant reduction in normalized volume change over those optimized schedules, really non-significantly different over the different Optima that we have found.

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Both in terms of total cell number and in this nesting positive subset of these more radio-resistant SARS.

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And so the sample size calculation is based on the hazard ratios that we scaled from the mouse model that we are so used to come up with the sample size calculations for our first clinical trial.

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Suggested we need maybe around 75 maybe to be safe, 85 patients to enroll in this patient.

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Trial. And so we're working on implementing this currently. If anybody's interested in the multi-institutional trial, please let me know.

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Make accrual much faster, but of course the bureaucracy are a little bit more complicated. This is in combination in collaboration with Stan Tangathari and Daphne has Cogan.

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At the Brigham and MGB. The next step then is concurrent therapy where we started thinking about temuzolomide and radiation just to address the newly diagnosed setting in the beginning. For that, we need to move to especially explicit model because unlike radiation, which is a field effect, so it doesn't really matter where

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Cells are localized, how much radiation exposure they get as long as they're within the target planning volume of radiation therapy. Now, timosolamide diffuses away from the blood vessels, so therefore the exact localization of the tumor cell population matters for exposure of the concentration.

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Of timosolamide that's present within the system. So what we have here is a cross-section of an agent-based spatially explicit model.

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That has these tumor blood vessels coming out at us like that. So the endothelial cells are the red cells and then our stem-like size, the SN positive cells are blue, and the differentiated cells are.

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Are green. And so according to the rules that we again parameterize this on this mouse model, we can now run forward stochastic simulations to identify optimum dosing sketchers in this increased setting.

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That might be a little smart to read, but where we have a radiation response and in addition a chemotherapy response that can either kill tumor cells or if not the age by one.

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So that we can keep track of not only the spatial organization but also the age structure within the cell population.

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And so what we did there was both optimize radiation scatters keeping the timosolomide administration schedule constant.

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But also keeping the radiation schedule constant and then optimizing the offset between temosauramide and radiation administration at standard fractionation.

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Thinking that that might be actually easier to implement as a clinical trial.

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And what we found here is that the optimum offset between timosolomide and radiation given at standard fractionation would be for mouse PK parameters of temozoramide, 41 minutes.

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For human parameters, 57 minutes. Before radiation, chemosomide should be given.

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And that also validated in a mouse trial where the suboptimum was given, tumosome at eight hours post radiotherapy.

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As opposed to the optimized where temozolomide would be given 41 minutes.

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Before radiation. And that leads to a significant survival difference. So that now is the question that we've been discussing with Chen and others, whether we should implement this as a clinical trial as well and what other evidence there is.

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Just by natural variation within different radiation oncology groups, whether they recommend tumothoramide to be given before radiation or maybe at bedtime or at a non-recommended time.

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And whether we could potentially do retrospective analysis. Of these data sets to see whether there would be a difference in outcomes depending on when timosolamide is given.

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We've also been working on optimum combinations with radiotherapy and PARP inhibitors in combination with one of my previous postdocs and also Anthony Chalmers group in Scotland.

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And also collaborating with Chen about the ATM inhibitors that he will talk about later today, and then we will also discuss tomorrow during the site visit a little bit more.

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And then the last aim is to look at patient specificity, where the goal is to adapt these models using patient-specific either biomarkers or ex vivo tumor assays that could be used to then come up with a patient-specific optimization for each individual patient.

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That can take into account also what these patients have already received up to the point of optimizing radio combination strategies. For instance, whether they're already at 60,000 or maybe they're already at 75 grand, so we only have maybe 35 or

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Or 40 grade left to optimize. We can also take real-world constraints into account. For instance, transportation issues or what if the bus is late, should we update the schedule later in the week?

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And so hot off the press is something that's also Chris Gassa's work that I wanted to show here as an example of how we can use in vitro data sets to try to come up with personalized optimizations.

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This is not specifically for GBM. But derived based on cell lines of a variety of different cancer types. But the question here is what if irradiation actually differentially impacted patients with respect to how migratory cells are that have been exposed to radiation.

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And so if that's true, that there might be a subset of patients that had positive correlation between those exposure and migration kinetics, then that's an interesting trade-off between how much radiation we should give to maximally inhibit the tumor site population

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But also maybe induce migration which could be related to metastatic behavior. So that might be a dangerous thing to do. If you have a patient where there's a negative correlation, then maybe that's a less important consideration because then the optimum for depleting the tumor cell population would also be the optimum for actually minimizing migration.

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And so we could imagine that if we had measurements such as those for a diverse patient population.

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That we could come up with a patient-specific optimization schedule to create individualized treatment plans for each individual patient.

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In collaboration with Khalid Lahave's lab at the HMS Systems Biology Department.

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Be characterized a number of different cell lines in terms of their migratory behavior in response to radiation exposure.

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Here on the x-axis at a different gray of radiation that were administered to these in vitro cell populations.

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And then we measure speed of displacement. And this is Brownian motion. So it's not a directed migratory behavior. It seems to be just random migration.

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But you can see that in some of those, this is a lung cancer cell line, for instance, there's a positive correlation between radiation exposure and migration.

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Which is interesting. So then we built this spatially explicit model based on the model that I showed you before, where we can now also take into account whether the cell population migrates outside of the target planning volume as defined for the radiation dose administration schedule.

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And we can then count how many of these escape tumor cells we have. The goal being that we now have another goal for the optimization.

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To minimize the escaped tumor cell populations if there is a direct correlation between these escaped tumor cell population potentially migrate… metastases or just in the brain as we've seen in previous talks, movement to other areas that would then lead to outer field recurrence.

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And so for one of these cell lines for this lung cancer example here, we can identify an optimized schedule, which is a de-escalating schedule over here.

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Which as opposed to the standard schedule, very much decreases both the escaped tumor cell count And the primary tumor cell count. So the prediction would be that would lead to better survival and also lower rates of distant metastasis.

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If this migratory behavior is really predictive of this metastatic behavior.

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And we can then read out for these different schedules that we have created here, the overall survival for the different patient populations.

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And then finally, using this. In silico clinical trial data set of 1,000 patients.

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We can then that are parameterized using distributions of radio sensitivity and also those speed coefficients as identified based on this in vitro cell population.

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Parameters. We can then use this in silico longitudinal data set, which only gives us based on these input parameters, the number of the

primary tumor cells and the escape tumor size.

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And we can then train a transformer on this longitudinal thousand patient data set.

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To back out the parameters that we can't measure in patients, but that we know what the gold standard is in this patient cohort because it's simulated.

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We can identify the migratory and radiation response parameters based on this machine learning approach, and this is work by Manuel.

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Shirk, who's also here, with the ultimate goal then of having a proof of concept of imagining a patient population where we have longitudinal measurements, for instance, of the primary tumor.

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And some metric that would be correlated to these escape tumor cells.

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For instance, proportion of CTCs in response to radiation. So if we treat a patient with standard fractionation, for instance, for the first, whatever, week or two of radiation.

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And measure longitudinally what these volumes are. We could then use this pre-trained transformer to identify the parameters of that individual patient, of the parameters that we can't measure in vivo.

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And then optimize given those parameters, a patient-specific optimum schedule for the remainder of the treatment administration schedule.

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And so that's just a proof of concept of how we are thinking about personalizing treatment.

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The problem, of course, is that it's very hard to get access to data sets like that. But that's why I'm so happy that you are all here and maybe you have some data sets that can help us solve this problem going forward.

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And with that, we would love to collaborate if you have any thoughts or any ideas for this. Big shout out to the lab.

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Big shout out to the CSPC. That's been super fun and also all the collaborators over the years that have enabled us to do this.

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Thank you.

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Questions? You know.

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I've always been very bad at math, so I'm happy somebody understands it.

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Two quick questions. One is a comment. You know, we think that we think that having more, enriching for stem cells is actually bad. We want to reach for differentiated cells.

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But you show it's the opposite, actually, really. And what, are you surprised by that? And if you want to comment on that.

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And the second question is, I was thinking that the other FDA approved treatment where this may be really helpful.

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I know we don't use it that much at Inafar, but it's the TTF fields. Because there it was sort of like empirical that patients had to use it for 18 hours at a certain a certain alternating current. It just seems to me that that's just like radiation. That's a device that's giving a physical

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Something. I'm just wondering whether you've been thinking about adding that in your model. Yeah, that would be great. We need some data to parameterize models like that. So I'd love to discuss where we could get access to some of those.

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The first question, yep. We don't do it very much, but there are other places that do a lot of it.

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Language classes? I'm trying to think. A lot. A lot of places around the area.

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At Mayo Clinic, don't you use a lot of TTF? We use it, but it's an offer to everybody.

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Probably Northwest won't be the best place, right? A lot of places they use it. Yeah, that would be great. It's best if we have detailed quantitative longitudinal data that we can use to paramilize these models.

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Sometimes the human cases are not detailed enough because maybe just given that one fractionation schedule or one combination therapy schedule.

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That would be interesting. And your first question, totally agree, and it surprised me. I think the reason is that they proliferate less quickly.

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So if you're only interested in delaying the time of the progression of disease, then that might be the way to get there. But it depends on the exact interplay between the growth rate of the differentiated and the nesting positive size.

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In this mouse model, it looks as if the NASM positive cells grow less quickly.

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Even after radiation has been discontinued. And that's the mechanism here.

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It seems. But of course, that's totally Probably mouse model, maybe even PDX line specific.

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And I wonder whether we could measure that somewhat to then again come up with a personalized schedule depending on what the ratio of growth rates is.

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Both before and after radiation. It's great work. I'm just sort of wondering if, similarly to you, you showed a bunch of data saying that we had these optimized schedules for tumor response. Have you looked at treatment-related necrosis, radiation necrosis, and other things with this? And there are these two things that you're balancing back and forth as you change the schedules? In the human schedule? Well, either, but in patients particularly.

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Yeah, so I mean, for the humans, the only readout we have is I guess we have some patient characteristics at the beginning, and then the readout is progression-free and over our survival. But the trial that we're designing now will also have longitudinal MRI

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Maybe even with some of the more modern methods that we have at the farmer now that could give us a little bit of functional readout as well.

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And I think that would be much more interesting because then we could even incorporate maybe some diversity measures from radiomics readouts.

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But I think at the end of the day, it's a problem of getting access to enough quantitative longitudinal information. It's even hard for us to get the MRI for everybody to be That's been seen with a longitudinal readout just to see whether the ratio of rates that we estimate from the mouse model

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Are correctly scaled for the human case. So for us, it's been a little bit of a problem of getting access to clinical data. If anybody has some, I would be very happy to.

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So take a look. Hey friends, it's a really nice talk. I guess the question I had was.

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About the generalizability of the model built on the gem. Generally, when we think about 10 gray given as a single fraction versus 10 gray given in five fractions.

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A single fraction is going to be much more effective. Just because of the linear quadratic nature of the survival curve.

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But in your, I think if I understood from the data, there wasn't a huge difference between a single fraction and fractionated And so I guess I'm wondering, like.

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How do you think about that as far as generalizing to a population of patients?

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This one here, right? Yeah, I think that's a good question, right? And that's partly why we're interested in looking at all of your data.

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To see whether we can write the goal for the first ATM, the AZ inhibitor project, right, is to take this model parameterized on that mouse model update with your data and see whether that's predictive for a a newly designed mouse survivor study.

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If that works, I think then that works That's the proof is in the pudding that that is generalizable.

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We haven't tried for many other mouse models. Apart from this one here, we have a few other RKS TVA driven systems with different molecular alterations. For instance, we tried this with non-inquire RF nuddle backgrounds and just PGFA overexpression.

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And that seems to have similar responses. But I think at the end of the day, the question is also going to be how much do we actually believe in these subtypes?

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Which is one of the questions I wanted to ask you, Antonio. We have all of the TCGA determined subtypes, and then we have your subtypes and then we have Brad Bernstein's data that shows that everything is everything anyways.

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So I wonder a little bit how, right, if we have to have a bespoke solution to each individual patient's tumor or whether it's just a very noisy system And the subtypes are maybe less important than we thought in the beginning. And I think that will also be part of the answer to this question here. And I don't think we know that yet.

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At least I don't know that yet. Thank you, Francisca. That was great. I was curious about the observation that you saw when you saw recurrence in the clinical trial. It was typically outside the field of treatment.

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I was wondering, could you go back to the pretreatment MRIs and examine those regions to see if there's any evidence of lesion or abnormality?

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That could have been spotted prior to treatment and maybe then actually treated? Yeah.

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They didn't look very carefully at all of them, but they checked a few.

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And there didn't seem to be any evidence. But I guess that doesn't surprise me all that much either, right? Because if you look at, right.

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One of the Antonio's talks and also this mouse model here, where if you actually labor the tumor cells. They're already over the entire hemisphere anyways, even though the contrast enhancing region would be a small subset of that.

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So I think the story is more like It's already everywhere before you start treating.

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You're just so happy to sterilize the region that you're treating with this multiple fraction high intensity.

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Fractionation schedule. If you could do that everywhere, maybe that would be one solution but that's too much radiation everywhere. That would be stereotactic whole brain.

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And I think that's the problem. So then the question is.

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How do we make it more efficacious? And then we are talking about radiation sensitizers and maybe cut turning it from a cold to a hot immune environments. And so I think at the end, maybe something like that in combination with

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Some of these other strategies to make each fractionation of radiation more effective.

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Should get us somewhere. Yeah, I'm kind of new to the field, so this could be a naive question, but I assume that the radiation and chemo also has effects on the macrophages and the myeloid cells in the tumor compartment.

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So I was just curious if you saw any differences in those cells with the optimized schedules and if those differences have been or can be incorporated in the mathematical models. Yeah, that's part of the goal of our CSVC. We don't have any data yet that would allow us to do that.

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I think some of the co-culturing experiments that Forrest was talking about.

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Which is also why I'm so interested in all the other co-culturing constellations that we can think about.

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Would help us with this. The mouse model is not super helpful. The human data is not super helpful.

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So I don't know what other quantitative data we could use to actually really parameterize this accurately. But it's probably going to be super essential.

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Godspeed to forest. Excellent. All right. Let's move on to our next speaker.

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All right. So next up, we have Natalie Artsy, who's an associate professor at the Brigham Women's Hospital, Harvard Medical School, also with an appointment at the Institute for Medical Engineering and Science at MIT.

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Her work focuses on development of new treatment modalities. For solid tumors, including GBM.

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Thank you very much, Forrest. And I'm so excited to be here today.

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My lab really focused on GBM starting about seven years ago when we realized that we've been working on very important problems, including many peripheral tumors. But in fact, the tools that we developed in the lab, some of the biomaterial-based tools are best suited to make a significant impact on tumors where barriers to delivery are so significant. So today I'll tell you a little bit about that story.

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And what we do in the lab in the context of GBM. But one thing that I think you should pay attention to, and it will be the theme across my talk, is that we really care about the time parameter.

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You've heard many talks today where we try to kill aggressive tumor cells, and that's the short term or the progression-free survival, perhaps. But we do need to train the immune system to help us eliminate the tumor. Maybe that will be then the long-term overall survival that we'll be seeing.

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So we try to use materials to control not only the presentation of drugs, but also the duration, how long the drugs will be in the brain.

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In order to educate the immune system and get some immune memory. So these materials can do a lot of things. One thing that they can do, and you can see here in green.

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Is a cross-section of an adhesive hydrogel we used to localize the delivery to the brain right after tumor resection.

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And in red or pink, you see here microparticles that break into nanoparticles that deliver, in this case, nucleic acid.

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And when you stain them nicely, you get a nice bouquet.

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And it's one of the things that we can do with those materials.

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So let me tell you a little bit about what we do in the lab. By designing and understanding the interactions between materials and drugs, we can develop better drugs.

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We developed different tools across length scales from nano and microparticles to macro scale materials like hydrogels that I'll tell you a lot about today.

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And as well as microneedle patches that are really cool. They are like a band-aid that you can apply with hundreds of projections of microneedles that are polymeric.

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They can release drugs effectively at a certain location. We use them both for internal organs as well as for skin delivery. And they're non-painful. Even when you apply them on the skin, because of the small micron scale, they don't reach nerves.

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So that we have a lot of things that we've done with those microneedles, both for delivery and diagnosis.

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But really, by understanding structure, function, properties of materials and their interaction with the immune system, we can engineer the immune system very effectively with the goal of training the immune system to get long-term memory, like a vaccine.

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So one of the projects that really represents some of this thinking in the lab is presented here. We essentially got an ARPA age grant last summer where we, in collaboration with colleagues at the Wies Institute, we discovered a drug. This is from Don Inberg's lab.

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An RNA drug that activates the innate immune system. It's an RNA sensor or the REGI pathway.

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Where we use DNA origami with William Shi's lab to present the drug in a certain way to better engage with the target.

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Then we use different nanostructures from my lab that in fact have different tropism to different organs and cells. And that allows us to shuttle the drugs to different areas and understand their mechanism of action. We may know what the pathway is, but where the drug goes and for how long it goes really affects the outcomes.

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And we don't leanberg, we really look early on at human chip models where we want to see what the effect on human cells is.

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So we leverage some of those tools now in the treatment of glioblastoma. And as you heard today, we know that glioblastoma following resection within six months despite radiation and temozolomide shows, again, usually recurs very close to the resected tumor. We also know that at the point where the tumor has been resected, if we use a different contrast, we'll see via MRI a hollow basically a halo that shows that

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The cancer cells are still there, but they infiltrated healthy tissue and cannot be removed.

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We also know that the tumor can be resected and still appear at another site.

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So the idea is that we probably need a brain-wide treatment in order to eradicate the tumor.

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But when we think about delivery to the brain. In order to get the drugs everywhere in the brain, we are really challenged with the blood-brain barrier.

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The other thing I want to highlight is the time period or the gap between the diagnosis to tumor resection And then from tumor resection.

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To the initiation of treatment. The six weeks gap between surgery and chemo radiation really facilitates the local spread of the tumor.

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And we thought, what if we could intervene early on just right after the resection of the tumor, we can apply materials before closing the patient. We can apply materials locally that will release the drugs Now we can release any drug of interest because we are not limited to the ones that cross the blood-brain barrier, and we can also prolong their release. And I'll explain to you why.

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So the idea is in addition to radiation and chemotherapy that are mainly cytotoxic drugs that are tasked with killing every cancer cell, we'll also leverage immune therapy.

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By training the immune cells to identify and eliminate the cancer cells, those cells can then circulate in the entire body, or in this case the brain.

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In the entire area and eradicate the tumor. So that was the premise of the work that we probably need to combine chemotherapy to eliminate the tumor as much as we can as this is a moving target, but also start to train the immune system.

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And since the training takes time, again, the time parameter, we need to combine them to get rapid killing as well as prolonged education and then elimination of the tumor.

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Some of the tools we developed in the lab, again in the nanoscale and then the hydrogels, are very different than other materials. Our materials are designed to be dynamic.

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To sense their environment and respond to it. And we believe that this enables better precision medicine.

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Here we design nanomedicines that are cell-responsive that were designed to deliver the stimulator of interferon gene or the sting agonist.

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You may know that the seaga sting pathway gets activated every time we get an infection.

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So we thought to leverage this mechanism to now alert the immune system, to recruit cells, activate them, and train them to eliminate cancer cells.

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But then these particles, while can be delivered systemically to treat peripheral tumors.

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Can be combined with the chemotherapy locally in our adhesive hydrogels to treat brain cancer.

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And that's what we thought to do. When we think about sting agonist delivery that you can see here on the left, as well as other nucleic acids, we know that those drugs cannot be delivered in a free form and be injected to the bloodstream because of rapid clearance and degradation and inability of these drugs to really get into cells.

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So in the field of nanomedicines, we use the negatively charged of these drugs to interact with positively charged particles.

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And these electrostatic interaction is usually stable enough to shuttle drugs effectively into cells.

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However, with the sting agonist, because it's such a small molecule, there's not enough ability to create a stable structure.

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So we created new structures where we chemically conjugate the drug to the particles.

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That renders it very stable. However, such a small molecule, any modification that you do will render it inactive.

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So we designed actually the particle to have a cleavable linker that is responsive to cellular cues.

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It can be pH, redox sensitivity, or in this case, we use the catepsin sensitive linker such that enzymes in the immune cells in the cytoplasm can cleave this bond and release the CDN drug, the cyclic dinucleotide, which is a sting agonist drug.

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So that's how it looks. It can be any core particle. In this case, we use polybeta aminoester, which is a very biocompatible material that can condense RNA or DNA molecules very effectively.

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And we use the catapsin cleavable linker such that in this cell it will release the sting The CDN will activate the sting pathway, resulting type 1 interferon release that then recruits a lot of our immune cells. And that's how the structure looks.

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A drug that we use, the Takeda drug, it was a collaboration with Takeda, a linker that is sensitive to the enzyme And in this case, the core particle was based on PBA, but it can be replaced with other cores as well if desired.

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So you don't need to understand all the chemistry, but just so you understand how long it takes to synthesize such a particle and drug.

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Indeed, what we need to do is to modify this CDN by protection and deprotection group to include the catapsin cleavable linker and then another set of deprotection and addition of malamide containing linker, we now have a group here, the malamide, that can click into a particle of interest.

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And this chemistry enables us to stabilize the molecule on the surface of the particle.

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But this needs also to be degraded. So we designed a cleavable linker to degrade on its own, and I'll show you how it's happening.

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But then we synthesize the polybeta amino acid particle. This is the synthesis that results in interaction of amine groups and acrylate groups with modification to include Tiol N41 groups.

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To then click the malamide modified drug to our particle of interest.

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Now we have a very potent nanomedicine. But it needs to release the drug. So we designed a cleavable linker to have two-step self-imulating degradation to have this scarless form of the drug.

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To make sure that it remains active. So all of this process Does it result in better efficacy? So to really look into that, we published a paper on melanoma, breast cancer, colon cancer, and I'll show you one result in a melanoma model that I think that really exemplified the importance of the structure of the particle

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In dictating efficacy. And you can see that this is the untreated mice in black. And when we delivered the free sting agonist, which is extremely potent, we push the survival just a little bit.

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The complex that has just electrostatic interaction improves the survival some more.

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But all of those mice succumb to the tumor. When we use the conjugated form that is cell responsive with the catapsin sensitive linker, now it's the same drug and the same dose.

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We now cure 100% of those mice. So we went on and took this drug and wanted to see how we can leverage that to treat glioblastoma.

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We developed this adhesive hydrogel that over the years we used to deliver a range of drugs. There are not many materials that you can

deliver small molecules, antibodies, nucleic acid within the same hydrogel.

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And the hydra just sticks to the site of interest, which becomes very important, especially in the brain.

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We could deliver chemotherapy antisense DNA, microRNA, sting agonist, anti-PD1, And let me show you what we've done in terms of the brain.

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So the idea was following resection. We can spray coat or inject this adhesive hydrogel to the brain. It will contain all the drugs of interest.

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It will adhere to the tumor cavity to the tissue following the resection.

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And right where all the leftover cancer cells are likely going to be, we start to release drugs to eliminate the tumor effectively.

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The idea is that it's easy to administer. We don't have to wait close the patients and initiate treatment after four or six weeks.

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The material can adhere to a range of tissues and control the release of the drugs.

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And we believe that the controlled delivery is actually critical. The material is composed of two polymer dextrin that we oxidize to form this aldehyde group and dendrimer that has 128 amine groups.

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This multi-valency enables very nice interaction and binding between aldehyde and amines to form the gel.

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But also, those anhydes can interact with the mines on the surfaces of tissues to form the adhesion.

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But the key is that we still have a lot of access functional groups that can interact with drugs.

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And that is what enables us to sustain the delivery of drugs over the course of weeks rather than a few hours.

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So the work that we've done in the context of glioblastoma was done by a very talented PhD student, Michelle Dion, who actually just graduated from the HSD MEP program here at MIT, who's done a fantastic job.

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She leveraged these tools to release drugs of interest, but to really use that to understand the mechanism of action of these drugs.

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And create some design rules by which we can train and educate the immune system to eliminate glioblastoma.

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What I'll show you here can probably be relevant to many other drugs, but we focused on a combination of drugs that we thought would be very interesting.

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One is doxorubicin. It's a very potent chemotherapy that when delivered systemically at a certain dose can cause cardiac arrest and toxicity.

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But what if we can use hundreds of these doughs because we now localize the treatment to the brain But really leverage the fact that it's 750 times more potent than temozolomide.

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The other aspect is that this drug can result in immunogenic cell death of the cancer, which means we expose all the antigens of the cancer cells which we believe is important in order to activate the immune system and synergize them

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Following the release with the activation of dendritic cells by the sting agonist. This is the same nanoparticle that I showed you before with the catepsin sensitive linker that can activate dendritic cells The first responders that we want to identify the tumor antigens

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Trained T cells to then become effector cells, activate them, and eliminate the tumor.

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We also use anti-PD1. And compare systemic versus hydrogel mediated

local delivery of anti-PD1, to maintain the activation of the T cells.

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And let me tell you already here that a systemic delivery of anti-PD-1 was not effective while the local delivery really enhanced the outcomes we've seen with the two drugs here, doxorubicin and Sting.

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But you can think about other combinations of drugs. The key here was to eliminate the tumor as much as we can because it is so aggressive and start to train the immune system.

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Have rapid response as well as prolonged response. So are these if a hydrogel contained the three drugs. And this is the premise of the work. We really need this sustained release. We call it phase one, two, three, where in the first phase

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There's rapid release of doxorubicin to kill the tumor cells, expose antigens, with CDN being released to start to train the immune system and recruit the immune cells to decide to make it more hot.

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While we do that for the next two to five days, we really want the drugs to be maintained over a week to make sure in particular that anti-PD-1 will be there to prevent evasion mechanisms from kicking in and generating immune memory.

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So the idea is to convert this immune suppressed microenvironment to immunoactive CDN high release and dox release will begin this process. Dendritic cells will present antigens to T cells. But another thing that is critically important is that CDN can potentially also recruit and activate natural killer cells

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And natural killer cells can work in a complementary way to that of the sting agonist because it's non-antigen mediated elimination of the tumor.

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Especially in glioblastoma that is heterogeneous, we want to make sure that we don't rely only on antigen presentation.

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We know that there's also downregulation and evasion of this mechanism. So having another way that is not antigen dependent these natural killer cells can sense stress on cancer cells, particularly following doxorubicin delivery, and eliminate those particles.

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In the last phase, we want to make sure we prevent immune escape. So we still want some of the CDN, but make sure also anti-PD-1 is there.

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To maintain their action and also generate immune memory. In our mind, the generation of immune memory is the whole reason why we use immune therapy.

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Like a vaccine. If we still have only transient response, we haven't done much.

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So indeed, with the hydrogel, we were able to include all these drugs and prolong the delivery of the drugs and retention in the brain that we believe is key in the outcome of what we've been seeing.

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I will skip the GL261 data because we got very good results in GL2 and GLP treatment. So now I want to focus on the CT2A model.

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The immune suppressed immune desert model that we, as expected, see that there is resistance to any of the monotherapies Whether it's doxorubicin, the CDN nanoparticles, or the anti-PD-1. But as we expected, when we combined the dual therapies, we start to see very nice increase in survival.

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But what was really cool is that the three-pole therapy that really made sense to us in terms of the immunity cycle and the long-term anti-tumor response indeed push the survival in such an aggressive model to 80% of the mice.

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The mice are completely cured. And we ask one, will they survive post-wheat challenge?

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To see if we have immune memory. And the other question we ask.

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Is it really that we need the prolonged delivery via the hydrogel or could we just take these three drugs and deliver them directly to the tumor intratumor Lee and get the same effect So the answer is the hydrogel is absolutely necessary. Intrato injection of the triple therapy resulted in only 30% of the mice surviving.

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But the most important point is that we challenge. When we now go and we challenge those mice, after 90 days, contralateral rechallenge.

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Most of the hydrogel group mice survived, while almost all the IT delivered drugs in a group succumbed to the treatment.

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So we see that indeed the prolonged delivery is important to educate the immune system eliminate the tumor, but also resist re-challenge because of immune memory.

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Now we probably have hundreds of graphs of immune phenotyping I'll just show you a few today to make the point that we really believe that we need almost all the immune cells in the brain to engage and work in concert in order to eliminate this aggressive tumor.

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We see that in response to our treatment, we have many more CD45 positive immune cells They go up in the CDN nanoparticle treatment group.

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But primarily in the combination groups. When we look at CD45 high, which are mainly the microglia, macrophage, and microglia, we see that indeed CDN and combinations increase those as well.

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What's interesting is that these macrophages and microglia were converted or polarized more so into the M1 phenotype. We just heard that more than 30% of the tumors contain these macrophages and microglia that support tumor growth.

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So the ability to convert them to the inflammatory phenotype is critical.

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We see less of the M2 phenotype by the reduction of the CD206 positive signal here.

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But the one thing that was really curious If we look already, it's only three days after the initiation of treatment. We see that these active microglia and macrophages contain PD-L1.

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So this means that we have a potential immune regulatory mechanism,

which makes sense. We know that it happens, but very early on, which really motivates the combination with the anti-PD-1. Otherwise, PD-1 on T cells can interact with the PD-L1 and become ineffective.

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And to remind you, we really want them to not only help us eliminate the tumor, but also result in immune memory.

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So that really showed us the importance of the combination that we chose, but also the sustained delivery.

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Just to show you briefly, dendritic cells indeed were activated, as we see in the combination treatment and the CDN.

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We see CD86 positive dendritic cells showing their activation markers, primarily high in the combination groups.

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We also saw that indeed natural killer cells, we had more of them and they were active.

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In response to the combinations and the CDN, but higher in the combination.

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And let me tell you, before we go, instead of showing you all of the characterization that we've done.

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We would expect that macrophages will play a role Because they're significant in the tumor microenvironment. But what about the scarce natural killer cells and T cells?

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And to really investigate their role, we eliminated, depleted those cells What was really interesting to see is despite the fact that we activate all of these different immune cells Depletion of just the CDI T cells, for example, eliminates the efficacy of this triple treatment.

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It really shows us that every immune cell is almost as important in this treatment efficacy.

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Same goes with natural killer cells. They're so scarce in the immune

microenvironment And yet, when we eliminate the natural killer cells, we eliminate the treatment efficacy almost altogether.

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So we suggest that rather than looking only at the dendritic cell T cell axis, as we tend to look at the immunity cycle.

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We now believe that macrophages, especially in the brain, play a significant role, both by direct tumor killing As well as by releasing chemokines and signals that will recruit effector cells Like T cells and natural killer cells. And those really help us because of the difference in time scale of their activity.

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Natural keto cells can rapidly eliminate cancer cells in the non-antigen-mediated manner. They don't need to be trained.

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So this can happen fast. As we start to train the T cells to recognize and eliminate the tumor and create immune memory.

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So we believe that this modified immunity cycle is important to take into account and can inform some of the selection of treatments that we choose to treat tumors and glioblastoma in particular.

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I just want to mention some cool projects that now we collaborate on with the forest.

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And Yufei here, Michelle worked really hard with Yufay to start and understand how the treatment of doxorubicin and CDN together enhances potentially antigen presentation or diversify the antigens.

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And can we use that actually as a way to provide also a vaccine or these peptides using, for example, mRNAs to encode for those to further eliminate the tumor to result in complete elimination and memory, anti-tumor memory. We're very excited about this potential, and especially in heterogeneous tumor, and one of the things we really need to consider, as Forrest mentioned, is which peptides actually are we going to choose

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It seems like doxorubicin CDN does a pretty good job, particularly probably for the tumor cells that share these peptides of interest. So the question is, should we target the more scarce one maybe we enriched for over time.

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So this is something we're very excited about. With that, I would like to summarize. I showed you that biomaterials can really be designed and leveraged to respond to tissue and cellular cues to maximize therapeutic efficacy.

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By designing nanomedicines that are cell responsive, we could enhance the therapeutic window and understand the mechanism of action of the drug.

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By chemically releasing those drugs from hydrogel locally. We could very effectively eliminate glioblastoma, but more so expand the therapeutic window of those drugs such that we can understand the mechanism of action. And because we start with a much lower dose.

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And can sustain the release, we really get a much safer and efficacious therapy.

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With that, I'd like to thank you for your attention. To the funding sources, the collaborators. Michelle Dyon, who led this project, and some of the other people in the lab.

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We helped her with some of the surgeries and the flow and really getting a ton of organs from hundreds of mice to be analyzed. That was really almost a lab-wide effort. So thank you very much for your attention and I'm happy to answer questions.

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Okay, questions?

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Thanks. I'm just interested. You showed the model, plasticized model, of a resection cavity and filling the resection cavity with your polymer and that's going to be your model for delivery. Is that what happened in these mice or was this a direct injection of the mice? Is there a… an actual mass effect from the injection itself that needs to be taken into account and then

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Do you have data on how far the drugs diffuse out into the mouse brains after these injections?

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Yeah, thank you for the question. So now in the mouse models, we did

not resect the tumor because it's very easy to cure the mice if you also resected the tumor. So we injected intratumorly.

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The volume of the gel is very small. We're talking about microliters.

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So there was no mass effect. We could inject it easily.

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Ultimately in patients, we could use it in a tumor for the non-resectable tumors, for sure. But the idea would be following resection during the surgery to apply it to the patients as well.

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So that was the illustration of what we ultimately imagine we'll do.

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But I think one thing that would be very curious is in a window of opportunity trial, following a biopsy just to analyze if this is GBM, now you can get results within a few hours. You could go in and inject directly and then a few weeks later when surgery happens, you can take the tumor and actually learn a lot from it.

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And that will be a second opportunity for us to then apply the material post resection And for newly diagnosed patients, I think it can be phenomenal because before we really have all the immune evasion mechanisms, this can really change the landscape of the tumor.

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You're relying on diffusion to get the drugs out. Yeah, exactly.

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You're targeting a very different part

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Yeah, so we did see there is some diffusion outside. I don't think it's a very significant, but that's the reason we're training the immune cells to then circulate everywhere in the brain to eliminate the tumor.

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And the fact that when we challenge the mice and we inject the tumor in the other hemisphere.

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And the mice can reject that was a good indication, at least that that process works well.

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Okay, other questions?

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Thank you for a very interesting talk. I might have missed this, but what happens to the hydrogel? Is it biodegradable or is it… So does it degrade over time and that would probably increase the diffusion I'm assuming from the previous question. So the hydrogel degrades over time. This imine bond that forms is hydrolytically degradable. So it will degrade, and that's one of the mechanisms by which we release the drugs over time.

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And indeed, the more degrades, you have less gel to prevent the drug from going in.

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By the way, when we also kill the tumor, especially here in the tumor, when we did not resect it, there's less extracellular matrix and less inhibition for the diffusion. So indeed, initially it's diffusion. It will be enhanced over time as the gel degrades.

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And you don't need to then, of course, eliminate the gel or another procedure to remove it. It would just really degrade over time.

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Thank you very much for your presentation. Is the idea that these tools also target metastatic node?

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Yeah, it's a good question. So since we train the immune system, as long as we trained it broad enough And the metastatic cells won't be very different from the ones in the tumor in the primary tumor.

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Then it should work. In cases where there are clear metastatic lesions, one could consider injecting to primary lesion as well, a big metastatic lesion as well.

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To better train the immune system to eliminate those as well.

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Yeah, so for example, if it's antigen mediated, then all the cancer cells that were killed and antigen exposed from the primary tumor, as long as they exist in the metastatic cancer cells.

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Will be eliminated potentially using this treatment. If they are very

different, and that's part of why we want to look at those peptide vaccines in combination with this treatment.

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To account for those that maybe are slightly different in the primary tumor.

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Thank you. Really cool and interesting. I didn't really get what's still missing to try this in humans. So what are the next steps?

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Would human cells be viable in this hydrogel? Would you be able to deliver cells as well, like CAR T cells, for example?

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Yeah, so a few questions. So in terms of getting these to patients, there are a few sting agonist drugs that are still in clinical trials, not for glioblastoma, but for other tumors. Actually, there's one now, Northwestern is doing for a glioblastoma.

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So we're looking into those and it's very interesting what they'll get. Again, usually they use the free sting agonist No vehicle, so we know the results can be very different, but still interesting to see what they're getting. Doxorubicin is also used by Northwestern

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It's delivered systemically though with opening of the blood-brain barrier via focused ultrasound.

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Another very relevant trial for us to watch. We believe the combination of everything we're doing should be better, especially because of the sustained delivery that we show, at least in the mouse model, is very, very important.

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And then the second question that you had? Yeah, cells can live in the hydrogen, not necessarily this formulation. We have many other formulations.

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So if you think about CAR T or Karen K or any other idea, those potentially can be delivered using the hydrogels.

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In combination with other therapies. Okay, so we've got a couple of online questions. So Monica Zamish asks, very nice work. Two questions. Do you know the antigen specificity of the immune memory

cells?

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So you want to answer that one first? Yeah, so in terms of antigen specificity, we're now running OVA models to look more into that.

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But then the work that we do with you also, Forrest, will shed a lot of light on that.

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So yeah, hopefully we'll have more results soon. And her second question is, do you think this combination therapeutic approach could be useful in the context of other aggressive cold cancer types like PDAC, for instance? Yes. The answer is yes. We believe it will work in other tumors as well.

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We worked on breast cancer, breast cancer, colon cancer, and melanoma. Breast cancer is probably the colder out of those. And even just the CDN with the anti-PD-1 worked pretty well. We believe that when we add other therapies like chemotherapies and others.

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It will work even better. And something that we're really interested in is to understand how this works with radiation and other clinically available modalities.

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That can expose, again, more antigens and recruit more immune cells to the site.

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Rakesh Jane says, congratulations on developing this elegant approach. A major problem with the Glideau wafer was that the drug was washed out rapidly from the blood vessels around the post resection brain cavity.

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Do you anticipate this problem with your gels? Yeah, thank you, Rakesh. And that's a very good question.

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We've done some of this work in collaboration with Henry Brem from Johns Hopkins.

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And he was excited about this collaboration primarily because we showed that with the hydrogels, we can now prolong the release rather than get an immediate washout, almost like a bolus injection of the

drug.

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That happened with the Glia del wafer. So that's why these chemically conjugated drug And the fact that we have a gel that has so many functional groups that can hold these drugs a place and as it degrades, it will release the drug. It's critically important in the design. Otherwise, we won't see

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Much of an improvement like we've seen here in the IT injection of the combination.

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Excellent. So last question is from Divya Sinha. And she says, thanks, Natalie. As you mentioned, the blood-brain barrier is a barrier, sorry, is a hurdle to GBM therapeutics compared to other tissue tumors.

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Can you review if and how this is addressed in the nanomedicines hydrogel cell responsive structures developed in this work? And could the application from in situ to IV depend on this and affect the response? What was the second part? So how does the blood-brain barrier affect basically your therapy?

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And yeah. Yeah, so to really overcome the need to cross the blood-brain barrier, we simply place the hydrogel right where the tumor was.

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So that eliminates that. But the presence of the BBB will also affect the ability of peripheral immune cells or other factors to potentially enhance outcomes in the brain.

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So that was nicely exemplified in some of the work I didn't show today, that systemic delivery of anti-PD-1 that we thought can enhance migrating T cells from the periphery to be more active.

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It didn't really pan out so well either. So we definitely think that the brain is almost like a a separate box with its own immune system, the cervical draining lymph nodes are very important and probably communicate there between the results in the communication between the brain and the periphery, but still the periphery is so much more isolated.

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And again, if we think about combination of systemic therapy with some

of the local therapy.

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We'll probably need to open the BBB using focus ultrasound or lead technologies and some other ways.

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To leverage both compartments. Awesome. Thanks. Thank you. All right. So let's thank the speakers from this morning again.

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And we have a lunch for the speakers in the back and another lunch upstairs on the third floor for the trainees.

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If you're not a speaker and not a trainee, there's a bunch of restaurants in the local area. All right.

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If you sit down so that we can start and stay quiet. So I am very pleased to introduce our next speaker, Dr. Stephanie Spranger. She's a professor in the Department of Biology at MIT and her lab focused on tumor immunology, dendritic cells, and the interaction between tumor cells and immune cells. And the forest has been instrumental in hijacking her.

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And making sure that now she becomes excited in working on GBM. This is our goal towards Stephanie.

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Thank you, Antonio, for this. Great introduction. And it's really a pleasure being here at MIT. And Antonia's right. Forrest got me excited. And then I got Amy, a trainee in the lab, excited to work on GBM. So we're now shifting towards this very, very horrible disease.

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I will, for disclaimer, mostly talk about non-GBM related work. And mostly talking about immunology. So I will be going slow because I know cell-cell interactions of the immune system can be daunting. So we in the lab think very heavily about

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Anti-tumor immune responses along the cancer immunity cycle. And I want to introduce on how we think about this.

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We think initially cancer cells, dying cancer cells, cancer cell debris has to be sensed by innate immune cells.

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Specifically, a very specific subset of innate immune cells called cross-presenting dendritic cells.

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And these cross-presenting dendritic cells infiltrate the tumor mass. They sense dying cells. They pick up tumor cell debris, and that induces an activated and migratory profile in those disease.

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That then allows those centritic cells to go to the closest secondary lymphoid organ.

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In most cases, this is the tumor draining lymph node. In the tumor draining lymph node.

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T cells that see the cognate peptide MHC complexes that are now cross-presented on the surface of the dendritic cells with peptides that are derived from the tumor.

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Are activated, they expand, and they differentiate along certain very preformed differentiation trajectories.

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Once activated and fully expanded, those CD8 T cells then hone back into the tumor microenvironment. And in many cases, the homing signals of those effector T cells to go back into the tumor are also dependent on Dendritic cells and sometimes myeloid cells that sit within the tumor microenvironment.

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And then once they're in the tumor, these CD8 cells have the capability to recognize antigens on the cancer cells and eliminate cancer cells. And this is where the power of immunotherapy and the immune system Recognizing cancer really comes from.

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If this would work, as I just described it, cancer wouldn't be a clinical problem because our immune system could get rid of the cancer.

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So one often targeted, therapeutically targeted example of how the immune system fails to eliminate cancer is T cell exhaustion.

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This is not, as I depicted it here on the slide, a one-time switch in

the tumor microenvironment. This is actually an entire trajectory that happens as the T cells are being activated.

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But once they reach terminal T cell exhaustion, the T cells are long-lived. They stay in the tumor, but they can no longer kill the tumor cells.

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And that's where most current immunotherapies across many, many different cancer types are actually acting on to prevent this terminal T cell exhaustion from occurring.

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The first cancer type that I want to talk about is non-small cell lung cancer, which if you take a 3,000 foot view is actually an immunotherapy sensitive cancer type.

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However, if you look really closely. Only in about 30 to 40% of patients is dual check pumpkin immunotherapy consisting of an anti-PD1 and an anti-CTel A4 antibody.

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Actually efficacious in the long term, which is certainly not enough. And we as a group wanted to understand how can we get this further up. And this is just another way to illustrate this. And fairly early in our studies, we wanted to compare this across different cancer types.

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Because our hypothesis was, is there maybe something about how the immune system is engineered For each specific cancer and each specific tissue site that prevents responses towards immunotherapy.

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So Maria Cerugalier, a previous student, went through many clinical data sets where cancers were treated with dual checkpoint locate immunotherapy, so similar to what I showed you on the previous slide. And here just reported the objective response rate following this dual checkpoint locating immunotherapy.

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And what struck us was the two most responsive cancer types were Merkel cell carcinoma and cutaneous melanoma.

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Merkel cell carcinoma, much less known, is a skin cancer, cutaneous melanoma as a skin cancer.

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And then everything else sort of triples off with the high mutational burden tumors obviously rising somewhat more to the top.

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And non-small cell lung cancer actually falls somewhere in the middle of the responsive cancer types.

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And that really solidified our hypothesis that maybe there's something about the specific tissue Architecture and the immune system for each tissue that mediates response or non-response.

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And in addition, if we zoom in clinically into what is known about the archetypes that are within non-small cell lung cancer patients, we can stratify them into four different discrete populations.

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With this one here being the best understood, we have T cells, we have exhausted T cells, and we have, and this is important, upregulation of PD-L1 on the tumor cells.

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And PD-L1 is a downstream target of interferon gamma. And interferon gamma is made by CD8 T cells that are properly activated. And that, if you put these pieces together, means There are productive T cell responses in those tumors if we have PD-L1 upregulation.

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The other cancer type that is far less understood but clinically reported is termed non-functional T cell response.

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And these cancer patients have non-small cell lung cancer patients, have T cells in their tumor mass, but they lack the upregulation of PD-L1.

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And this was what we thought maybe The tissue-specific immune constraints might play into Because why are these T cells not properly activated? Why are they not doing what they're supposed to do?

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So first, Brendan Horton and then later Maria Surugalia worked on this problem.

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And the way Brendan approached this is he used a cell line that is derived from a tokenostrum model driven by KRAS.

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Mp53. And then simply implanted those cells orthotopically. We can do this by tail vein injection or intratracheal injection. We get the same phenotypes. Or subcutaneously, which is an absolutely artificial site.

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But it models the immune response towards a cutaneous tumor. And then treated with dual checkpoint locate immunotherapy, similar to the patient data that I showed you.

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And we were quite surprised that the lung tumors were absolutely resistant to checkpoint locate immunotherapy, while the flank tumors reduced in size by about 50%.

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And this was even more stoic when we looked at actually what happens in the tumors themselves, because we found quite a lot of T cells in the lung tumor lesions.

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But they failed to expand following checkpoint blockade immunotherapy. Versus in the flight tumors, we had fairly low at baseline, but they drastically expanded following checkpoint.

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And that gave us the indication this is probably a T cell intrinsic defect to respond to therapy and not something the tumor imposes on the T cells.

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So we then took an unbiased approach to understand how are these two T cell responses different.

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We used single cell RNA sequencing in collaboration with Chris Love's lab. And these two clusters down here are the activated CD8 T cells.

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We then stratified them back on which tumor site did they come from. Cluster one.

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Is mostly from flank tumors, while the lung tumors mostly had cluster two T cells.

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And then… Of course, this is a polyclonal T cell response, so we then engineered in a model antigen, which allowed us to really go after The

tumor reactive T cells, and we saw no differences.

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And then when we looked at what are the differentially expressed transcripts, we found that the cluster one T cells have all the markers that we would canonically associate with an exhausted T cell response.

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And in contrast, and we're still trying to piece this all together, the cluster two T cells have some markers of tissue resident memory T cells.

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Or central memory T cells, or tolerized T cells. But they're not effector T cells and they're not exhausted T cells.

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Is this relevant for humans? We can generate signatures based on these differentially expressed genes.

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And then impose them onto non-small cell lung cancer patient data sets and actually see that in the large majority of the T cells, the till that we find In the non-small cell lung cancer patients themselves have this dysfunctional phenotype as we called it.

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Well, only a small fraction of the T cells have this truly exhausted T cell response.

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And that indicates to us, even if the patients have a mixed population, we might not be leveraging all the T cells that are in a non-small cell lung tumor patient.

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With current checkpoint blockade immunotherapies. Now, the lung is a very different site than the flank for tumors to grow. So we wanted to understand when in the T cell activation cascade does this difference occur.

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And for that, we used a trick. We used, again, this model antigen called SIY, and we used T cells that are transgenic for a T cell receptor that is specific for this one peptide.

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We label those T cells with a proliferation dye and then transferred them in and let them be activated for three days in mice that have

tumors.

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And then look specifically in the tumor draining lymph node. And what we found is that the activation profile here read out by dilution, because every time the cell divides, the dye gets diluted, we see roughly similar kinetics of T cell activation over time.

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But then when we pull out the activated T cells from those lymph nodes and do sequencing on those T cells, and I'm still blown away by the number of differentially expressed genes that we found.

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Just three days, 72 hours after we put them in, we found over 1,000 differentially expressed genes.

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Indicating that this bifurcation of taking an exhausted T cell differentiation versus a dysfunctional tolerized T cell activation happens very rapidly.

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As the T cells are being activated and probably within the first three to four cell divisions of T cell activation.

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Two of the very relevant functional markers that we used for subsequent studies are CD25, which is the high affinity IL-2 receptor, as well as granzyme B, which is a cytotoxic molecule. And these are higher upregulated in the inguinal lymphoma and the T cells become activated.

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Maria then really wanted to understand those dynamics in the lymph node, like what imposes this tolerance promoting microenvironment that pushes the T cells down this absolutely non-effective differentiation path.

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So to first establish, she identified that cross-presenting dendritic cells indeed are the cells that drive T cell activation in both lymph nodes. And then she looked at the activation signals that these DCs provide to the T cells.

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The first thing we looked at was just antigen density and antigen availability. And for that, we used a trick where we engineered the tumor cells to have Z is green, which is a pH stable fluorophores so we can track it in the dendritic cells.

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And when we looked then in the dendritic cells in the corresponding draining lymph nodes, we find that the mediastinal lymph node has more antigen.

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We looked at various different ways of how you can sparse this out. We always found more antigens. So we excluded signal one as the main problem.

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But then when we looked at signal 2 and 3, meaning co-stimulation as well as cytokine profiling, we identified that there's far less co-stimulatory signals that push T cells into an effector state in the mediastinal lymph node.

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And far less IL-12, which is a key cytokine associated with cytotoxicity in CD8 T cells.

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So that by itself. Led us to hypothesize, well, maybe the dendritic cells are intrinsically different between the two lymph nodes. And Maria has done a lot of experiments disproving this hypothesis.

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And then we were looking for a third cell type in this interaction that would actually or could actually impose this immunosuppressive feature on the dendritic cells.

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And we ended up hypothesizing that potentially regulatory T cells could suppress dendritic cell activation very specifically.

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And to first test that, Maria established an in vitro co-culture assay.

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Where we sort out dendritic cells as well as regulatory T cells from the same lymph node.

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And we specifically used dendritic cells that have taken up antigen.

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And then co-culture them with tumor reactive T cells. Either in the presence or absence of regulatory T cells.

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Here on the left, you see that indeed dendritic cells intrinsically are not dysfunctional. If you only have DCs and CD8 T cells, we get really nice effector T cell differentiation.

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If we add regulatory T cells back in, and this is a very, very low ratio of regulatory T cells, we get very significant suppression of effector function, but not proliferation. And this was exactly what we saw.

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In vivo in our mouse model. We did a lot of other in vivo experiments that I'm not going to show for time constraints, but to summarize, we have established that the cross-presenting dendritic cells that sit in the tumor draining lymph node interact with both regulatory T cells as well as the CD8 T cells.

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And the regulatory T cells via an MHC class 2 dependent interaction.

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Inhibit the upregulation of CD80 and 86, as well as the production of IL-12, and by doing so, suppress the CD8 T cell activation.

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So why can regulatory T cells in the mediastinal lymph node do this better?

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Compared to Tregs in the inguinal lymph node. That was our next question. So we then thought, well, maybe it's density of regulatory T cells or number of regulatory T cells. Neither of these was the right answer because both of these are about the same between the two lymph nodes. And then we again collaborated with Chris Love's lab

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And did single cell RNA sequencing paired with TCR sequencing. And that was really key because we were able to understand by clonotype analysis which part The population of the T-Rex is actually expanding in response to cancer.

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And quite nicely, we found that it's the same population of Tregs that are responding to the cancer. They even have shared clonotypes, meaning they're seeing the same antigen.

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But when we compared exactly this population down here. For their differentially expressed genes, we found the Tregs that sit in the

mediastinal lymph node are more skewed towards the Th1 phenotype.

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Allowing them to be potentially more suppressive for CD8 T cells and cross-presenting dendritic cells.

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I'm going to show you the flow validation for this. We find more CX03, which is a key Th1 marker, as well as TBET on those regulatory T cells in the mediastinal lymph node.

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Th1 is downstream of interferon gamma, so we then hypothesize Is there a higher level of interferon gamma in the mediastinal lymph node?

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Maria established an ELISA to actually assess this, and indeed there was more interferon gamma present in the mediastinal lymph node. We then said, well, there's more natural killer cells responding to the cancer that we put in. Maybe that's a consequence of the tumor growing in the lung.

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We looked in naive animals and actually identified The difference was bigger.

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In the naive lymph nodes, indicating this is a true tissue specific constraint or manipulation of the immune system.

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And because… The lung is a mucosal associated lymphoid or mucosal associated tissue. We hypothesize this might be a consequence of having commensal bacteria somewhere in the animal. So we looked in germ-free animals and indeed we identified that having no commensals reduces the levels of interferon gamma in the mediastinal lymph node specifically.

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And this is for recurrently following up on. So to summarize this part, what we identified here is that the regulatory T cell suppressed DCs very specifically, and by doing so inhibit the dominant CDA T cell responses in this setting.

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But how can we therapeutically fix this problem? And this is something where collaborations with engineers is really an amazing tool to get at those mechanistic insights. So we found in our differentially expressed gene list that cytokine receptors like CD25, so the high affinity IL-2 receptor, and IL-12 were differentially regulated.

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So we collaborated first with Dane Whitrub and later with Forrest on this.

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To understand can we use half-life extended cytokines to actually push these T cells into more effector-like phenotype.

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And indeed, we can. We can upregulate CD25. We can upregulate granzyme.

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In this very nicely translates into a very significant survival benefit.

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When we combine these two cytokines together. And then Alicia and Forrest's lab really worked with Brendan on this to understand it's not just 2 plus 2 is 4. It's actually 2 plus 2 is 10. Because IL-2 by itself changes the signaling in the T cells a little bit. Il-12 changes the signaling in the T cells a little bit.

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But the both together really drastically change what is being phosphorylated in the T cells, amplifying the immune response quite substantially.

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So now I talked a lot about non-small cell lung cancer.

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What about cancer types where we don't have any immunotherapy responses, much similar to GBM? I'm slowly going to walk my way to GBM, trust me.

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The next cancer type that we got really excited about was ovarian cancer for many, many reasons, but one of them was it's a very dismal disease.

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Immunotherapy is not working, very similar to GBM. And it's mostly not working because we don't understand how the immune system really is is built normally to fight peritoneal tumors.

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So what we're doing here is we use a cell line that was originally derived in Bob Weinberg's lab.

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Which we call CPAC, and it's using the mutations or overamplifications of cyclin A1P53.

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Akt2 as well as KRAS, and it models a homologous recombination proficient high-grade series of ovarian cancer.

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And this is one of the most dismal diagnosis you can have if you have ovarian cancer because not even the PARP inhibitors actually do anything to your survival benefit. The only thing that does work is frontline chemotherapy four out of five women actually recur within

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Three to four years. So first, coming in with the same premise, can we build a differential model system to understand what is not working about the immune response to ovarian cancer in the peritoneum.

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We first tested. Is immunotherapy, are these cancers really immunotherapy resistant? Fiona, the graduate student working on this, put the tumors into the peritoneum, treated with dual checkpoint blockade immunotherapy, and this is the survival curve. You can see immunotherapy doesn't work.

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When she does the same thing and puts the tumors in the flank now.

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And treats with immunotherapy, there's two things to note here. The tumors grow, and then even without therapy, there's a period where the tumor is actually being somewhat controlled.

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And when we add immunotherapy on top, we really see this reduction in tumor control phase and then slow escape and we think this is actually an antigen loss phenomena that we see here.

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So yes, we again build a model system where the flank tumors are sensitive to immunotherapy.

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And the orthotopic tumors are not. And this is ongoing research. This is just a flavor of what we're doing here.

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Here we then looked at the T cell responses and we find again that

there's more T cells in the IP tumor lesions fewer in the sub-Q tumor lesions.

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But the ones in the IP tumor lesions have more markers of activation But less markers of function. This is very reminiscent of what we've seen in the lung tumor setting.

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And we still have to do more work on understanding what the T cell phenotype really is.

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But then Fiona also asked the question. What about the dendritic cells that sit in those tumor lesions?

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As I told you very, very beginning of the talk, dendritic cells are important to re-stimulate T cells.

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As they enter the tumor. And what Fiona observed was There's an accumulation of a very unique dendritic cell subset and without Going too much into the specifics of dendritic cell biology, typically we would expect dendritic cells up here in this flow cluster and down here in this one, and we would not expect this double positive population.

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With one exception, the mesenteric lymph node and the Lamia propria, because these double positive dendritic cells have been reported to induce tolerance to food antigens.

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In the gut. Yet in these ovarian cancers, what we find is that over time we see an accumulation of these dendritic cells. And our working hypothesis now is these dendritic cells might induce tolerance in the tumor to tumor-derived antigens.

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And that's what we're pursuing. And we think here in the ovarian cancer space, we might actually have a dual hit where the cancer evades somehow productive priming.

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And then also whatever is being produced is being further shut down in the tumor microenvironment.

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Now to GBM. Can we also use the same principle to understand why

immune responses against glioblastoma are not working, and what can we do to then make them work.

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So Amy used a saline C22A. She engineered them to express the ZS green fluorophores, so we can track dendritic cell biology, as well as synthecal ova antigen.

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And then treated them with dual checkpoint locate immunotherapy. And this here is the flank tumor setting.

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So you can see when we put the tumors into the flank.

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By itself, without therapy, these tumors are somewhat controlled. But when we layer on therapy, we get 100% cure rates. But again, this is flank.

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But it gives us the lever that we have, a productive immune response against a GBM cell line.

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When we put these exact same cells, exact same cell number, intracranially into the brain.

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Immunotherapy doesn't work. So we again have a model system where we can really understand what works against a subcutaneous flight tumor And what is missing in the cancer immunity cycle in the brain.

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And again, some early indications that we have is that something very similar might be true, as we see in the ovarian cancer space, where priming might already be very dysfunctional, but then also in the tumor microenvironment, the antigen presenting cells that the T cells see might also be dysfunctional. And to follow up on Natalie's talk, that merits really a multi-layer therapeutic approach where we enhance systemic priming.

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And then we layer on a remodeling of the tumor microenvironment.

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And with that, I would like to thank the people that have done the work. This was really a team effort. Brendan started this. Maria followed up.

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This is all the lung cancer team. Fiona is working on the ovarian cancer together with Brett and Grace and Amy and Heidi are our GBM.

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Star team. And with that, I would like to take questions.

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Thank you, Stephanie, for the this beautiful talk. Any question?

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I was just going to ask, can we speculate a little bit evolutionarily? Why is the brain such a cold place?

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Is it just because it's so dangerous if something gets up there and therefore everything that could possibly be, right? Yeah. I think the brain… The three organs that we're looking at so far, I think, are cold for different reasons.

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I think the brain, you cannot allow any swelling or any inflammation because we need the brain to live.

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So we need to protect it. And only if we really have an insult that is high enough do we get inflammation in the brain?

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The lung is a single layer epithelium, so if you get too much cytotoxicity, you're going to kill. So in this case, I think it's more the immune threshold to trigger is high.

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When it triggers, it triggers, but then the second layer immune suppression is also really high. And that's what we have to combat in lung cancer.

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And I think the peritoneum is generally super prone for inducing tolerance.

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And I think we're going to see very different mechanisms at play for all three. And so then isn't it Isn't the way to solve this problem then to look what the epigenetic differences are that regulate the coldness of these different must be epigenetic, right?

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And then try to see whether you could re-engineer whatever that

epigenetics are. I'm not sure it's going to be epigenetics. I think it's most likely going to be cytokine mil years that determine thresholds for immune activation. And then also how many tissue specific regulatory T cells are around that can suppress. And this is very cancer specific, I think.

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Infections don't typically have self antigens that they bring along. Cancers have plenty of self antigens that they bring along. So you will always have DCs that have Selfantigens plus antigen CD8 T cells can see. So the Tregs are just right there to say, no, no, no, no, no. This is something we don't want to kill.

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And I think… the opposite of cold brain is a hot brain, which is multiple sclerosis. You know, demyelinating disorder, multiple sclerosis, we just have lots of inflammation. And I think that just tells you why you have to keep the brain cold.

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That's just as devastating a disease as Encephalitis is the other example. Any swelling, any killing of neurons in the brain will have devastating effects.

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Thanks. Great talk. I was wondering what the antigen presentation networks look like inside the brain. Is it different types of dendritic cells?

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How do T cells circulate in the brain environment? I just don't know as much about it.

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Very good question. We're learning as we go. The first flow plots I've seen, I'm like, Amy, are you sure this is how the dendritic cell populations look? By now, we've done enough. There's an incredible skewing towards DC2s in the brain.

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Even just naive brains. This might actually be amplified with a tumor there.

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What we're missing for the brain is an actual infection model or an MS model where we see In a hot brain, does the ratio of dendritic cells really change? The draining part is another really interesting one, and Amy is working really hard with Heidi

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Find out where does priming really happen, because that's still not fully clear.

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Stephanie, we have a very long question from the audience here. Can you read it? You're the chair. It's too long to read.

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The TRX density density

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Okay, the question is about Treg density in the mediastinal lymph node.

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The Treg density was the same, but the proximity of the regulatory T cells to the DC1s was increased in the mediastinal lymph node versus the regulatory T cells were further away from the cross-presenting dendritic cells.

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In the inguinal lymph node. So we think it's a proximity and actual cell-cell interaction. And Stephanie, I have a question, actually. I was wondering if in the lung cancer model, now instead, rather than implanting orthotopically lung cancer tumor, right, in the in the lung. You would implant a different type of tumor let's say

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Breast cancer right there or anything. Would you find the same type of tolerance In other words, do you think that you are detecting here tolerance because you are using orthotopic model or is it really an innate state of tolerance of those tissues? We've done

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A few studies using melanoma. To address that early and it's not fully fleshed out but It depends on what you compare it to. So we use B16 to see lung tumors versus flank tumors.

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The immune response against the melanoma metastases in the lung is weaker compared to the primary subcutaneous melanoma. But it is stronger compared to a primary lung tumor.

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So it's somewhere in the middle, which I think brings me back to there is a component of antigen-specific succession. That's exactly what I was thinking about. But then the other interesting experiment that we did is we put a lung tumor in first.

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And then challenged for the subcutaneous tumor. The original priming of the CD8 T cells is dominant.

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And we cannot reverse course by putting in a second tumor in a more immunogenic cycle. Which is the more natural history. It's a mix of components.

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Okay, I guess if… I don't know, there are more questions. If not, we… Thank you, Stephanie. We move to… The next speaker who is… My good friend, Dr. John Sarkary.

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Professor of radiation oncology at the Mayo, where he runs the traditional neuro-oncology lab and he has a major role in the National Resource Hub, the generation And the characterization of GBM PDX that all of us have been using.

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I know he's a long-term collaborator with the forest but I don't see us working on mouse models without John.

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All right. Well, thanks, Forrest and Francisca, for inviting me. To talk about some stuff that I think is really cool. I did my postdoc at Mayo and developed the kinase assay for ATM.

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And now we actually have drugs that can hit ATM, so it's super cool and it's kind of a nice merging of pharmacology and radiation oncology, which are the two things I really like.

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I work with a lot of people. And I was told for an ASCO presentation I should have the key points first. So here are my takeaway key points.

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Wsd is an ATM inhibitor from Weishine Biopharma. It's a very effective radio sensitizer.

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But as I'll talk about, careful integration with with radiation therapy, planning techniques, and pharmacology is really going to be important to move this forward in clinical testing.

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So ATM was discovered by analyzing a number of children like this. This child got a fairly low dose of radiation, 30 gray and 19 fractions with cobalt.

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For Hodgkin's lymphoma, which she ultimately was diagnosed with ataxate telangiectasia syndrome.

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Which is a homozygous inactivation of ATM. And that led to this discovery of the biology of ATM, which is a simplified version is shown here on the left, which So it's really a key orchestrator of DNA damage response.

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To ionizing radiation, especially double strand breaks related to ATR and DNepk as other family members.

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But is a central And super important pathway.

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And so now we have… the kinase we've identified drugs that can hit this kinase. And so essentially we're going to use a drug to cause what is happening with this child. We're going to inhibit ATM completely.

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And give radiation there. So it's something that needs to be thought about fairly carefully.

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Here's just data with WSD 0628. You could change the name to any other ATM inhibitor. You'd basically see the same data. On the western blot, you can see that we're robustly inhibiting autophosphorylation of ATM.

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That gets induced with radiation. And then suppressed by about 10 to 100 nanomolar. And CAP1 is a direct phosphorylation target of ATM. And so you can see loss of CAP1 phosphorylation.

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That's induced by radiation and suppressed by the drug. On the top right is a classic clonogenic radiation sensitizer study where 30 nanomolar has really robust radiosensizing effects.

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To give you some perspective, clinically used radiosense studies like cisplatin.

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Would be not even close to what 10 nanomolar is showing. So this is, you know.

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As a radiation biologist looking at this curve, you're like, dang.

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That's something that's pretty rocking. And then this is the bottom curve is really important just to understand When we give a drug to a mouse or a human, it doesn't stay at a constant level like it does in the dish.

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So you kind of need to understand, well, how long do I need this drug around to do its thing?

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And so here we dose these cells with the WSD compound and then radiated them and then wash the drug off at different times after And so that's what you're seeing on the x-axis, the time the drug was washed off after radiation.

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So you can see if you wash off the drug at four hours versus 12 hours.

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There's more killing in the red line with the radiation plus WSD.

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If the drug's on for 12 hours and so on to 16 and 24. So really kind of providing us a target. Hey, we want to maintain The equivalent of 30 nanomolar in cell culture for 16 to 24 hours to get really nice effect in animals and in humans.

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And so this was a drug that was engineered to be a brain penetrant.

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And we kind of superimposed a target based on free drug levels. But basically, you take my word for it in that 6.5 nanomolar, that's kind of our minimal target.

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And with dosing, we should be able to achieve that for at least 12 hours.

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And then in these studies here, the A through C is 2.3 gray times 10, so this is a reasonable fractionation schedule of radiation and you can see radiation placebos in black, radiation alone is in gray, and radiation plus drugs in red. And I would say that's a pretty kick-ass response.

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Like if I had a tumor and I was going to be treated, this seems like it would be a good drug combination.

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But, like we showed you with that child. Atm inhibitors can cause a lot of toxicity.

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We actually were having problems with our mice dying when we were giving the radiation to the brain. We figured out, well, we're skimming along the top of the roof of the mouth.

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And they were having severe toxicity and weren't eating and losing weight. So we kind of said, well, let's kind of study that a little bit more directly.

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And so here we actually have a model where we're reading the entire oral cavity of the mouse. You can see that yelled out areas where the radiation beam is coming in the the upper and lower incisors are shown in white there. So we're ridding that entire oral cavity

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On the bottom there, we're basically giving the radiation and then weighing the mice daily after radiation. And a week after radiation.

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They get essentially a sunburn on the inside of their mouth and they stop eating and drinking, lose weight.

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And if they lose more than 20% of their body weight, then we euthanize them.

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So you can see with three fractions of 5 gray, the blue lines.

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The mice are fine. They don't lose any weight. This is exactly what we'd expect from human experience as well. When we double up and go three fractions of 10 grade, they all get sick.

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Has to be euthanized. And then with the lower dose of radiation plus drug Yeah, they get sick.

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You can see that what happens in the middle there with H&E where you have a nice stratified epithelium on the top, but with the drug plus radiation you lose that epithelial covering of the mucosa.

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And the dose response is quite dramatic. This is a plot where Each dot represents five mice that are radiated and we're measuring how many of those have significant body weight loss.

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And so you can plot this and kind of figure out like.

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What dose of drug causes what kind of change in toxicity And the notable thing to say is, well, okay, with no drug.

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The dark blue line, it takes about 27 gray and three fractions to give this severe body weight loss that requires euthanasia. Whereas when you're at five milligrams per kilogram or 7.5 milligrams per kilogram.

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You only need around seven or 10 gray of radiation. That's a dramatic difference when we think about it in the clinic, we're going to do this.

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That could be a significant toxicity. I think it's interesting.

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We see a fairly similar dose response relationship to improve survival in the animals. So this is an orthotopic GBM model.

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And we're dosing the mice with drug just before a single acry fraction of radiation.

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And similarly, like at 1, at 2.5 milligrams per kilogram, we're seeing a doubling in survival with radiation. That's pretty good.

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It's pretty similar to what we were seeing with the oral mucosa.

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So… That gets us to this dilemma.

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The best radio sensitizer I've ever seen. Any ATM inhibitor, it's not just WSD.

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But you have a lot of risk. And so how do you balance the risk versus the benefit? And that's really kind of what I want to talk to you about today.

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So first, when we're thinking about just generically developing a radio sensitizer.

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It better be worth it. If you're going to take the risk of being like that child.

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It better be worth it. And so in recurrent GBM, We saw earlier today the risk of death is about 50% at six months.

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And it's almost universal by a year. That's pretty worth it, especially when you're in your mid-40s or mid-50s and recurrent GBM.

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Yeah, I'll take that risk. But then how do you minimize that risk?

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And part of the thing that we really want to figure out is what do we need to radiate?

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Defining exactly what to radiate is super important. So we've done a lot of studies with MRI compared to F-DOPA PET, which is a large amino acid.

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That's taken up, it's FDA approved for Parkinson's disease. Imaging, but also useful for GBM.

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And you can see the MRI scan is on the far left.

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Let's see if we can find this. This is the F-DOPA PET scan. And a lot of times we use the contrast enhancement to define where the tumor is.

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But you can see in this example in a recurrent GBM, there's a lot of tumor that's lighting up outside that contrast enhancement. And this is just a summary of 20 patients we did on a trial.

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The yellow is the overlap between those two signals. One imaging modality is not enough.

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I think it was, you know, so if we really want to define our tumor better, we better use both.

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Really just to illustrate this point, I imagine there's not many radiation oncologists in the room, so I thought I'd go over like, hey, how do we use this?

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So a newly diagnosed GBM patients, we typically would just use, historically have used just the MRI And we'd outline, as shown in blue here, where the contrast enhancement is And one thing that's notable is there are some regions that are really bright and easy to see.

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And there's what we call wispy enhancement, which is pretty wimpy. And then we'd expand that volume by one to two centimeters and we'd have, this is our target.

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And if we did that without thinking about the PET scan, yeah, there's There are some areas that they're not the greatest, but it's pretty good.

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Well, as a physician, pretty good is not good enough. And certainly if you were a patient and you said, well, it's pretty good.

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You know, that's not going to really go over very well. We really want to know where it is exactly. It becomes really important for recurrent disease These patients have already gotten 60 degree radiation. And so we're very tight on our margin. So if we're using contrast, we're just expanding by a couple millimeters

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And going to target that region. Well, you can see like If we just did the MRI in this patient, we would miss a lot of tumor.

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And there's no surprise here, if you treat half the tumor, the tumor is not going to do any good. The tumor's coming right back.

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And so targeting is very important. The reason that targeting is

important is that we're defining our target. Okay, we've used both imaging modalities. We've defined our target.

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But then we develop our radiation plan. And this patient here, standard plan, we'd bring an arc, we'd come from the front and bring that machine and come over to the side and then we'd come with a mohawk and come up over the top

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And that's going to, you know, x-rays go through and through, right? And we're having the high dose region here where we outline the target, but there's this lower dose wash that It's just not particularly useful. We're really potentially more toxicity and you can see

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From that mohawk beam We're having dose that comes into the optic nerve.

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And so just to kind of, again, kind of tell you how we think about this, this is a very small portion of our prescription for radiation therapy.

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And we define various parameters about here's how much dose we want to the target.

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How much we're willing to accept it, not get exactly the dose that we prescribed.

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But then we also talk about normal tissues and we said, okay, well.

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This region around the optic nerve, we want it to be less than 20 gray and less than 25 gray on the right side and the left side. And you can see, oh.

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Dang, here, we're well above our target. We accepted this plan because we're Maybe we're going to be on this optic nerve a little bit high, so the risk is somewhat high that we'll have blindness in one eye.

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But this other eye is well below our target. So unilateral blindness.

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Is a risk that we're willing to take. It's not a given.

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But we've got to accept that risk. You think about it, as you add in a radio sensitizer like an ATM inhibitor.

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What is that risk? Without the radius instruction, we have decades of experience. We understand that risk very well with an ATM inhibitor, we don't understand that risk very well. And so there we're thinking, well, let's Maybe there's a different strategy we can use if this is our target volume

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Is there a way to do this better? And it really kind of goes to radiosurgery type techniques as we bring more and more beams in.

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We can spread the low dose out better and have a much tighter high dose region and limit the the normal tissue that's going to be exposed to radiation.

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And that, I think, is going to be one of the important things as we think about just generally these really potent radio sensitizers is doing a better job. And so I think…

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These are known risks. We know this is going to be a problem.

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Definitely strategies we can use to take care of this. I think I've seen several clinical trials with very potent radisins that did not think about this.

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And they failed. They were unable to achieve are clinically meaningful concentrations of drug, and those drugs were discontinued with external beam radiation.

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So I think kind of thinking this through is super important.

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Anyways, the data that I just presented earlier about the ATM inhibitor, that led us to this clinical trial that we're running at Mayo Clinic.

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It's the first in human evaluation of this ATM inhibitor in recurrent

GBM. Again, we chose recurrent GBM because the risk-benefit ratio is a little bit more acceptable when we have no idea what the drug is going to do.

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This is a pretty standard radiation regimen that we The first day they're getting drug only and then the second day they get drug plus radiation And they go on for 10 days of radiation.

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We're right now in the dose escalation cohort and then We'll talk about… One thing, and we'll talk about tomorrow, is the tumor penance cohort and how that could play into some of the work that we're doing.

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Because there we're going to give drug and radiation and then harvest the tumor with Ian's help.

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So just a schematic again for the non-drug developers. Generally what we do is we start out with a dose of drug that we think is going to be completely safe. But we also think it's probably not going to be that useful. So we want to escalate through these low doses and get up to higher doses where we think we're

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Going to be more effective. And so far we've accrued on these three dose levels and we just opened to this dose level here.

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But the question is like. If I do a really good job as a radiation oncologist and I don't have a lot of toxicity because I'm avoiding the skin and other mucosal sites and stuff like that.

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The drug inhibiting ATM should be very well tolerated for a short term.

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These children grow up, yeah, they have some problems, but for several years of life they have no symptoms.

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Two weeks of inhibition of ATM should be completely well tolerated for on-target drug effect.

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So what's the effective drug dose level? How do you think about doing that? Part of it is like, okay, yeah, if we run into really bad toxicities.

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That's something that tells us we can't go any higher. But these are going to be toxicities from most likely off-target effects of the drug And so in this case, we've had a few, one patient with liver enzymes elevated.

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And then one patient with some cerebral edema that increased, but they had a rapidly progressive tumor This happened early on in the course, and we think that was not drug related.

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But I think kind of what we think is going to be useful is looking at the pharmacokinetics in the plasma And then correlating that with the pharmacokinetics that we get in our animal models.

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And so the only take home here is that this looks like a real drug. It has a half-life that's on the order of several hours.

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So we should be able to inhibition for several hours after radiation. That window of 16 to 24 hours is probably realistic.

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With even a single dose of drug, but if we had to, we could give a second dose of drug five, six hours after the radiation.

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But this is what I want to really think about. If you remember that one graph I showed where we were looking at the efficacy extension with radiation in the orthotopic tumors.

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And this is the plasma profile that we were looking at.

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One mic per kg wasn't very effective. 2.5 mg per kg was pretty effective.

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5 and 10 was even more effective. So that kind of says, okay, in the mouse.

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The free drug levels in the plasma we probably want to be, just empirically, we'd say we want to be right here.

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And in the tumor, we understand partitioning in the tumor. So we could say this is what we measured in the tumor in those mice.

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It all makes sense. One mg per kg didn't work, 2.5 did.

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If we take this now and just put it over on the human data, where are we?

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And so we're not quite there yet. These are the different dose levels, five milligrams, 10 milligrams, and 20 milligrams in patients.

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We're starting to get close to the target, but we really want to be above that. We probably want to be a log higher in plasma concentration.

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You know, we're thinking like… Yeah, we may be getting there, but we're actually amending the protocol to go higher than that original dose escalation that I had shown.

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Just because I'm nervous that, you know, will we get above, will we get, you know, have a reasonable duration, I'd like to get something like this, like five or 10 megs per kg exposure inhuman, so we need to clearly need to go higher.

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But we have an interesting anecdotal response. We all get to show the anecdotal, hey, this looked awesome. This was a patient with recurrent GBM.

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We gave radiation plus drug. She had a really nice response, regression at one month and Even more impressive at three months.

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And if we really hadn't thought about the pharmacokinetics and didn't measure the pharmacokinetics.

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You know, the unbound tumor concentration is a little bit low, you know, but if we… If we look at total drug concentrations our maximum drug concentration not shown here, is above what the IC50. So if we were just saying, hey, what's the IC50 in cell culture and what did we achieve in humans, we'd be like high-fiving all over, like, yeah, this was awesome.

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But if we start thinking about, OK, well, in our animal models, we needed this unbound concentration that's kind of in that 10 to 100 nanomolar range We're just not there. And so this is probably, even though unusual, just a really awesome result from radiation only.

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And I think it kind of illustrates the importance of thinking about this and modeling that a little bit more carefully about the pharmacokinetics.

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So just in general for this clinical trial, we're continuing our dose expansion. Like I said, we're going to add a few more dose levels.

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Then once we identify an effective dose level, we'll go to a include patients that are going to the OR for resection.

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They'll get the drug, then they'll get gamma knife radiosurgery And then we'll resect the tumor so we can look at both drug levels in the tumor, but also look at some of the pharmacodynamic markers for target inhibition.

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One of the strong reasons why we included Gamma Knife in this, which is not our standard of care at Mayo Clinic.

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We felt that the patients needed to have some benefit. And just getting an ATM inhibitor And that's it in a surgery really provides no benefit to those patients.

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Whereas the gamma knife, the high dose region is going to be resected anyways as part of the resection, but potentially that low dose bath There's going to be some low dose from the gamma knife.

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And would we have some benefit there for those patients? And then we have plans to expand this into newly diagnosed once we are comfortable that it's reasonably safe.

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And that's all I got to say. Thanks so much.

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Thank you, John. So if you amend the protocol to go higher in the dose

escalation, so now you have three patients with grade three or larger toxicity.

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Go up, right? How will we manage that? Sorry, I didn't catch the… You said you want to amend the protocol to go higher in the dose. And so for the for the patients that you've dosed now, we have three grade three or four

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Toxicities, right? That's then going to go up as well or is there a way to circumvent There were three grade three toxicities. Two of them where liver enzyme elevations that happened in one patient.

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And then another was a cerebral edema that happened we felt would have happened whether they got radiation or not. They were rapidly progressive tumors probably patient we probably wouldn't.

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In retrospect, enroll on the trial just because they're not in great shape.

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We didn't feel those, those didn't meet the criteria for being dose-limiting toxicities. And so we're comfortable with the escalation. There's a whole schema about Prioritizing how many patients are evaluated for toxicity if they develop a toxicity, then we potentially add more patients

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So there's like a, and then if you have toxicity at one level, then you drop down to a lower level So it's kind of a whole system that…

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Thanks. So Jan, this stuff is really exciting. One question I have for you in the development of these drugs, though, and you talked about this.

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The other option for the therapeutic window would be to lower the dose of radiation.

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And so you really, dose finding to elevate the dose of the drug But you also could potentially lower the dose of radiation. And as we, I mean, you and I have all seen these patients who go on to these radio sensitizing studies where the toxicity has been absolutely terrible, just like that patient that you showed.

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So how do you envision that? That becomes a much bigger matrix of searching around for dosing in patients. How do you envision that?

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Working. Right. Yeah, I think it's challenging to significantly dose reduce the radiation because you have the proven therapy And it's There's no expectation that every patient will benefit from a novel therapy.

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Generally, maybe 50% of patients would benefit from a novel therapy And so you're kind of saying, well, I'm going to roll the dice and 50-50, you're going to get a subpar therapy because you don't see sensitizing effects with the radiation that's now at a lower level.

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So the way I would envision it in a different way would be to say we give There's really very few drugs that we give daily as a radiosensitizer in, say, lung cancer. It's commonly given you give chemo with radiation, but we typically give it

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On one week every three weeks or something like that. So there's nothing that says you have to give it a radio sensitizer every day.

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And so I would rather… have, say, a two-week or six week course of radiation and give radiation full dose of drug, if you will, one or two weeks out for maybe a third of those treatments.

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And then the other days not have drug versus giving a low dose of the drug and have kind of subpar sensitizing effects.

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But that's actually stuff we were just talking with. Francisco about like, can she model that?

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Gentlemen, I have one question. You showed really beautiful data in the mouse. There you showed, I think, a couple of models probably. And I was wondering how different is the response, right, from one model to another because going back to the issue of

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Diversity between patients. I am sure that there are tumors that can be identified before you enroll them in the trial, potentially, that may be completely resistant to this, right? Typically what we see in the lab when we treat them with radiation cypress, radiation are the

mesenchymal, right?

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Gpm tumor. Those are the most resistant. And they are, I personally would not want to enroll those type of patients, right? Because I think it's the wrong therapy for them. On the other hand, those in the neurodevelopmental axis might be really exciting opportunities where you can also probably go down, right? With the drug, right?

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Have you thought about that? Have you seen anything? I know we spoke a little bit, but have you seen anything in the mice?

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Or even in the patients, right, where you may have data to correlate the status of the tumor with the response.

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Yeah, it's a great question. There's a reasonable amount of data before our lab published it as well as something we published with another ATM inhibitor that PC mutation.

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Those tumors tend to be more sensitized with the drug, with an ATM inhibitor.

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With radiation. But to address that question directly, we're doing a screen with 40 different PDXs so we'll do intracranial implantation randomized to placebo radiation or radiation plus drug.

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Across 40 PDX models. See what the spectrum of response is.

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And hopefully identify predictive biomarkers. I would love to work with you on that. That would be really exciting. Okay, fine.

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Thanks. Yeah, Jan, I was just thinking about, given Natalie's talk earlier and just thinking about sort of local delivery versus systemic delivery.

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And is there an option in your mind for trying to, instead of giving the ATM inhibitor sort of systemically to try and give local delivery where you're going to irradiate Yeah, yeah, for sure. I mean, I think… two strategies might be two strategies might

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It's challenging in a GBM, but in other sites, potentially an anti-drug conjugate or similar strategy.

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To have a tumor specific delivery of your radio sensitizer. And to try and limit that normal tissue toxicity.

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Some type of depot nanoparticle formulation or what have you that would dispersed drug over time.

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There's some challenges, obviously, having uniform distribution of that drug throughout your tumor volume.

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And the residence time of that drug staying in the volume But those are, I think those are interesting questions. I mean, radiation is inherently a local therapy.

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And so combining two local therapies is very reasonable.

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Yeah, for sure.

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Thank you. I just had a question about with the increased cell death that you're seeing in the mouse models, are you also seeing increased immunogenicity and have you tested this with any immune therapies? That is a great question. So we just did our first cholinogenic assay with CT2A and GL261.

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Just to see in vitro whether it's a radio sensitizer for those lines.

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It looks like it is, and so we're going to look at that in the syngeneic model and then talk to you guys.

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Thanks, Jan. Just curious back to the local therapy ideas. Have you thought about or used a gamma tile approach to deliver the radiotherapy?

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And combining it with the drug? Yeah, we haven't. I think… Yeah, we're not a gamma tile.

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Group, I think you'd hand the microphone to Ian and he could talk to you more about why we do or don't use gamma tiles. Well, we don't because you guys don't want us to.

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I give it back to you. No, we've not done that. We've debated doing that. There's been, of course, various types of brachytherapy for gliomas and various flavors for a long time, 40 plus years.

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This is the latest flavor. We haven't pursued that, but perhaps… the context of a radiation sensitizer, we could really look at that.

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Okay, I think if we have no more questions, thank you, Jen.

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And… And… We should go now with… talk from Dr. Michel Mongi, who will be online.

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Hi. Nice to see you.

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Oh, here she is. Hi, Michelle. Nice to see you. And I'm happy to introduce Michel Monge from Stanford University, where she is a professor of pediatric neuro-oncology there.

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And she has done a pioneering work in the characterization of the connectivity between tumor cells and the normal brain. We are very excited to hear your talk.

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Wonderful. It's so nice to join you. Thank you for letting me join you from my office.

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Thank you, Michelle. Wonderful.

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I'm going to talk a little bit about the neuroscience of brain cancer.

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I'm a pediatric neuro-oncologist, and as I think many people in the audience likely know, cancers of the central nervous system in children happen in particular locations at particular ages.

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And this is especially true of gliomas. In fact, I remember as a

trainee in the partners program.

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Or what used to be called the Partners Program, now MGB, and attending saying to me, you know, if you tell me where a child's tumor is, I'll tell you their age.

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And I remember being just really struck by that and started paying attention in adult glioblastoma to the location predilection kind of overlapping with regions of the nervous system that we know to be relatively plastic and adaptable.

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And so together, this suggested to me that Gliomas in particular, but perhaps brain cancers in general may be diseases of dysregulated both neurodevelopment and also neuroplasticity.

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And just to introduce the audience to some of the tumors that I'm going to focus on in this talk.

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This is a low-grade NF1 associated optic pathway glioma. This is a tumor that tends to occur in fairly young children. And I'm showing you an MRI scan here from a four-year-old.

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It's a low-grade tumor, doesn't tend to transform. It is a survivable malignancy but often causes really severe disability and often blindness.

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In contrast to the low-grade nature of this tumor, here you see a diffuse intrinsic ponting glioma, also called an H3K27M altered diffuse midline glioma of the pons. This is one of the most aggressive imaginable cancers with a median overall survival of only 11 months.

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And a molecularly related disease that happens in slightly older children, DIPG tends to happen in mid-childhood around ages six to seven.

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In slightly older children peaking at incidents around age 10, H3K27M diffuse midline gliomas of the thalamus tend to occur. And again, very diffusely infiltrative There is no central mass, unlike GBM. There is just this diffusely infiltrative component that affects the entirety of the structure, in this case, the brainstem, in this case, the thalamus, often both thalami.

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And then in adolescence and young adulthood, hemispheric hygricliomas, including kind of the pediatric version of GBM, tend to occur.

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And when you take a step back and think about the developmental processes that might correlate with the time and place incidents of pediatric gliomas.

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It strikes you that developmental myelination is happening in the times and place that these tumors tend to form. And as I think probably many in this audience know, myelin fascinatingly is develops chiefly postnatally in a process that spans about 30 years of neural development.

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From just before birth until late 20s, early 30s, myelin continues to develop and it develops in predictable chronological and topographical patterns.

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Such that at a time when there is a discrete wave of developmental myelination in the brainstem and more particularly in the ventral pons and the corticospinal tracts.

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This is when diffuse intrinsic pontine glioma tends to occur in mid-childhood between ages of six and seven.

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Similarly, at a time when there's a discrete wave of neocortical and cortical projection fiber myelination, this is when hemispheric high-grade gliomas of childhood, including pediatric glioblastoma, tend to occur. And these observations are concordant with findings from my laboratory and from many others that

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Origin of these high-grade gliomas in children, but also in adults is frequently cells that are in the oligodendroglial lineage, in the myelin-forming cell lineage, either bona fide oligodendrocyte precursors or OPCs or just earlier neural precursors that are committed to go down this oligodendroglial differentiation pathway.

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And so we may learn really important lessons about gliomogenesis by better understanding what normally regulates the behavior of these healthy glial precursor cells. And so when I started my lab now about 15 years ago, one of the first questions we wanted to ask was what

does regulate?

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The proliferation and functional differentiation of oligodendrical lineage cells. And one idea that was in the literature at the time and that I found really intriguing was the idea that neurons themselves may regulate the extent to which their axons are myelinated, that they may do this in an activity

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An experience dependent way thereby kind of tuning the dynamics of a neural circuit by modulating the myelin and therefore the speed of action potential conductance.

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So we wanted to understand if neuronal activity promotes changes in the oligodendrogly lineage, particularly OPC proliferation, and whether in general myelin might be plastic and adaptable.

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To experience an activity. And what we found was that indeed, in the healthy nervous system, particular neural circuits, particular neurons exhibit this very clear plasticity of myelin.

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Fine-tuned changes that happen in the oligodendroglial lineage initially with proliferation of OPCs in an activity-dependent way through activity dependent paracrine factor secretion those proliferating OPCs then generate new oligodendrocytes, which in sheath regions of axons or entirely unmyelinated axons. And that change in myelin alters the sort of tunes, the dynamics of a circuit

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In a way that in the healthy nervous system promotes coordinated circuit function and contributes to learning and memory and other brain functions.

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So then the question was, if neuronal activity promotes the proliferation of these healthy oligodendroglial cells, what happens if there's an oncogenic mutation in that OPC?

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Could this really powerful activity induced regulation of the normal precursor be hijacked and subverted in the context of glial cancers?

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And so now about 10 years ago, we leveraged tools of modern neuroscience like in vivo optogenetics to ask if we modulate the activity of a neural circuit of a particular genetically encoded

subtype of neuron, does that influence a glioma that may be growing within that circuit?

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So we performed optogenetic stimulating initially these cortical projection neurons and the motor planning area. This evokes some complex motor output from the mice. So we know that we've successfully recruited activity in that circuit. And then we can ask this really straightforward question about how Other cell types respond to changes in neuronal activity. And we did this in the context of a diffusely infiltrating pediatric cortical glioblastoma from one of my adolescent patients.

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And what we found is that when we stimulated these cortical projection neurons in the premotor planning circuit, that there was circuit specific increases In glioma cell proliferation. So just like their normal counterparts, OPCs, these tumor cells increase the rate of proliferation and that this results in an overall increase in tumor burden

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Specifically within the stimulated circuit. So this was the first demonstration that brain activity can influence brain cancer growth.

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More recently, and in collaboration with David Gottman's lab at Washington University, we leveraged a genetically accurate mouse model of NF1 associated optic pathway glioma that the Gottman lab has spent many years developing and optimizing.

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In this mouse model, the tumors in the optic nerve form very consistently at precisely nine weeks of age.

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And we can ask questions about the activity of the optic nerve and how that might influence the tumors that ultimately form there. So again, using optogenetics, we found that if we stimulated the optic nerve.

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From six weeks onwards and then looked at the object nerves at 16 weeks.

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That the tumors that formed in the stimulated mice were much larger than those that occurred in identically manipulated litter mate controls in whom we simply did not turn on the light.

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Now, this opt-in pathway glioma model is a really exciting opportunity to ask questions not only about tumor growth but also about tumor initiation, because again, we know exactly where and when these tumors are going to form in the optic nerve.

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And we don't really need to use optogenetics. It's the visual system. We can just change visual experience to modulate activity in the nerve.

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And so if we put mice into dark rearing conditions. We thought maybe in the beginning we could just put cool glasses on them. That doesn't work.

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I'm kidding. But if we put them into 24 hours of darkness around the time that these tumors form, or even just afterwards.

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So putting them into dark rearing conditions at nine weeks of age or at 12 weeks of age.

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And then examining the optic nerves at 16 weeks of age. When we examine the optic nerves, we find that mice that were dark reared to decrease visual circuit activity had far fewer and much smaller tumors compared to litter mate controls that were simply raised with normal visual experience with normal

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12-hour light and 12 hour dark cycles. And when we did a control experiment to say, well, is it that we messed up their circadian rhythms we could Control for that by light entrainment, 15 minutes of light at their waking and 15 minutes of light at their transition to night periods of 7 a.m. And 7 p.m.

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To keep their circadian rhythm synchronized we saw no difference in this effect. We still did not see tumors in the absence of normal visual experience.

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If instead we put mice into complete darkness at 12 weeks of age.

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Prior to the initiation of this tumor, that when we examined the mice at 16 weeks.

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There are absolutely no tumors compared to 100% tumor initiation. In litter mate control mice that were raised with normal visual experience. So there's some really powerful interactions between neurons and nerves and brain cancers.

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And over several years, we discovered that one major category of mechanism is that there are neuronal activity regulated secreted factors, paracrine factors that signal between active neurons or other cell types in the environment that are responding to activity.

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And the cancer cells themselves. And those include some expected peregrine factors like BDNF, brain-derived neurotrophic factor.

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Is something that we found to be a key paracrone factor mediating normal neuron OPC interactions.

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We've also found a shed form of a synaptic adhesion molecule called neuroligand 3.

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This is a very crucial signaling molecule between neurons and glioma cells. We find that it's also an important signaling molecule in myelin biology.

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Now, this Neuraligand 3 discovery was kind of surprising. At the time, we didn't know that neuroligin 3 was any kind of mitogen in any kind of context, and we didn't even know it was cleaved or released. So we wanted to understand more about that.

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And in studying Neuroligand 3, we wanted to understand how relatively important it might be. So there are many mechanisms that are intrinsic to the cancer cell that promotes growth and survival. There are several microenvironmental mechanisms at play. We wanted to understand the relative importance of neuraligin-3. So we simply

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And this is work that was led by Hamza Vengitesh, who's now in your town, on the other side of town and the Longwood campus, running her own independent lab.

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And what Hamza did is she took these patient-derived brain cancer cells and xenografted them into the environment of either the neuroligand 3 wild type or neural ligand 3 knockout brain.

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And what we found was absolutely unexpected and kind of stunning to us In the context of the Neuroligand 3 wild type brain of a normal mouse brain, these GFP labeled patient derived, in this case, glioblastoma cells.

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They expand, they don't they infiltrate the cortex, they invade across the corpus callosum, they act the way GBM cells tend to act in both patients and in mice.

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But in the absence of Neuroligand 3, those very same cells just stagnate.

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They persist. These cells were xenografted six months prior, but they don't expand. And you can measure that in real time using in vivo bioluminescent imaging. And you can see here that this pediatric glioblastoma model just did not expand over the course of the experiment.

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Kind of surprising and apparent dependency of these gliomas on microenvironmental neuroligand 3 was conserved across multiple different glioma types, including DIPG and adult IDH wild type gbm We also found that this was a key mechanism in that optic pathway glioma model.

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But we did not see this surprising dependency extend to a patient-derived model of breast cancer brain metastasis, suggesting that while neuroligin 3 is a really important mechanism across multiple different glioma types.

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It may not be conserved across all cancers growing in the brain.

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So why is Neuraligand 3 this shed postsynaptic adhesion molecule having such a profound effect on glioma growth?

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In trying to understand the processing of neuroligin 3 and its downstream signaling pathways, we found that Neuroligand 3 is shed in a strictly activity-dependent way through the enzymatic activity of a metalloprotease called ADAM10.

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It then binds on the glioma cell to a proteoglycan called CSPG4, also called NG2, similarly binds to normal OPCs through the same mechanism.

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And then this recruits numerous oncogenic signaling pathways. There's early and upstream stimulation of focal adhesion kinase.

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Downstream sark, RAS, and M3 kinase mTOR pathways and that helps us understand the sufficiency of Neuroligand 3 in promoting the proliferation of glioma.

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But it doesn't at all explain this unexpected dependency. And so we then dug a little deeper and looked at the gene expression changes attributable to neuraligand 3 binding and found that there were a number of synapse-related genes regulated by Neuroligin 3.

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Looking at the whole cohort of really interesting gene sets that are regulated by Neuroligin 3, We noticed that actually Neuraligand 3 is regulating a number of different processes, including many ways that we now know neurons and cancer cells are interacting.

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But we really focused at the time on these synapse associated genes. And you'll see that there's a feed forward effect of neural ligand 3 binding on its own expression, so the cancer cells start to express neuroleigin-3 once they're exposed to neuroligin.

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There's upregulation of the gene encoding the receptor for BDNF, TREC-B or NREC2.

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But in addition to that, there are a number of AMPA receptor subunits and other synapse associated proteins that are being regulated.

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By neural 3 and that raised kind of a lot of questions in our mind. Number one, we wanted to validate that this was really happening in patient samples. So we collaborated with Mario Suba's group across town and found that in single cell data sets of each major form of glioma, including H3K27M mutant diffuse mid-langliomas, IDH wild type.

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Glioblastoma and IDH mutant glioblastoma. That in the malignant cells, there's robust expression of AMPA receptors, of neuroligand 3, of PSD95, and other structural and neurotransmitter related proteins.

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If we look at that data from kind of a cellular architecture standpoint and think about the cellular hierarchy in these tumors, here you're seeing data from the H3K27M data set. You can see that the cancer stem cell-like cell, which is in the case of DMGs, is an OPC-like cell.

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Is strongly enriched in the synaptic gene expression. And actually, that's really interesting because it turns out that normal OPCs communicate with neurons not only through these activity regulated paracrine factors, but also through functional neuron to OPC synapses.

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And so then I started to wonder if there are neuron to glial synapses in the healthy nervous system, might there also be neuron synapses forming on these malignant cells that are very OPC-like.

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And when we look, although it seemed like a crazy idea, when we look by immunoelectron microscopy, we see very clearly these synaptic structures between presynaptic neurons, and you can see these synaptic vesicles and the synaptic bouton.

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A clear synaptic cleft. And then this postsynaptic density on a cell that is immunogold labeled, that is a malignant glioma cell.

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Testing the idea that the idea Neuroligand-3 may be functioning perhaps in its most fundamental way to regulate these neuron to cancer synapses we find that far fewer of these structural synapses form in the absence of neuraligin-3 from the tumor microinver.

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But are these neuron to glioma synapses just a shadow of the cell type from which we believe that these tumors emerge, or may they actually be electrophysiologically functional?

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So to test that, we performed wholesale patch clamp electrophysiology of the tumor cells, these GFP labeled tumors.

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That had been xenografted to a well worked out and very experimentally tractable circuit in the hippocampus. By the way, DMG very frequently spreads to the hippocampus. So this is also kind of a clinically relevant paradigm.

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When we patch clamp these cells in the CA1 region of the hippocampus.

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We can at the same time electrically stimulate the axonal afferents into this region, the Schaefer collateral afferents.

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And when we do that, we see very clear postsynaptic currents in the cancer cell.

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These are dependent upon action potentials. They're blocked by the voltage-gated sodium channel blocker tetrodotoxin.

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They exhibit multiple electrophysiological characteristics of bona fide synapses, including paired pulse facilitation.

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And single vesicle events called mini EPSCs. And this first kind of synapse that we identified are mediated by calcium permeable AMPA receptors.

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Now, normal AMPA receptor mediated synapses in the healthy brain and in between neurons are regulated by activity. Their strength can be changed in response to neuronal activity. They exhibit what we call synaptic plasticity. And one mechanism by which activity of a circuit can regulate the strength of a synapse in that circuit is through neurotrophin signaling, through BDNF signaling.

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And looking at the gene expression profiles of these tumors, we thought that perhaps BDNF to TREC-B signaling was one way that synaptic plasticity might be at play. So Katie Taylor, when she was a postdoc in my lab, she's now leading her own lab at Memorial Sloan Kettering.

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When she was in my lab, she thought, well, let's see if there's synaptic plasticity at these malignant synapses.

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And we find that when we repeat that experiment, patching onto a malignant cell in a xenografted slice.

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And then perfuse BDNF over the slice, there are clear increases in the amplitude of those malignant synaptic currents. This depends upon expression of the BDNF receptor TRECB if we CRISPR delete the gene

encoding TREC-B, encoded by NTREC2, then BDNF has no effect on the synaptic current.

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We determined was happening is that Neuronal activity, which results in the release of BDNF, increases the trafficking of AMPA receptors to the postsynaptic membrane in the cancer cell.

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So the cancer cells are hijacking this classical mechanism of postsynaptic plasticity to increase the magnitude of the malignant currents.

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We wondered if there were other kinds of synapses, glutamatergic neurons are the most common.

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Neuron subtype in the brain, second only to, and secondarily are GABAergic.

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Neurons. And we wondered whether GABAergic neurons and especially GABAergic interneurons might be promoting the growth of some kinds of cancers. And we looked at GABA-related gene expression.

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We found really interestingly that there was robust GABA-A receptor subunit and other GABA-A associated genes expressed in diffuse midline gliomas in H3K27M mutant diffuse midline gliomas, much less so in IDH wild-type glioblastoma.

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And so we wondered whether there may also, the AMPA receptor dependent synapses, I should say, were present in DMGs, present in pediatric glioblastoma, present in adult glioblastoma.

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Replicated at this simultaneously in Frank Winkler's lab. We found the exact same thing.

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These GABAergic synapses we thought might be more tumor specific. And if we look at the DMG cellular hierarchy, then we find that just like synaptic genes in general, the GABAergic genes are somewhat enriched in this OPC-like compartment.

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So when we look for GABAergic synapses, again, using immunoelectron microscopy, and we did this in this case by fusing a GFP to a GABA-A

subunit. So then all the immunogold labeling in the tumor cells are indicating a GABA receptor. We see very clear neuron to glioma GABAergic synapses. You can see this through high resolution microscopy as well.

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Cultured patient-drived EMG cells that we've engineered to express a GFP tagged GABA-A subunit, GABR-G2.

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And then we co-culture them with these red neurons. And I'm showing you here neurofilament stained in red, together with immunolabeling of a presynaptic marker called synapsin 1.

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And you can see in this 3D reconstruction very clear co-localization of the glioma expressed GFP tagged GABA-A subunit and the presynaptic neuron expressed synapsin 1.

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So structural GABAergic synapses are forming between GABAergic neurons and DMG.

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And we wanted to know if those were electrophysiologically functional. So they are. We see very clear GABAergic currents in the tumor cells. These are GABA-A dependent. They're blocked by picrotoxin. They're also blocked by a different GABA-A receptor inhibitor called biculine.

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And interestingly, we find that these GABAergic synapses in DMG are depolarizing.

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Now, we think about GABA as an inhibitory neurotransmitter, and it is for mature neurons, but whether GABA causes hyperpolarization and inhibition or causes depolarization or excitation.

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Is just really dependent upon the intracellular chloride concentration of that cell. Gaba is just a simple chloride channel. And so depending on the intracellular chloride concentration.

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Chloride ions will either flow in or out of the cell, depending on, you know.

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The gradient there. So we find that diffuse midline gliomas exhibit this depolarizing current in response to GABA.

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And I just want to point out here that IDH wild type glioblastoma shown in blue has essentially no current or a very, very small current in response to GABA. So again, this kind of tumor specific difference in the electrophysiology of H3K27M mutant. Dmg and IDH wild type.

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High glioma. Importantly, positive allosteric modulators of GABA-A receptors like benzodiazepines.

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Strongly augment the strength of these GABAergic currents. So we wanted to know if a stronger current might differentially affect the tumors.

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And what we find, and I think this is a really important clinical point, is that in H3K27M mutant DMG, quite specifically, we don't see this in GBM, There is benzodiazepine induced increase in tumor cell proliferation rate.

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In vivo when MICs are administered, in this case lorazepam or Ativan.

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That results in an increase in overall tumor growth and a decrease in mouse survival. So just as we need to understand the neuroscience of these tumors so that we can identify medicines we may be able to repurpose to disrupt neuron glioma interactions. We also have to be really mindful of the tumor specific neuroscience of each of the cancers we treat and avoid medications that might be inadvertently.

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It stands to reason that if GABAergic signaling is depolarizing and promoting the proliferation of these tumors that GABergic neurons are also promoting the growth of DMG. So we tested that again using in vivo optogenetics.

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And we see that when we optogenetically stimulate GABAergic interneurons in the tumor microenvironment, this increases the proliferation rate of the cancer cells.

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So I've told you now that both glutamatergic and GABAergic neurons can influence gliomas. I'm a pediatric neuro-oncologist focused on DIPG, so I study diffuse midline glioma.

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And in that disease context, both neuron types are promoting growth. For adult GBM, glutamatergic neurons play a larger role between these two.

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We've now looked at many other neuron types, again, especially the diffuse mid-lang gliomas occur in midline structures. And we find actually that every neuron type that we've studied robustly promotes the growth of these cancers. Cholinergic neurons, serotonergic neurons, noradrenergic neurons.

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So there's a lot of neurons influencing glioma. Does that mean that there's some convergent mechanism? Is there some way that all of these neuronal inputs are ultimately affecting the growth and progression of the cancer.

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Well, all of these different ways you know for the tumor cell in a neuronal activity dependent way to depolarize is a super metabolically expensive thing to do.

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And that suggests that maybe this is the convergent mechanism. Maybe membrane depolarization alone is promoting glioma growth and progression through voltage sensitive signaling molecules or voltage sensitive signaling pathways.

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That would make some sense when we consider that actually in the developing nervous system, in the prenatal brain, neural stem cell niches.

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Exhibit synchronized waves of membrane depolarization and consequent calcium transients throughout the germinal zones. And that seems to be important for normal corticogenesis.

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So we tested whether membrane depolarization alone was sufficient to promote glioma growth.

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And we did that again using optogenetics, but this time we expressed these light sensitive opsins, not in the neurons, but rather in the tumor cells. And in this way, we can use light to depolarize, to optogenetically depolarize the cancer cell.

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And we can do that in an in vivo setting. So if we depolarize a

xenograft.

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That a glioma xenograft that is alone sufficient to promote cancer cell proliferation.

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Now, we're not just seeing these gliomas undergo membrane depolarization, but they're recruiting mechanisms of adaptive plasticity increase the magnitude of the depolarizing current. So maybe the more depolarizing it is, the more growth promoting it is. Well, we can test that again using the same system

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But just flashing the light to open the light sensitive, in this case, channelrhodopsin 2 cation channel.

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For different time periods, which can cause either a medium depolarization of the cancer cell or a larger depolarization of the cancer cell. And when we do that.

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We see that there is a depolarization magnitude dependent effect on cancer cell proliferation in vivo.

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We can visualize this electrical activity in the tumors using genetically encoded calcium indicators like GCAMP6S. So this is GCamp Success expressed in DIPG cells in vivo. And you can see really clearly What has been for me a very startling new understanding that these cancers

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Are electrically active tissues. And that's not the way that we have been approaching either understanding them or treating them.

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And so now as a field, we really need to understand the mechanisms of malignant circuit assembly, plasticity and evolution over the disease course.

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To discern the granular details of these voltage sensitive mechanisms of cancer cell proliferation.

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This will give us both insights for patients therapy As well as insights for normal neural development and plasticity viewed through the magnifying lens of these glial cancers.

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What I'm telling you in summary is that GBM, DIPG, gliomas in general integrate.

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Into the circuits that they invade. They do this through bona fide neuron to glioma synapses that are then elaborated and amplified through mechanisms, hijack mechanisms of adaptive plasticity.

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They also do this purely electrically through activity dependent potassium evoked currents that are then amplified in a tumor to tumor cell gap junction coupled network.

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We know that this happens not only in mice, but also in patients. In collaboration with Sean Herbie Jumper at UCSF, he measured using intraoperative electrocorticography and MEG imaging The functional connectivity between adult glioblastoma, IDH wild type glioblastoma, and the rest of the brain.

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And the degree to which the tumor is functionally connected to the rest of the brain is a robust predictor of overall outcome with more highly functionally connected tumors or tending a much worse prognosis.

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We can begin to think about how we can target this therapeutically, disrupting neuron cancer interactions.

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Every way we do it in the lab slows tumor growth and progression.

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So we can think about neuroscience inspired targets that include neurotransmitter receptors, neurotrophin receptors.

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Synaptogenic factor signaling axon pathfinding molecules, synaptic adhesion molecules, ion channels, and gap junctions.

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There are excitingly, clinical trials that are now beginning or have begun, including one that is collaborative between Stanford, MGH, and Dana-Farber.

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There's great therapeutic potential of disrupting these neuron glioma

interactions. As one example, if we disrupt AMPA-dependent signaling, either pharmacologically or in this case genetically by expressing a dominant negative version of the AMPA receptor GLUA2 in the glioma cells This has a stark growth inhibitory effect on the tumor.

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So there are very clear mechanistic parallels between normal neuron to OPC signaling and malignant neuron to glioma signaling.

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So much so that it really underscores the extent to which these cancers are simply hijacking mechanisms of normal neural development and plasticity and demand that we begin to approach these cancers from a neuroscience perspective.

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I want to introduce you to or make sure I highlight the people who have been involved in the work that I've just shown you. Saveenkatesh, Ewan Pan, Katie Taylor.

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Tara Barron, Richard Drexler, and Avisha Gaviche. Many, many people to thank past and present collaborators here and many in Boston. And of course, patients and families whose donation of tumor tissue enables all of the work that we do. Thank you so much for your attention and hopefully we have some time now for questions.

05:09:38.000 --> 05:09:45.000
Thank you, Michel. I'm sure there will be a lot of questions for your exciting presentation.

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Jen.

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Michelle, great talk. Thanks so much. I'm curious how, in the light of your work, how do you think about tumor treating fields and the mechanism of how those might be providing some benefit?

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Yeah, I would love to know how

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Yeah, I would love to know how tumor treating fields influences the activity of the neuron and the depolarization of the glioma cells.

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I think I don't know the answer, but it does bring up the wider concept that neuromodulatory devices and approaches to perhaps drive a depolarization block or modulate this malignant circuit activity is

something we should all be thinking about very hard.

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Yes.

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Thank you, Michelle. That's really super exciting. I'm wondering… So obviously, if you have a brain match from a different histology that takes place takes root in the brain, right? Obviously, the synapses won't be exactly the same because it's not a brain cell. It's like whatever, a breast cancer cell or a lung cancer cell.

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But do you have any hunch or evidence or is there any data out there that maybe the surrounding neurons around the mat then would also have similar upregulation and activity that could then influence the growth of the MET?

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Yeah.

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Yeah, actually, we've studied this. So this is collaborative work, Holm says now leading her own independent laboratory at Dana-Farber, work she began before she left my lab and that we've continued together collaboratively.

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Together with Julian Saj and work led by, I just have to say, an absolutely brilliant medical student who just matched at Brigham and Women's Hospital. So well done. You get salsa Sabchuk, who's completely brilliant and led all of this work.

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We find that not only does neuronal activity drive small cell lung cancer brain metastases.

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But there are synapses. Now, this is a neuroendocrine tumor, so maybe that's not so surprising.

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But when these cells get to the brain, they make both glutamatergic and GABAergic synapses with neurons and that very robustly drives their growth through what appears to be very similar mechanisms.

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Yeah, so… Yeah.

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And do you think that the pharmacological inhibition of what you're talking about with GBM cells would then also apply to brain matter?

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Wow.

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Yes, I do. Yes, I do. And Frank Winkler's group has has shown we both have these bio, they're preprints that are up on bio archives. You're welcome to read them. Frank Winkler's group has a um studies showing very clear EM evidence of synapses between

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With breast cancer, with melanoma, and I think with non-small cell lung cancer.

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So I do think when tumors go to the brain they adapt to take advantage of the currency of the realm, if you will.

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And i do Sure.

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So I'm so sorry. Then I have another follow-up question. So then the data that you show about lorazepam.

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That would then apply to mats from other indications as well, right?

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Yes, yes.

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So the GABAergic synapses that we observed, as I mentioned, were present in DMG, not present in GBM.

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We do see GABAergic synapses with small cell lung cancer, and those experiments with lorazepam are ongoing right now.

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I think it's important to identify which tumors are safe to be exposed to which symptomatic medicines.

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And we don't know, but we have found some of the other strategies disrupt those interactions that work in GBM also work in small cell lung cancer.

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And then, of course, I'd be super curious what happens in melanoma brain meds and breast cancer brain meds.

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Yes, Frank Winkler's group has this preprintout that shows synapses in both of those disease types.

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And in breast cancer, I should say, in addition to direct neuron to malignant cell synapses.

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There are also malignant cells that are sort of more astrocyte like when they get to the brain and form perisynaptic context, a sort of pseudo tripartite synapse that was described by Doug Hanahan's group.

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And that paper is published from 2019. In that case, glutamatergic signaling through NMDA receptors on the breast cancer cells drive breast cancer brain metastasis growth.

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Yeah.

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That was an awesome talk, Michelle, and thanks for joining us today. So I had a quick question about sort of trying to treat the tumor and the tumor neuron interface without affecting normal neurological function because many of the glutamatergic receptors that we might want to inhibit here are also present and important for normal brain function. How do you think about that?

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Yeah, and actually we can take some key lessons away from anti-epileptic medicine use.

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It's surprising that people tolerate those medicines as well as they do, but they do tolerate them well. They're designed to get into the brain.

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And when we repurpose medicines, for example, like the AMPA receptor targeting parampenil.

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We see that that slows glioma growth pretty robustly. And there are clinical trials that have already started using rampanil together with standard GBM therapy to see if that can augment the therapeutic

outcomes.

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Okay, we have one question from an anonymous attendee online. And they say, what are your thoughts on synaptic upscaling potentially playing a role in glioma progression?

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Synaptic upscaling. Just to be clear, can the person who asked that question clarify a little bit what they mean by that? One thing that I will, while perhaps we wait for that answer.

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One thing that I'll mention is that there is really, really clear effects, not only of neurons and glioma cells but Conversely, and I need an hour-long talk to talk about this too, but there's conversely, effects of glioma on neurons. Harold Sondheimer originally showed this. Ben Deneen has done beautiful work.

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Following this up, we have found the same thing happening in DMG.

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There is a profound effect of gliomas on the excitability and structural and functional circuit dynamics of normal neurons and neural circuits. And that contributes to tumor associated seizures, but it also augments the cycle, the sort of vicious cycle by which neurons drive the tumor cells, the tumor cells.

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Increase the excitability of neurons. They do that through secreted factors that include synaptogenic factors.

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Glipicans, thrombospondens. That increase the excitability and change the connectivity of neural circuits that increases the input to the tumor.

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All right. They haven't posted anything after, but thank you for that answer.

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Thank you, Michelle. I think I can ask you the last question. So you have really focused on the growth of the tumor cells as your readout for the excitability coming from the neuron.

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Yes, absolutely. Absolutely.

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In some work we did with Frank Winkler and Varuna When Kataramani, we also showed that there is an effect on invasion of the tumor set. Do you think that these phenotype is also mediated by the same factors that you showed, neuroligand 3 or

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Bdna for track B, or do you think there is more to discover there?

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Yeah, so two points. I think there's just like growth can be stimulated both by synaptic mechanisms and by paracrine signaling mechanisms.

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We found that there's invasion encouraged by neurons both through both kinds of mechanisms. So the 2022 cell paper that Varun led, beautiful work showing that the glutamatergic neurons synapsing onto the tumor cells can promote invasion. We've also found that there are secreted factors

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Present in condition medium from cortical slices regulated by neuronal activity that promote invasion. We published that together with Ben Deneen and You know, we spent a long time trying to identify the factors. It's not BDNF and Neuroligin 3. There are unique factors.

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That are promoting invasion of glioma cells that are secreted. We actually haven't identified them yet.

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But we know that there are both paracrine and synaptic mechanisms. And it's certainly a very important part of the effects of neurons on the tumor and is probably why, I don't know if the other clinicians in the room have also seen this, but at least in DMG, I tend to see

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No? No.

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Cancers invade along circuits, like not in a just extension kind of manner, but for example throughout you know limbic circuitry, including crossing the midline and following the circuits to the the next synaptic partner. I think activity clearly is influencing invasion and activity is also probably influencing

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Treatment efficacy. I think it's promoting survival in the tumor cells. That's less well studied. But Frank's work shows at least

through the gap junctions.

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Between the tumor cells that there's treatment resistance.

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Yeah. Thank you, Michelle, for the great presentation. I think we can stop here. Thanks a lot.

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Okay, sure.

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So we've got a short break and maybe we can come back in about 10 minutes or so for Dave Ode's talk.

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I encourage you all to take your seats and we got our final presentation here.

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It's my pleasure to introduce Dave Odie, who I've known for quite a while.

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Does undergraduate work at University of Minnesota, went to Rutgers. For his master's and PhD and then came back to University of Minnesota, I think in 1988.

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99. Yeah, I graduated in 1988. Oh, yeah, that's right. I went back there in 99. Back in 99 as a system professor and as a ranks.

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He's an expert in modeling the mechanics of migration and then validating those models in real measurements. So awesome. Thanks for joining us.

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Look forward to your talk. Thank you, Jan. And thank you, Forrest and Francisca, for inviting me to participate today.

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And we've heard earlier about the invasive nature of glioblastoma, and that's a major focus for my group is How do they do it? How do these cells crawl through the brain tissue in order to spread the disease?

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And the mechanics of that. Have time, I'll tell you about the mechanics of immune cell migration as well.

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I decided to squeeze in the middle between those two stories.

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A crazy pitch for a clinical trial just to see where it lands. If you shoot me down fast enough, I'll just go right on to the immune cell mechanics. It's a talk that has three halves to it.

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I decided late to add one half in the middle because I was a mistake.

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But we'll see. Okay, so I have a wonderful group at Minnesota on the left here, and I've been really mentored and encouraged by two key people at the bottom here. I'll specifically mention Steve Rosenfeld, who's at Mayo Clinic Jacksonville.

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And Steve's the one who told me I might have something to contribute to the brain tumor space. He's a neuro-oncologist.

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And a molecular biophysicist, and he saw what we were doing with modeling neuron growth and mechanics and thought that could be applicable to brain tumor invasion and mechanics. So Steve's been encouraging me for the last 10, 15 years to try to

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Our imaging and modeling to brain cancer. And being at Minnesota has introduced me to Dave Largo Spada in the lower left, who's the associate director of our cancer center and an outstanding cancer geneticist.

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Expert on brain tumors. And other tumors as well.

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And of course, benefited from the generous support of the people of the state of Minnesota and the people of the United States who have supported us continuously and generously through the National Cancer Institute for many years now.

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So thanks to all of you. And we heard earlier about the invasive nature of glioblastoma, and you can see it in the sequence for this one patient, which was one of Steve's patients.

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Who presented with the disease on the left. And underwent surgery.

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Radiation chemotherapy. And as Jan mentioned earlier, almost inevitable recurrence which you see in the middle image, only three and a half months later.

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And that recurrence in this case was very close to the original lesion.

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And over the next two months, despite entering a phase two clinical trial for a new immunotherapy.

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This individual progressed even further. To the point where the whole left hemisphere is basically taken over.

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And two weeks after this last scan perished from the disease.

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Six months from being like you and me sitting here feeling pretty good to being deceased from this terrible disease.

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I thought I'd just mention the invasive nature here specifically and being able to disrupt the cell migration might be a therapeutic opportunity to limit the disease's spread, keep it more local and confined.

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And compact tumor. So that local therapies like surgery might be more effective at at limiting the tumor mass and invasion.

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And also focused radiation might be more effective as well, as long as we can keep the tumor more local and keep it from spreading further into the brain.

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So that's motivated us for a while now to try to figure out what are the mechanics of how they do that. And then does that give us clues on how to disable it?

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And at the same time, we're interested not only in the glioma cell.

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Migration, we're interested in immune cell response and how immune

cells navigate through the brain in order to effectively In the case of anti-tumoral cells.

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Like cytotoxic CD8 positive T cells. Find the cancer cells and then when they get there, kill them.

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So it's a tough finding problem because the diffuse nature of the glioma means that there's big gaps between one cancer cell and another cancer cell.

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And so you're asking T cells to go from one to another. It takes time to find the next cancer cell.

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So maybe the time to kill it maybe isn't that long, but the time to find the next one can take up a lot of time.

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Meanwhile, time is ticking by and the cancer cells keep growing and they keep moving and they keep dividing.

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So they can't afford to spend so much time looking for cancer cells.

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And maybe they're not all that well equipped to do it. And so we want to understand the mechanics of those, particularly for cell-based therapies, to make sure that those cell therapies that we're introducing actually are capable of navigating through the brain.

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Which they have to do in order to go from one cancer cell to the next in order to keep serial killing.

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So we're interested in both of these aspects of migration, both the cancer cells and the immune cells. I'll focus first on the cancer cells.

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And just showing a histology of, again, the diffuse infiltration. And the surgeon colleagues that I have say that they're comfortable resecting up to this If they can find it exactly this kind of margin here.

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Where there's a tumor mass that's clear that they can identify beyond

which is tissue which has clearly been invaded by cancer cells, but is still more or less functioning neural tissue.

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So they risk by resecting that functioning neural tissue, they risk severe debilitation or worse.

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For their patients and they're not willing to take that risk.

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And therefore, they view this surgery as largely a palliative treatment that is worth doing because it improves quality of life because it debulks the tumor.

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But by itself and even with other combinations and standard of care do not cure people.

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So that's the problem is these cells are being left behind and they continue to keep invading and progressing.

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And they're kind of sparse as you get out to the edges, making it hard for the immune cells to find the last few.

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That are out there because there's such great distances between them.

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So if we could keep them from doing that, we might be able to keep a more compact tumor. So once immune cells do get in there, they just keep killing locally because they're all right there.

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So here's what it looks like more in a live using confocal microscopy imaging of Here we've induced a tumor using a method that Dave Largospata's lab developed.

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And fluorescently tag the glioma cells with GFP. And you can see individual green labeled glioma cells invading into the brain parenchyma to the upper right and away from the tumor mass in the lower left.

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And they go at different speeds which we can measure. So we can start to see the speeds at which cells are actually migrating in brain tissue in these ex vivo brain slice assays.

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And that gives us a handle to start to look at what the genetics and the the drugs are that we could explore to understand the mechanisms by which they're doing that.

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And you can see this not just in brain tissue, but in brain-like tissue.

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Now using human IPSL-derived brain organoids as the platform. And interfacing that with green fluorescently tagged glioblastoma cells that can attach to that organoid, ingress into it, and migrate through it and make movies of those as well. And you can see these

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Long, thin extensions that these glioblastoma cells extend into this brain-like tissue.

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Enabling them to explore their local environment and potentially generate pulling forces to help pull themselves through that environment. And I'll show you more evidence of that later.

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And you can also see cells dividing. There's a cell that right here crawls up, divides, boom, now there's two cells.

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And so we can see the whole processes of migration and proliferation playing out in these brain organoid structures as well.

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And we can recapitulate this even in a 2D assay, which is more traditional type of in vitro assay. But here we've engineered the substrate to be a hydrogel.

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That has a Young's modulus that's more closely matched mechanically to what the Young's modulus of brain tissue is.

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More in the 5 to 10 kilopascal range. So these cells also have these long, thin extensions.

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That reach out into their environment, enable them to get grip in their environment and pull themselves through it.

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And so this kind of… retraction of protrusions is a key process that we'd like to understand in order to disrupt the invasive nature of these cancer cells.

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And so I'll tell you a little bit about the work that my former student Clarence Chan did when he was in my lab.

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Which is developing a mathematical model for this process of those protrusions.

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Which are driven by F-actin self-assembly at the leading edge to push the membrane forward in a ratcheting type mechanism to advance the leading edge and cause a cell to move in that direction.

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But confounding that movement at the same time our myosin 2 molecular motors depicted on the left here that pull on those actin filaments and pull them retrogradely back into the middle of the cell away from the leading edge.

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And then the third factor are these adhesive bonds, so-called molecular clutches.

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That like integrins and things like that that can create mechanical linkages between the affect and then the extracellular environment.

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And they transmit force as the F-actin moves to the left under the influence of the motors.

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And engage with a substrate that can get stretched to varying degrees and start to generate transmit mechanical forces to a compliant extracellular matrix or extracellular environment.

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And so we have equations for those processes for both the motors And for the clutches. Oops, sorry, jumped ahead there.

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For the motors and the clutches. Which is that the motors have a force velocity relationship. This should be fairly familiar from just going to the gym.

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And lifting weights. If you go to the gym, you start lifting weights, you pick up a light weight, it's very easy. You can lift it about as quickly as you can can contract your arm muscles so that it's like almost

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No resistance there. But as you increase the weight that you're lifting, you eventually get to the weight that you can barely lift or can't lift at all.

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That's the stall force of your muscle. And this is well known for ensembles of motors like skeletal muscle, but also true even down to single molecule level measurements of force velocity for single molecules of myosin and other motors like kinesin and dynein.

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So it's a well-established principle for motors. It's just true of motors generally.

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The more load you put on them, the faster they go.

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So the second part is what's the deal with the clutches? They engage and disengage spontaneously But once they're engaged, they can start to come under load which can then potentiate their off rate because they're being stretched. And that's kind of like pulling the bond apart.

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So there's an offer rate that becomes force dependent, so-called Bell's law. It's well established going back to the 1970s that we also employ in this model. And with that all together and then allowing stochastic bond formation and breaking in a load-dependent way.

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We can simulate the formation and breaking of the clutch bonds.

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Which are these small springs that you see in the middle here.

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Transmitting force to the underlying substrate, which is this big spring.

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All the springs are color coded according to force. So if it's blue, it's under no force. If it's red, it's under a lot of force. And you

notice how they kind of warm up.

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Notice how the big spring, the substrate loads up from blue to green to red.

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And repeats this cycle over and over. It's because the… This adhesion here that's formed with this ensemble of clutches is transmitting force from the myosin motors on the left that are moving the F-actin conveyor belt to the left.

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That's transmitting force to the underlying substrate and stretching it, and that force increases until it gets so great that the bonds are forced to fail because the loads on them are so great. And once a few fail.

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The rest start to fail in like a cascade domino effect.

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And that's a basic feature of the motor clutch model. So you can think about how If this is the mechanism that glioma cells use to invade into brain.

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You might ask, okay, so what is the clutch that they're using? What's the adhesion molecule? Perhaps we could target it and block this process and then they wouldn't be able to get grip on their environment, then they wouldn't be able to migrate. So we've tried to address that question.

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Which I'll tell you a little bit about today was really largely the work of Becky Clank Markowitz when she was in my lab.

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And Becky did these computational models that built off the earlier model that Clarence developed.

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Where instead of having just one protrusion. She'd have three, four, five protrusions all kind of linked together mechanically in the middle, like the middle of the cell.

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So that all those protrusions are pulling on each other at the same time, all trying to go in different directions.

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And then it becomes a tug of war and a force balance. And eventually one of them wins out.

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And that causes the cell to displace itself in the direction away from the losing side.

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And you can see in these three different scenarios, cells move either well, like in the middle, or poorly, like on the left or the right.

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And what we've changed in the three cases is just simply the number of clutches that are in each of these simulated cells.

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So that on the left, there's very few clutches. Which means as these protrusions get sent out and the myosin pulls on them, there's almost no resistance to pulling them back in.

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Because there's very few adhesion bonds there. The few that are there.

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Break right away under load because there's so few of them they can't share the load effectively and they get pulled back in. So they get these kind of retracted cells that aren't very motile.

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On the right is the opposite scenario. Too much adhesion. So, so many adhesions that these protrusions go out and they basically get stuck And the myosin can't overwhelm all those adhesions and they pull like crazy, but they don't actually retract anything and the cell just gets stuck like it's in mud.

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It also doesn't move well. In between is kind of a good balance between just enough grip to move, but not so much that you can't move on.

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Which this cell moves pretty nicely off to the right within its frame.

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So that predicts that there's a biphasic speed dependence as a function of how adhesive the environment is.

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If it's too adhesive or not adhesive enough, cells won't migrate well.

But if it's in between, they'll migrate really well So that's the prediction we would make that we want to test experimentally.

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Ideally at a… in vivo or disease relevant setting. It's been previously established in vitro using a variety of cells Doug Lauffenberger's work actually was very seminal in this area in the 1990s.

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But to our knowledge, hadn't been applied in an in vivo setting in a disease relevant situation.

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So that's what… We undertook to do with… Working with John Olfest.

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To investigate CD44 as a potential clutch in glioblastoma. The idea being that CD44 has the right properties and that it spans across the membrane.

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Has the ability to bind to F-actin on the inside of the cell via adapter proteins like Ezra and Radixon or Moesin.

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And combined to hyaluronic acid on the extracellular matrix side. Where hyaluronic acid is relatively abundant in the brain relative to other ECMs like collagen and fibronectin, which are not so abundant in brain.

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So working with… John, who unfortunately passed away from metastatic melanoma at age 35, Which was terrible.

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Jim McCarthy, who's a CD44 expert, and John's student, Stacey Decker Grunke.

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Using a mouse model that John and Dave Largaspada had developed using the Sleeping Beauty Transpose system.

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Introducing oncogenic drivers in brown into neonatal mouse brains by injection of a plasmid cocktail And using Sleeping Beauty that can recognize this transposed lace can recognize these black triangles.

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Inverted repeat direct repeat sequences in the trans genes as well as

the host genome.

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To insert these transgenes into the host genome. And fairly reliably drive gliomogenesis in these mice.

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And you can pick oncogenic drivers that correspond to the human glioma glioblastoma.

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Genetics, and so it has that advantage. It's immunocompetent animals so you can You have the immune system in play too.

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And you can use pretty much any mouse you want, whether it's wild type or mutant. So by using a mutant mouse of CD44, we can start to control the CD44 gene dosage in the system.

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To go from… to use knockout background to get to Essentially.

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Zero CD4, no CD44. Intermediate level of CD44 in the wild type case or start to overexpress with a very active promoter overexpressed CD44 in both of these two backgrounds to get high or very high levels of CD44 And what we would predict based on our biophysical model is that the intermediate level of CD44

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Should be the fastest migrating, most invasive phenotype. Because they have the right balance of motors and clutches. And if you go too high or too low, they'll migrate poorly.

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And the mice will do better. So that was our prediction. And then we tested that using, I showed you the movie earlier. You do this experiment for each of the four cohorts to see what the speeds look like and what the survival looks like.

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And what we found was exactly what Becky's model predicted based on the motor clutch model that the wild-type intermediate level of CD44 had the fastest cells and the poorest mouse survival.

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And so you see this biphasic curve, both for migration and then upside down survival the corresponding inverse of that poor survival at intermediate level of CD44.

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Which prompted us to look at human CD44 mRNA expression to see if they similarly exhibited this kind of biphasic trend.

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And indeed found that was the case in this cohort. And so that intermediate level of CD44 correlated with worse outcomes relative to somewhat better outcomes at either low or high levels of CD44.

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So to kind of summarize that whole story, it's basically that we had evidence that CD44 has the right properties to be a clutch for glioblastoma cells in their invasion into the brain tissue.

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Such that there's a… There's just the right balance of motors and clutches.

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Of myosin motors and CD44 clutches. So we've done a lot of other studies on the motor clutch model, and other groups have used our model two to make predictions that led to new discoveries.

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And I focused on the migration of brain tissue just now. But we've also used it to discover negative And other… aspects of cell biomechanics and migration in complex environments.

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So what I'd like to tell you about next is can we find direct evidence that they're actually using this motor clutch mechanism in brain tissue And this is the work of my former student, Sarah Anderson.

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Who made movies of cells migrating through brain tissue and considered a competing model to our model called the osmotic engine model, which was developed by Constant Constantopoulos, Sean Sun and Denny Words at Johns Hopkins.

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The idea of this model, which is quite interesting, I think, is that there's a net There are electro-osmotic pumps at one end of the cell.

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That drive ions in. Which then in turn draws water into that side of the cell, which pressurizes the cell and causes the water to flow out On the other end of the cell.

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And due to that imbalance of electro-osmotic pumping, you get a net

flux of water through the cells.

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And by conservation of momentum and mass, the cell moves to the right along with that. It doesn't need to touch anything.

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Cruise through the environment. As long as it has that asymmetry.

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Although it's helped by having a seal along the sides to maintain the asymmetry.

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So it's a plausible biophysical model, in my opinion. We wanted to take it seriously because maybe because they had proposed that glioblastoma cells might actually use this mechanism to invade through brain.

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So taking their model seriously. Considering what the predictions would be between the two models.

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The motor clutch model on the left, as I mentioned, predicts these pulling forces on the environment. They're pulling in on the environment as they grab it.

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So if you were to sit and watch a cell as it's moving, it's going to pull on the environment, deform If it's sitting next to like a blood vessel.

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It's going to grab onto it and pull it and deform it. And you should see the deformation pulling towards it.

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As one side fails just because that's what happens when the forces build up, one side eventually loses.

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Then the cell will move in the opposite direction. And so you'll see at the leading edge of the cell.

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The vasculature would move towards the cell as it invaded in that direction of the vasculature.

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That is in contrast to the prediction of osmotic engine model, which predicts that as these fluxes pass through the cell as the water fluxes through the cell.

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The cell moves autonomously. And any adhesions that get made.

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Would be basically resisting that motion and would get dragged along with the cell as it moved to the right in this scenario.

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So that would predict that if you could visualize the deformations in the vasculature, they would move along with the direction of the cancer cell as it moved to the right, an opposite prediction. So one model predicts that they move towards each other.

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The other one predicts that they move together in the same direction.

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So Sarah tested this experimentally. By fluorescently tagging the vasculature using isolectin B4, which also happens to tag the microglia in the environment.

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And green fluorescently taking the glioblastoma cells. And observing as they migrated in a perivascular manner, this cell moves down and to the right along this stretch of vasculature indicated by the Magenta Arrow. And what she predicts is that it would pull it towards it as it moves to the right if it's the motor clutch model, which

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A little light in here, but maybe you can see it.

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You can see that stretch of vasculature just get sucked in, pulled in by the glioma.

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Glioma cells that moves down into the right. You can make a chymograph in that region there and track the features of magenta and green. And they exhibit this kind of convergence that you'd expect from the motor clutch model.

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They tend not to exhibit this kind of pushing dynamics that the osmotic engine model would predict.

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So you see this kind of V shape in the chemograph of the green and the magenta coming together.

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And… She did this for a number of different cell lines too, including ones from the Mayo PDX Collection, so we're grateful to Jan and his team for providing us with the cells to examine the brain tissue.

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And the same features consistently across all these different lines see this kind of pulling behavior. You can see the magenta getting pulled down to the left in the great most movie.

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So Sarah went through and scored all those events to see if there's examples of pushing or pulling.

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And pretty much almost every single time, but not totally With one exception, she saw one example of what looked like a pushing event.

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Here, but otherwise all the other events of deformation that were associated with movement were these pulling type events that I just showed you examples of.

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So to us, this is direct evidence of what you'd expect from a pulling type mechanism.

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Without evidence to support evidence of a pushing or osmotic engine type mechanism.

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And yeah, I'm going to skip this real quick. She showed that she could block CD44 and that suppressed the ability of the cells to move in the brain tissue.

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And kind of interestingly, didn't really adversely affect the T cell migration.

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Which I probably won't get to those movies today. Next 10. But it actually potentiated slightly the T cell migration in brain tissue.

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So overall, just to wrap up this first part here. The glioma cells seem to use a motor clutch mechanism in brain tissue that is CD44, and

I didn't mention it, but also integrin and myosin 2 dependent And they don't seem to exhibit behavior that's consistent with osmotic engine models. So we disfavor the osmotic engine model

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Favor the motor clutch model for invasion and CD44 playing an important role in that.

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So then we started to think, well, how can we target glioblastoma cell migration. Can our model predict what the best things to target are and what the not so good things to target are?

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And so my former postdoc, Jay Ho, who's now assistant professor at Brown University.

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Started to simulate what would happen if you changed any one of the parameters in the model to see how that would affect how fast the cells migrate.

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And that's shown here is that this cell migration simulator that I showed you earlier When you change any one of the parameters.

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Shown here in the rightmost column. You see an effect on the random motility coefficient or speed of the cell But the biggest impact on that was largely the F Act and assembly dynamics, V. Poly.

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If you increase that, you can increase the speed a lot.

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And so that's shown here as the The RMC in the top row here as you go from low, medium to high actin polymerization rate goes from relatively low speed to relatively high speed.

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And… So that made us think, well, rather than targeting the clutches.

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Maybe we should target the F-actin assembly. And so… Or really, you could target any part of this potentially and have an impact negatively if you target it the right way.

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On the random motility of the cells. But the standout champ was the actin dynamics.

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So my current student, Katie Vopat, started to think about ways to target effect and dynamics.

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And we're particularly struck by the feature of these spiky projections that glioma cells send out into the brain tissue which are very much like reminiscent of so-called Philipodia.

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Which are parallel bundles of F-actin. That are cross-linked together. I think I have the next slide.

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By bundling proteins such as this protein fashion, which is the yellow circle here.

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Bundles these together into a nice bundle. And keeps it together so it has a nice spiky, pointy protrusion and those things can poke into the brain tissue and help the cells generate protrusions that go through it and help them navigate through the brain tissue.

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So she was intrigued when she found a paper that said that an antidepressant called amipramine can actually block fashion one interaction with F-actin and can block this bundling process.

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And so we were intrigued because if that's true, then it might be able to block or suppress glioblastoma cell migration and invasion And here we'd have a drug that's already used clinically. It's brain penetrant because it's an antidepressant.

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So we thought this would be an interesting angle to pursue potentially.

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Katie started to look at imipramine as a as an anti-migratory drug and found that Maybe let's restart this here. These cells initially have These long spiky protrusions, which when she has amipramine on the left versus control on the right.

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Those rapidly disappear, so you lose those spiky protrusions. The cells kind of retract and round up, and they become relatively non-migratory.

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In the presence of amipramine. And this is very preliminary and early. That's why I hesitated to show it. But I thought, let's just take the chance and roll the dice here and talk a little bit about possible drug that we could use. And this is a very inexpensive drug. It would cost about $10 for a course of treatment.

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I looked it up on Mark Cuban's gooddrugs.com. And still early days, but so far it looks like it's statistically significant.

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Decrease in cell speed at even like 10 micromolar mippramine.

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So we've got more work to do, obviously, but we've tried to test it in vitro on hyaluronic acid gels to be more closely mimicking brain tissue.

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And we've tested in the brain slices too. You're not seeing the red vasculature too well here, but you can see the green Glioblastoma cell when it's treated with imipramine, I'll start it over again. You see the long.

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I can't see it that great, but these long protrusions here.

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Adamipramine, and they basically go away. And the cell becomes non-motile, kind of stuck right here.

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For extended period of time for several hours. So it's showing evidence of anti-migratory activity in brain tissue as well as in vitro.

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So we think it might be an intriguing drug to try.

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As an anti-migratory, anti-invasive drug for glioblastoma. There's actually evidence that is anti-invasive in glioblastoma based on earlier work by Jenny Munson, when she was a PhD student in Ravi Bellam Konda's lab at Georgia Tech.

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And found that, as you can see here on the imipramine treated case, a decreased number of invasive cells in this wrap model for glioblastoma and evidenced here as well in this histology.

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With quantification here in the top row. And they tried to work out the mechanism, but it was a little vague.

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In their final interpretation, they thought it was targeting this NADPH oxidase, NOx4.

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And eventually… perhaps signaling through SARC.

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And eventually affecting actin. So I guess what I'd say about all this is a lot of question marks here, so not really completely worked out.

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How it works as an anti-invasive, but it had that effect in this rat model for glioblastoma.

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And it seems to end up involving actin again. So that's consistent with the article I mentioned that Katie found.

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Of the group that found that imipramine blocks fashion. The F-Actin bundler.

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It has this actin-directed effect that can affect invasion. So can we do an imipramine clinical trial for glioblastoma?

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I'm currently discussing with our with our clinical team at Minnesota trying to get a protocol together to do this.

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The two clinicians that I talked to, one is human treats human patients. The other one treats canine patients. So we have a We have one of the top canine clinics in the United States formerly by Liz Pluhar, now directed by Dr. Susan Arnold.

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And Dr. Arnold's comfortable with trying to use imipramine with the dogs.

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Who have spontaneous gliomas. They're not experimental animals. They're pets that need treatment.

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And she uses it already in the clinic for dogs. It's already used clinically for dogs.

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To treat separation anxiety and things like that. And urinary control.

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So… She's comfortable with exploring the idea And I appreciate getting feedback from this group. If you think this is a terrible idea.

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Or there's reasons why it's not going to work. Now's the time to tell me because I think we're going to kind of keep pursuing this as long as the preclinical data keeps supporting it, and the clinicians want to go along for the ride and feel like it could help their patients and not harm them.

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I'll just mention, too, it could have pleiotropic effects, and this has been investigated by Doug Hanahan's group in La Which is that it seems to have other effects on the immune system.

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Including macrophage reprogramming and inducing recruitment of CD4 and CD8 positive T cells that are beneficial to suppressing tumor growth.

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So it doesn't rule out these other pathways, which how that works, they didn't fully work that out either, although they suggest autophagy as a mechanism by which that happens.

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When he started interfering with actin dynamics, all kinds of things can break loose in the cell.

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That may manifest as autophagy or signaling alterations and things like that.

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So there could be pleiotropic effects. And there actually are clinical trials open to test imipramine for glioblastoma. One is in Texas at UT San Antonio, MD Anderson.

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Branch in San Antonio. And testing it in recurrent glioblastoma. And another one is in Spain.

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Which is motivated by the group that discovered that imripamine blocks fashion activity.

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In Cartagena. And so they're enrolling triple negative breast cancer patients and colorectal cancer patients.

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And treating them with imipramine. And assessing their outcomes.

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So it's already happening. It's not like we invented this or came up with this idea. What we're excited about is the modeling that we're doing pointed towards effect and assembly dynamics as a key thing to target.

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And thanks to the group in Cartagena discovering that imipramine targets fashion and blocks bundling We seem to be backing that up with our preclinical data. So that's why we think it all kind of makes sense mechanistically.

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To go after glioblastoma with imipramine as an anti-invasive strategy. So I have my whole immune cell migration stuff, but I think I've used up all my time and then some.

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And Forrest is agreeing, so at least he's not reading the newspaper. So I'll stop there, and if there's time for questions, I'll be happy to answer them. And if you want to give me feedback on this idea, that'd be great. If you think it's terrible, let me know.

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I think it's great. Join us, cheer us on. Join the conversation.

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Questions?

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You said it was 40 micromolar that you were using of the imipramine to see the effects in vitro. Do you know… much about the pharmacokinetics of it and what concentrations you get in the brain and so forth. Yeah, I mean, we've started to look into the literature on that.

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Seems like one micromolar might be a reasonable, attainable. Concentration that's used for treating impression already. And we've taken it down to one micromolar. I didn't show the data, but that's where we're headed is.

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There is no drop off. I mean, the reduction in RMC was already fully evident at 10 micromolar and didn't get any There was no more suppression of migration when he went to 40.

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So it's already saturated out at 10. And Katie's seeing suppression at one micromolar in vitro as well. So we think we can see effects at one micromolar and we think that looks to be a clinically achievable concentration.

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David, what happens to the tumor cells after treatment with imipramine? In terms of, you say they don't invade, right? You block invasion.

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Now they stay there, right, in the original location. Do they proliferate more? Do they proliferate the rest? Do they die? I mean, I think answering this question is quite important. For example, I would test They respond to standard of care, right, to radiotherapy and chemotherapy. Are they becoming more sensitive, less sensitive?

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Because, you know, obviously invasion is important, but you also want to make sure that you don't make sales more aggressive.

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Yeah, I mean, we don't have those results yet, but the other… groups that I mentioned.

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Nelson and Bellum Conda and then the group in Spain They've seen that it has beneficial effects in the animal models that they've looked at.

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But you've raised an important question, I think. It's like, so what if you stop them from moving? They keep proliferating.

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But I would argue they proliferate in place now instead of moving out and proliferating. And I think there's this problem of I mean, one thing is what that does is it creates a very compact tumor that's jammed together.

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Which makes it hard for cells to proliferate that are in the tumor. Why? Because there's no space to put the daughter cells.

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So expanding for the cell to expand to get to to twice the size that it was initially as it goes through the cell cycle and we've been discussing over the last year or so with Scott Menalysis Group, the biomechanics of cells actually growing and the physics of that.

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You've got to have space to grow. So it's not a direct effect on proliferation, but you can't proliferate if you don't have space to grow into.

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And so that's one thing. Two, as I mentioned, is like the immune system has this big problem with diffuse tumors. When they're that sparse and spread out, you've got to… skip from one to the other to be a serial killer.

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You might spend like hours trying to find the next cancer cell Meanwhile, that cell's growing, as are all the other cancer cells.

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I think it's going to make the… it's going to make the tumor more vulnerable to immune therapy and normal immune response if we can keep it in place more.

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So those are two reasons why I think anti-migratory therapy is still exciting, even if it doesn't directly stop migration.

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I mean, proliferation, sorry, Antonio. Great talk. Yeah, I was just wondering, thinking about the implications of your model in this immunotherapy context.

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Would delivering an agent like imipermine Could it at all interfere or become antagonistic with lymphocyte migration throughout the tumor or perhaps have some on-target toxicity with the normal brain.

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Yeah, that's a great question. And it would have been touched on if I'd done the third half of my talk, but I'm not going to do that. So I'll just try to give you the short answer.

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When people look at T cells migrating and lymphocytes kind of generally.

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They tend not to have these really spiky protrusions like you saw with the glioblastoma cells.

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They oftentimes have this more amoeboid or rounded bleb-like leading edge in their migratory.

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And we've investigated that scenario now using a biophysical model for for blubbing too and amoeboid migration.

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And what we've concluded is that Cells should be able to move just fine and at very fast speeds if they use blebbing combined with adhesion.

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But blebbing dynamics alone without adhesion is ridiculously slow and can't explain what you see with T cells and brain tissue.

06:12:00.000 --> 06:12:04.000
I could probably just give you a little, I'll just show you a movie just to get you thinking about it.

06:12:04.000 --> 06:12:13.000
Here's T cells migrating in brain tissue. Same assay as before, but now we swapped in T cells instead of glioblastoma cells in green.

06:12:13.000 --> 06:12:21.000
And again, magenta's vasculature and microglia. And what you see is a lot of green cells that aren't moving. They're just sitting there.

06:12:21.000 --> 06:12:26.000
But the ones that are can move very fast. Like watch this champ here.

06:12:26.000 --> 06:12:35.000
Right there, back and forth, back and forth, then out. And when they moved, they moved really fast, like 10, 20 microns per minute.

06:12:35.000 --> 06:12:42.000
But a lot of cells aren't moving. We're concerned about the therapeutic cells that come in there and just don't do anything, just sit there.

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Time's ticking, you know? They got to go get killing. Don't sit there and wait.

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So we want to understand why they're waiting. We want to understand

what limits their speed.

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But they don't seem to really use these philopodia so much.

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They can do that. They're capable. We've made like a whole… in vitro, we can see all kinds of protrusions of T cells.

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Some of which look kind of spiky, like this guy here with with that thing. So if you treat these cells with fashion.

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They've got other options to go explore the space that don't require Philopodia and actin bundling to do it.

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So our prediction is that they won't be very sensitive to imipramine.

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In a negative way. But in fact, we need to test it. So that's on the list there.

06:13:31.000 --> 06:13:57.000
Thanks. So I had a quick question, Dave. That was an awesome talk, by the way. But I had a quick question about sort of other cytoskeletal events that might happen and thinking about sort of cells undergoing a mesenchymal transition, for instance, where you see a lot of cytoskeletal rearrangements. I don't know that those are driven by fasten, so maybe this drug wouldn't affect that. But have you guys thought about that or looked at that?

06:13:57.000 --> 06:14:12.000
Yeah, I haven't thought too deeply about it. I mean, Katie got me excited about like two months ago. What? Remember me? What is it? Sorry, first. I'll come back next week with a full report.

06:14:12.000 --> 06:14:24.000
Yeah, no, I haven't really thought about it too much, but certainly I… Once you change even just the shape of the cell, you've kind of changed everything. Yeah. Because you've changed the architecture of the cell and that affects function.

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It's very hard to have that not affect signaling, to have it not affect other parts of the cytoskeleton.

06:14:29.000 --> 06:14:37.000
Have it not affect metabolism? It's almost unavoidable. That's why I

think these pleiotropic effects probably kind of make sense that it's actually mostly targeting fashion.

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I'm not sure about that, of course, but it could be multiply targeting a lot of things.

06:14:43.000 --> 06:14:54.000
Seems more likely that it really targets fashion and then the downstream impacts of that play out differently depending on which cell they hit, whether it's a macrophage or… or a T-cell or a glomal cell.

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Cool. It'd be cool if all those ways have played out were all in favor of a better outcome in the club by someone.

06:15:04.000 --> 06:15:26.000
So have you thought about the mechanism of action of why the drug that you're focusing on inhibits the mechanistic action? Because it's a tricyclic compound and there's so many like diazepines or things that are already FDA approved, have those been looked at for migration inhibition or is it something about this like

06:15:26.000 --> 06:15:44.000
Moiety of this drug that specifically targets that you know migration aspect. We're following up on the fashion discovery from the, there's a group in Cartagena Spain that discovered this and I cited their paper very briefly Which is this paper.

06:15:44.000 --> 06:15:59.000
Which I didn't go into any detail about. But they actually did a docking study with these tricyclic compounds, including omipramine, and found that fashion was one of their top hits in a simulated You know, kind of docking attempt.

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I guess that's kind of what you might wonder, well, how does that explain its antidepressive activity?

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I'm not a… I'm not an expert on psychology or psychiatry, so I don't know.

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But what I might think, a reasonable hypothesis to me is that if it's interfering with F-actin dynamics Of course, the brain has its wiring is via F-actin protrusions to form dendritic spines and things like that.

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So if you interfere with that, you're going to interfere with the normal normal dynamics of brain architecture, and that may manifest as It's a reuptake inhibitor, which is how it's described in the literature typically.

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But at the mechanistic molecular level might actually be through fashion, probably no.

06:16:42.000 --> 06:16:52.000
Until someone finds other target. Thanks. Yep.

06:16:52.000 --> 06:17:01.000
All right. If there's no other questions, let's thank our speakers for the day.

06:17:01.000 --> 06:17:12.000
Thank you all for attending our first inaugural, I guess that's redundant, but anyway, our inaugural symposium for our center.

06:17:12.000 --> 06:17:22.000
Hopefully we'll see you next year at our second non-inural symposium. Cool. Thanks.