

2020-02-13 How to Detect Dormant Cancer Cells Surviving Microtubule-Targeting Agents

Michael David: Hello everyone and welcome to ACS Webinars, connecting you with the best and brightest minds in chemistry live every Thursday from Washington DC. I'm Michael David, and I am pleased to be your host for today's broadcast, which is being co-produced by ACS Pharmacology and Translational Science.

[00:00:22] According to the American Cancer Society, in 2019 over 1.7 million new cancer cases were diagnosed. And 600,000 people died due to that disease and today we are going to be hearing about a new tool that can be used in this fight. We will be joined by Lenka Munoz, the head of the cell signaling laboratory at the Charles Perkins Center at the University of Sydney.

[00:00:46] Lenka will be discussing the tubulin code and its impacts on the efficacy of microtubule targeting agents. Our moderator for today is Patrick Sexton of Monash University, who has made significant impact in the fields of pharmacology and translational science. He is a leading international researcher in the field of G-protein coupled receptors and his research crosses industry and academic boundaries through the elucidation of fundamental biology and the intersection of this with drug receptor interactions.

[00:01:18] And now I'm going to turn it over to Patrick to get us started for today.

[00:01:22] **Patrick Sexton:** Welcome, everybody from all around the globe and all sorts of time-zones. In Australia, here, it's very late in the morning and it's my pleasure to be here. So I'm editor in chief of ACS Pharmacology and Translational Science, which is a broad biomedically focused journal that looks at, research focusing on fundamental research and how that can be leveraged for improved treatments of disease. So I'm excited today that we have Lenka Munoz, who is going to talk about her research, recently published in the journal. So Lenka, got her Pharm. D. In 2001 from Comenius University in Slovakia, before moving on and doing a PhD in medicinal chemistry, graduating in 2005 from the University of Bonn in Germany and then did a post doctoral, placements at Northwestern University in USA where she moved into starting to look at molecular pharmacology. In 2011 she joined the University of Sydney where she heads the cell signaling laboratory at the Charles Perkins Center, and she's going to talk a little bit about some of the work she's been doing, applying her background in medicinal chemistry and the overlap with pharmacology.

[00:02:34] Lenka's research focuses on molecular understanding of the action of cancer drugs. And she's got a strong interest in developing effective therapies for oncology diseases such as glioblastoma. And today we'll be focusing, on detecting and targeting to them and cancer cells and discussing, relationships between microtubules, and microtubule targeting agents. So, over to Lenka.

[00:02:59] **Lenka Munoz:** Thank you, Patrick, for your introduction. And so as Patrick said, that I will be talking mostly about my research that is focusing on the microtubule targeting agents. So the webinar outline just to walk you through what I will be talking about in the

next 30 minutes or so is that the main focus will be the microtubules and microtubule targeting agents.

[00:03:23] However, I will also take a little detour to kinases, which are probably my first and biggest scientific love story, but working on one particular kinase inhibitor that I will introduce to you in a couple of slides. My research took an unexpected turn into the field of microtubule targeting agents, and during this transition I have learned the importance of using orthogonal inhibitors in cancer research. And I would like to share this knowledge with you. And then from the kinases, which will be just a little bit before we come back to microtubules the tubulin code and I will discuss in detail the pharmacology of microtubule targeting agents and how we arrived at Cancer dormancy.

[00:04:06] But before I start my scientific presentation, I would like to ask you a questions and I turn over to Mike for us, for a moment.

[00:04:15] **Michael David:** All right. Our first question for all of you is, have you worked with cells and or analyzed cell based data of drugs? Is it that you have never worked with cells and are not familiar with cell based data, that you've never worked with sales, but are familiar with cell based data?

[00:04:29] Or that you have worked with sales and are familiar with analyzing that sell based data?

[00:04:38] And Lenka, 44% of the audience said they have never worked with sales and are not familiar with cell-based data. 24% said they have not worked with cells, but are familiar with the data. And 32% say they have worked with cells and are familiar with the data. So with that, I'll turn it back over to you.

[00:04:53] **Lenka Munoz:** Oh, thank you, Mike. So I will maybe explain a few of the data in more details because half of the audience might not be able to follow when I talk about the surveys, experiments. And that helps me a lot to make this presentation enjoyable for everybody. So it was a little bit of introduction to the microtubules.

[00:05:13] So the microtubules are cytoskeletal elements of eukaryotic cells, and they have a diversity of functions. So for example, they provide mechanical support for cells, or in other words, microtubules are the walls that shape the cell. As you can see here on this image where we visualize microtubules with confocal microscopy and we got these beautiful images of glioblastomas cells.

[00:05:38] Microtubules are crucial for proliferation of cells because they are necessary to form the mitotic spindle in mitosis when the cell will separate the duplicated chromosomes and then it will divide into two daughter cells. And they also provide mechanism for cells to move and also to differentiate.

[00:05:59] Structurally, microtubules are dynamic polymers that assemble from heterodimers of alpha and beta tubulin. So the microtubules are phoned by polymerization of these heterodimers, but at the same times, it is very important for the microtubules to be

able to depolymerize because this dynamic polymerization depolymerization enables microtubules to, do their function.

[00:06:26] Now, microtubules are highly conserved in their 3D structures, so they kind of look the same. And the major question in the field was to understand how can microtubules, this part looking the same adapts to diverse functions in the cell. And what do we know now is that the function of microtubules is determined by the interaction with microtubule associated proteins and also by the tubulin codes.

[00:06:55] So what is the tubulin code? Tubulin code is a combination of eight alpha and nine beta tubulin isotypes that are variably decorated by post-translational modifications. These modifications can be the phosphorylation or acetylation that occur on other proteins or also that tubulin specific modifications such as removal of the panel of the last residue in the residue on the alpha tubulin called the tyrosination, and then removal of the penultimate glutamate, when we get that, they'll talk to tubulin.

[00:07:31] What we also know now, and this is a, there are several or many nice papers coming out, especially in the last decade, is how this post-translational modifications impact on the function of microtubules. So, for example, there is a very, very elegant studies showing that the tyrosination, detyrosination, is crucial for the guiding the chromosomes to the cell equator during the mitosis so the cell can divide. But what is less known is how the tubulin code impacts on the efficacy of microtubule targeted agents.

[00:08:08] So microtubule targeting agents, I don't think I need too much of an introduction because it is one of the most important and oldest cancer and class of cancer drugs that are used in the clinic to treat various tumors for a very long time. The first microtubule targeting agent, to my knowledge, was approved by FDA in 1963 and that was Vincristine. And the reason for this is very simple. Cancer is, first of all, a hyper-proliferative disease where excessive cell proliferation leads to life threatening tumors, and because the dynamic microtubules are crucial for cell proliferation, microtubule targeting agents by blocking polymerization or the polymerization of microtubules will disrupt the function of, of microtubules. And then, and then will then lead to the cell to start apoptosis. So if the cell doesn't have a functional or dynamic microtubules, it will not proliferate. And that will then cause, that that's how the therapy works.

[00:09:16] And with these microtubule targeting agents, they are classified based on whether they inhibit polymerization or the polymerization, and also based on their binding site. Well, what is important to understand that at the mechanistic level, it is disruption of the microtubule dynamics that leads to the apoptotic effect of these drugs.

[00:09:39] One more thing that I would like to have mentioned, because I will come back to it, is that in oncology, a microtubule targeting agents are considered a non-targeted or non specific chemotherapeutic drugs because micro tubules are expressed in every cell. And this is in contrast to the molecule targeted therapeutics such as kinase inhibitors, which are used in personalized fashion. So for example, EGFR inhibitors are used to treat patients with EGFR amplification and so on. Whereas microtubule targeting agents could be technically used for every tumor on every patient because microtubules are expressed in every cell.

[00:10:22] But now I would like to take the detour since I started talking about the kinase inhibitors and, and tell you a little story from my laboratory.

[00:10:33] So one of the first questions when I started my own lab here at the University of Sydney, I asked was whether MK2 kinase inhibitors really improved chemotherapy efficacy in glioblastoma, and this was based on several papers and drug discovery pipelines in pharmaceutical industry that have shown that targeting MK2 pathways improves efficacy of chemotherapy.

[00:10:58] And I will not go into the details of this signaling pathway because it is not relevant to the story, but I will talk more about MK2 inhibitors that we use to address these questions. So I ordered, all that time commercially available MK2 inhibitors. These were two ATP type-one kinase inhibitors, developed by Pfizer.

[00:11:22] And then I could also get hold of two ATP, non ATP-competitive for allosteric MK2 inhibitors. And so we started testing these inhibitors in our glioblastoma models, and what we observed very early on was that one of these inhibitors was behaving differently, namely the compounds one or CMPD1 and what we observed was interesting, CMPD1 induce, apoptosis in glioblastoma cells, but all the other inhibitors genetic knockdown or genetic knockout with a CRISPR cost platform did not induce apoptosis, which was then telling us that the efficacy of this drug is non-related to MK2. So we performed an extensive drug target validation studies, and what we identified was that the primary target of CMPD1 in the cell is not MK2 kinase, but it is actually tubulin.

[00:12:26] And that CMPD1 inhibits tubulin polymerization and the apoptotic efficacy of this drug results from inhibiting tubulin, not from MK2. And back, this was already a few years ago when people in the kinase field usually talked only about profiling kinase inhibitors within the kinome, so I was quite surprised to find out that kinase inhibitors can inhibit proteins outside of the family.

[00:12:57] And I would like to ask you another question, in relation to this, discovery. So I'm turning over to Mike again.

[00:13:05] **Michael David:** Our second question for you all is in your drug discovery research, what is your approach to off targets in relation to targeted protein families? Is it that you consider off targets only within the targeted protein family, that you consider off targets within and outside of the targeted protein family?

[00:13:23] That you rarely consider off targets in your research or it's not applicable?

[00:13:31] All right Lenka 53% of the audience said that it was not applicable to them, and the next highest was 30% that said, they consider off targets within and outside of the targeted protein family, and the rest got under 10% each. So with that, I'll turn it back over to you.

[00:13:46] **Lenka Munoz:** Thank you. Well, that's that's great that the majority of people does consider, off targets outside of the family because as I reviewed, in my paper published in Nature Reviews Drug Discovery in 2017 kinase inhibitors, especially are quite

promiscuous, and they inhibit many proteins, that are non-kinase proteins such as tubulins, bromodomains or IDO enzymes. So it's good to see that this word has got out and people are looking outside of their targeted family. And I would also like to just very briefly emphasize that the comprehensive drug target validation is very important, especially in the cancer research when we trying to understand how cancer drug works and to assign them the right molecular target.

[00:14:37] And this drug target validation studies, they can be quite comprehensive and take a long time. That's why sometimes people in the field take shortcuts and I would like to share a little trick that we have learned while working on this kinase inhibitor that inhibited tubulins.

[00:14:56] And the trick is to look at the morphology of the shape of the cells. Because as I said at the beginning, microtubules are essential for cell morphology. They shape the cells and what actually guided us in the CMPD1 investigation was the fact that cells very quickly changed their morphology.

[00:15:16] By very quickly, I mean, within 30 minutes to two hours. And so we worked with glioblastoma cells, which normally have these nice, star shape, a star shape, a large star shape. And when we treated them with CMPD1 they shrunk within 30 minutes. Because, I thought that this might be related to targeting the cytoskeleton.

[00:15:40] We tested another 17 cancer drugs in seven cancers cell lines to confirm this hypothesis and what we observed was exactly fitting our hypothesis, so cell drugs such as Tivantinib, which is another kinase inhibitor that also inhibits tubulin or Vinblastine, which is the prototypical microtubule targeting agents.

[00:16:03] They shrunk cells very quickly within few hours. So these are, the black arrows are showing you this is a healthy cells. These are kinase inhibitors results in [????] that do not target tubulin. And you can see that the cells remain their beautiful morphology. They do not change for several hours.

[00:16:23] Whereas the drugs that target tubulin, they cause this shrinkage. We confirmed using the Fucci platform that this shrinkage was happening to cell in any phase of the cell cycle. So the red cell, are the cells in the early cell phase, the G₀, G₁ cells. The green ones are nearly the mitotic cells, but it doesn't matter in which phase they are.

[00:16:49] They were all shrinking. Whereas the cells that have been treated with real kinase inhibitors did not show the changes in morphology. So this is a little trick that I like to share, and I always tell everybody working on concept drugs to make sure that they are aware of all the targets they are hitting. All you have to do is just to look under the microscope at the morphology of your cells.

[00:17:15] Now people in the audience might be wondering, why are we interested in this microtubule targeting agents and CMPD1 compounds since so many drugs, so many microtubule targeting agents already exist and are clinically used. And the question, and the answer for this question is very simple.

[00:17:33] The major hurdle in the neuro-oncology drug discovery is the blood-brain barrier. For a cancer drug to treat glioblastoma it does not only have to have apoptotic or anticancer activity, it also has to cross the blood-brain barrier. And those working in the space of CNS drugs know that the CNS drugs are usually smaller, less polar, and they cannot be P-gp substrate. And unfortunately all the clinical microtubule targeting agents are natural products or the analogs and that that means that they are large in size and they are highly polar and therefore they do not cross the blood-brain barrier. And cannot be used for the treatment of glioblastoma.

[00:18:17] So what I say, always very simple that of a lead molecule, CMPD1 works like vinblastine because it inhibits tubulin polymerization, but it looks like fluoxetine, an antidepressant drug is much smaller. And so we are now developing this class of small molecule tubulin inhibitors for the treatment of glioblastoma, but also of other brain tumors.

[00:18:43] And so while other medicine or chemists are moving forward in the drug discovery pipeline, we also tested the small molecule MTAs. We benchmark them against the clinically used tubulin inhibitors, Paclitaxel, Vinblastine, Colchicine, and Ixabepilone. In my laboratory, we work with a panel of glioblastoma STEM cell lines that present the glioblastoma subtypes and what we observed was a very interesting. We knew, and that was confirmed, that the potency of other drugs is weak because of the smaller size. We are losing hydrogen bonds with the target.

[00:19:25] But what I observed very early on was that, none of the curves, regardless of the potency, so even the most potent drug, Vinblastine, the curve was never coming all the way down, meaning that they did not hit all of the cells. And the, that was all the efficacy we could see in any cell line with any microtubule targeting agents was up to 30, 40% of cells remaining viable after the treatment. So because this, MTA efficacy was the same regardless of the potency, this was indicating to me that the problem is the targets, not really the drug.

[00:20:05] But then I had another problem. If microtubules are highly conserved in their 3D structures, and they are expressed in all the cells, what can cause this lack of efficacy? And that's when I saw that, it might be the significant molecular diversity or the tubulin codes. And I asked a very simple question, does the tubulin codes impact on the efficacy of microtubule targeting agents.

[00:20:32] So what we did next, we profile the tubulin codes in the panel of our 15 glioblastoma cell lines. So we work with, we included three standard cell lines. These are commercially available glioblastoma cell lines that are serum grown. And they fully differentiated. And then it's our panel of twelve glioblastoma STEM cell lines that we grow on the serum free condition.

[00:20:58] So they remain, they re-keep their STEMness and they do not differentiate. And what we observed was interesting. So the first important observation was that the tubulin code of the serum-grown cells did not represent those found in the STEM cells, and then we found quite a large diversity ranging from 20 to 130% in the tubulin code, which tells me that the microtubules are not the same in every cell. And this actually fits when I go back to

our confocal image, because in disease taking images, the colors are assigned based on the depth of the tubulin expression in the cell.

[00:21:42] And you can see that this is one antibody use confocal microscopy, and each cell has a different color, which means that the level of tubulin expression in these cells within one cell line is quite diverse.

[00:21:56] We next on move to determining, efficacy of, several orthogonal microtubule targeting agents in all cell lines.

[00:22:04] We performed viability assays, got the dose response curve and calculated the potency. IC 50 efficacy. So that's what I'm always most interested in my lab or in my research to see how deep the curve comes. And then we calculated the area under the curve. And also the hill slope gives you a lots of information about the cells to cell variability within your cell line.

[00:22:30] And what we've got was lots of data, cause this was six drugs tested in 15 cell lines. But the data was kind of a mess or unconvincing. And what I mean by that is that when we looked under the microscope, we could see that in some cell lines, the microtubule targeting agents killed the majority of the cells.

[00:22:52] Whereas in other cell lines, we had lots of viable cells after the treatment. But when we looked at the curves, and especially the efficacy, that E-max, and the values when nearly identical. So what did we do wrong?

[00:23:06] We did not look at the different proliferation rates of these cell lines. So cancer cells proliferate at different speeds, and this greatly influences the dose response curves, if you are testing antiproliferative drugs.

[00:23:22] So the quicker the product duration, the more cell cycle during the assay, the better will be the potency of your, of your drug. However, this will give you artifactual drug sensitivity. And as I said, we couldn't see differences in our panel. And to overcome this Peter Solgar's lab and the MIT and Harvard, they developed the GR calculator tool, which will adapt those response curves to the doubling time of those cells.

[00:23:52] So. What is also very good about using these GR or growth rate corrected dose-response curves is that they will also tell you whether your drug has a cytotoxic effect or a cytostatic effect. So what do we then did, we determined the doubling time for all of our cell lines, and we use the GR calculator, which is available online for free. And we re-calculated all of the dose-response curves, and we've got the gr, gr curves for old drugs and re recalculated, gr metrics as well. And then we moved on to correlating this data with the tubulin code.

[00:24:35] Now, because we also still had the relative metrics so the standard, I see 50 E-max and AUC data, we correlated that as well. And what we got was that there were only random correlations for individual drugs with some features of the tubulin codes. But interestingly, we've got consistent correlations with the gr metrics and specifically the GR

max value, which means the bottom of the curve of all drugs tested, negatively correlated with the total levels of alpha and beta tubulin.

[00:25:12] Which means the MTA efficacy is independent of the tubulin isotypes and post-translational modifications. But, it correlates with the total amount of the tubulin in the cells. So if there is less tubulin in the cell, there will be less efficacy.

[00:25:31] We moved on to validate this correlations. So the hypothesis or the correlations that we got was that MTA efficacy, declines with decreasing levels of alpha and beta tubulin.

[00:25:46] We picked four cell lines that had, so two of them, WK1 and RN1 were the most sensitive, and you can see that the cosine curves were going quite down into the site of toxic spice. And then we have two cell lines, JK2 and WK1 which are quite resistant. And here, you can see that the effect of the drugs was only cytostatic.

[00:26:12] We confirmed decreasing tubulin levels by several methods. One of them was immunofluorescence, so you see that the intensity of beta tubulin is decreasing. And what we also found out when we looked at our mRNASeq data is that this cell lines actually had an increasing levels of stemness markers. So this is interesting.

[00:26:34] The less tubuline, the most stemness markers, and we validated the efficacy with the longterm assay, or where we treat cells for two weeks with a very high dose of tubulin inhibitors just to see how many cells can be killed in total by these drugs. And you can see that the percentage of surviving cells after this longterm treatment is increasing.

[00:27:01] So we confirm that the less, tubulin in the cells also indicates less efficacy and apolplectic activity of microtubule targeting agents. Now, the questions that, of course everybody would ask, is this drug related? The answer is no. We tested many other drugs in these assays and also the clinical MTAs and unfortunately, even the most potent, and clinically used vinblastine, for example, also generates this surviving cells and unfortunately also in the sensitive cell line.

[00:27:39] One important step of validation was to exclude drug efflux pumps because microtubule targeting agents are often substrates for PGP and another drug influx pump so we can form this longterm assay where we co-treated cells with colchicine and drugs inhibiting the pumps.

[00:28:01] Yeah. As you can see is that the percentage of surviving cells did not decrease. It's actually increased, which I think is because some of these drugs induce proliferation. And we could also show a complete target engagement in every cell. And we did this by immunofluorescence, of tubulin network, and you can see that every cell in both cell lines shrinks.

[00:28:26] That means that the drug got into the cell and induce a tubulin depolymerization. So this confirms that the MTA efficacy is independent of the expression and activity of the drug efflux pumps.

[00:28:43] So next we were interested to characterize this surviving cells. And what we've done is we profile them by flow cytometry, because remember, these have only few cells left, after we kill them in the microtubule targeting agents.

[00:28:58] And what did we found out was that the surviving cells were expressing markers of dormancy. And so what is dormancy? Dormancy is a sleeping period in the organisms life cycle when growth, development and activity are temporarily stop. So this means that dormant cells, they are sleeping cells, they do not proliferate, but they also do not die.

[00:29:22] And so if it's sleeping is temporarily stopped, we wondered whether these cells will recover. So what we did next is we generated, we performed again the experiments. We kill the majority of glioblastoma cells with microtubule targeting agents. And then we removed the media and we were observing what was happening to the cells.

[00:29:46] And what we found out was that these cell started proliferating. The more of, the less sensitive cell line recovered quicker then the sensitive cells, but they all recovered and started proliferating again. And when we tested their efficacy against microtubule targeting agents, what we observed was identical curves.

[00:30:09] So this excludes drug resistance, but implicates drug tolerance. So what is a drug tolerance?

[00:30:17] Drug tolerance is an ability of cell to survive, but not proliferate in the presence of cytotoxic treatments. And this is transient, irreversible, and non mutational phenotype in most cases. And this is the difference between the resistance.

[00:30:34] So tolerance usually comes up in the first exposure of tumor cells to the antiproliferative drugs. This drug tolerant persister cells, that's what the cells are called when we talking about drug tolerance, they can either exist in the tumor mass before the treatment or the drug treatment causes proliferative cells to transition to a drug tolerant persister cells. And so what can happen after that is that these drug tolerant persister cells in drug holidays, when we remove drugs, resume proliferation, and go back to, the original, population, that's what we have seen in other hands. But sometimes these drug tolerant persister cells then can accumulate mutations and they become resistant and resistant cell means that the resistance cell proliferates also in the presence of anti proliferative drugs.

[00:31:33] This was actually well fitting with our observation because drug tolerance with very often driven by activation of dominancy mechanism. And before I briefly talk about how to target dormant cells, I have one last questions for the audience about the origin of the drug tolerant persister cells.

[00:31:53] **Michael David:** All right, so our final question for you all is, if you know the origin of the term drug tolerance persister cells.

[00:32:00] Did it come from neuroscience, microbiology, immunology, or none of the above?

[00:32:10] Well, starting off microbiology was taking the lead, but immunology eventually had the largest share with 43% and microbiology had 38%. And with that, I'll turn it back over to you.

[00:32:21] **Lenka Munoz:** All right, so there must be many microbiologists in the audience because microbiology is the correct answer. We borrowed this terminology from microbiology, where the persisters are on growing those slow growing bacteria with antibiotic tolerance.

[00:32:38] So what can we do with these drug tolerant persisters or the dormant cells? There are three strategies how this can be targeted. There is a so-called sleeping strategy that keeps the dormant cells in the harmless dormant state, and this is actually used in the clinic as well.

[00:32:57] For example, ER antagonists are used to maintain a breast cancer cells in dormancy. The advantage is that you stop reactivation or awakening of these dormant cells. But however, a patient is living with a minimal residual disease, and eventually, even if these dormant cells we say they dormant, they slow-proliferate and thus tumor mass will eventually grow.

[00:33:25] Then there is a bit of a controversial approach, which we call awakening strategy, and that means that we invite these dormant cells we use, in drugs that can reactivate cell cycle in dormant cells, and then you treat them with antiproliferative therapy which will then hopefully this assume would eradicate the tumor completely.

[00:33:49] As I said, this is a very risky approach and translating this approach to the clinic will be probably quite difficult because you wouldn't be making, tumors to grow faster in the first place before the treatment. And probably the safest, approach is so-called killing strategy, which is a two steps of the first approaches to kill proliferating cells with antiproliferative drugs and then target the dormant cells.

[00:34:18] And there is quite a lot of ongoing research now. Identifying targets in these dormant cells in order to develop drugs that will kill dormant cells. Because we have to remember that the biology of proliferating green and dormant purple cells is very different. So I just summarize my talk, with few points, then I would, like, as a take home message. So tubulin expression varies in cancer cells. And I think that the microtubule targeting agents should not be named non targeted chemotherapeutics because they follow the principle concept of pharmacology. More targets means more efficacy.

[00:35:00] Now, as I learned kinase inhibitors have non-kinase off targets and a audience is aware of this, which is good. And I always tell to my students, you have to think outside of the books and always look outside of the family that you are targeting. And I, I'm a very strong advocate for comprehensive drug target validations that should never go out of style.

[00:35:24] It is important in the concept drug, discovery to use the growth rate metrics rather than the relative metrics, because the proliferation rights will impact on the efficacy of drugs and to detect dormant cells. I think the easiest is just to look at the bottom of the dose response curve and in the GR dose response curve, whether your curve goes into the

phytotoxic space. It is also important to remember that cancer is not purely a proliferative disease and dormant cancer cells have been detected in many cancers.

[00:36:00] So I would propose that the war on cancer should also become more on sleeping cancer. I would like to acknowledge all my past and present members of my lab and collaborators and friends. And that brings me to the end of my presentation.

[00:36:16] **Patrick Sexton:** Hi, everybody. Thanks, Lenka. That was a really wonderful seminar.

[00:36:20] A brief note. So just to, people aren't really aware of the journal, it's a relatively new ACS journal, bridging that gap between chemistry and biological science and how that's translated into disease treatment. And, we publish in a whole range of areas, including oncology. And it just illustrates a few papers that have been, published in this area, including the one from, that Lenka was just talking about.

[00:36:45] And so I guess now I'm moving on to asking questions, a question, which I guess is a general question. What are the sort of major side effects that that one could expect from this type of treatment? And is there, you know, any difference in terms of the type of agents that, that you're looking at here versus some of those natural product agents?

[00:37:07] **Lenka Munoz:** So the major side effects of microtubule targeting agents, neurological. So there is a neuropathic pain and hematological. I'm not a clinician, but what I gathered from the literature, the hematological side effects can be managed. The neurological can be sometimes more severe. And I've heard it's reported that the treatment with a microtubule targeting agent had to be stopped because of the neurological side effects.

[00:37:35] But they're still widely used and to the other part of the questions. So, ours is, we don't exactly know, because we are in, in the early stages of the discovery. We have, PK and toxicity data in the animals with our class of drugs, and they will tolerate it. But, you know, it's still a long way to go.

[00:37:57] The only difference between ours and all the natural products and clinicals is really just in the size and the ability to cross the blood brain barrier. We are getting excellent brain uptake with our follow up compounds and that's what makes a difference. It's not really the side effect profiles or the, or the efficacy or the mechanism of action that is similar or the same, identical to what is out there.

[00:38:26] But what is out there doesn't cross the blood brain barrier and cannot be used in the treatment of any brain tumors.

[00:38:34] **Patrick Sexton:** I guess as an extension question from Peter about how you might assess those sort of side effects pre-clinically?

[00:38:42] **Lenka Munoz:** Again, I'm not a clinician and I will leave this to my colleagues once we get there.

[00:38:52] **Patrick Sexton:** Okay. Good. Mmm. So a number of questions, technical questions, Does the change some morphology related to polymerization or deep polymerization or the relative extent that this occurs. So it's a question, relating to the changes in some apoptosis and whether that was related, to the active polymerization or the deep polymerization or the rate with which that's in equilibrium, I guess.

[00:39:23] **Lenka Munoz:** Yeah. Good question. Both agents, both the polymerizing and those that maintain polymerization. So both paclitaxel and vinblastine, they both induce changes in morphology. So looking under the microscope will not tell you whether your drug is inhibiting polymerization or inhibiting that polymerization.

[00:39:45] But. I strongly believe that if the cell is changing the shape and early on, that it's important to understand that these changes are early because you cannot look under the microscope two days later when all the apoptotic machinery has been activated. So apoptosis will happen two, three, four days after you treat the cells with drugs.

[00:40:07] So then all the cells look sick. But. Morphology changes within few hours when apoptosis is not occurring. That's what indicates that the drug, and this is we tested kinase inhibitors, but I would assume that other cancer drugs will do the same if they target tubulin.

[00:40:26] **Patrick Sexton:** Great. Here's a question from Maria. She comments that, that she thinks that maybe what you're calling dormant cells, may be perhaps hypoxic cells and wonders whether or not combination therapies with hypoxia activated drugs might, be a mechanism that that could be targeted.

[00:40:45] **Lenka Munoz:** Yes, definitely in vivo or could be hard because hypoxia, cells don't have oxygen, obviously they will not survive and they might activate dormancy. But these experiments that I have presented were all done in the cell culture under the normal 21% oxygen. So that's a normoxia. We don't work or we do, but not in this project under hypoxic conditions. So hypoxia is not relevant to this set of experiments, but definitely relevant in vivo.

[00:41:20] **Patrick Sexton:** I guess sort of related to that in terms of, combination treatments, what would be the best combination that you found so far?

[00:41:30] **Lenka Munoz:** Yes. The best combination is to combine a drug that kills antiproliferative drug that kills proliferating cells. And that's other microtubule targeting agents because they do have so far the best because in glioblastoma models and we, when we combine it with drugs against dormant cells, and these are ongoing projects in my labs that I did not present for obvious reasons. But when we combine those two, we can nicely eradicate a nearly the end, I don't want to say the entire cell population because it's, some clinicians will tell you, you can never kill every single cancer cell, but we get much better efficacy and we do not get regrowth of the cells.

[00:42:15] So I believe in combining a drug against proliferating and a drug against dormant cells, just those anti dormancy drugs that's still, lots of science in making. There are others

working on that as well. And several drugs and targets have been identified, but, this hasn't moved past animal models.

[00:42:38] **Patrick Sexton:** No problem. Here's a question from Peter. How does the tubulin code in malignant cells compare to, you know, normal neurons in the various different types of neurons.

[00:42:48] **Lenka Munoz:** Ah, yeah, that's a good question. So, yeah, that, that's difficult, because neurons express, a lot of beta three tubulin, for example.

[00:42:58] So we were trying to compare our tubulin code to astrocytes because glioblastoma is a, originates from astrocytes. But that was quite challenging because we were getting, it's difficult to culture astrocytes. They do not proliferate in vitro. So, and you can get, astrocytes from animals, but not from humans because they do not proliferate.

[00:43:23] So we didn't have access to many and those few that we tried it unfortunately it didn't work. So that's why the paper doesn't have these non-malignant controls. But knowing that beta three tubulin is used as a marker, as a neuronal marker so for example, when we start the differentiation, which time for beta three tubulin, because that is highly expressing neurons, it is anticipated that that will also cause the side effects in the brain because it will target. So ideally we haven't cracked that yet. But if somebody could find that tubulin iso type or tubulin, post-translational modification that is only cancer specific, that would be the way to go.

[00:44:11] But unfortunately, we haven't succeeded with that because we couldn't generate a good controls.

[00:44:19] **Patrick Sexton:** So, I guess this, this question is an extension of this discussion around how you target dormant cells. So obviously most cells in the brain in terms of proliferation are relatively dormant. Mmm. And so how, how do you get selectivity for the cancer cells?

[00:44:38] And, I guess, you know, have nontoxic drugs, to kill the, the dormant cells as well as those proliferating cells.

[00:44:49] **Lenka Munoz:** Yes. Yes. A good question. What is important to understand is that dormant cancer cell is different from dormant noncancerous cells. So the noncancerous cells, when they dormant, they fully differentiated.

[00:45:03] It's a completely different mechanism and biology of those cells. So for example, targeting these dominant cancer cells, the targets that my lab has identifies and others, they are not relevant that much to the dormant nonmalignant cells. So that's where people. I often get this question, but it isn't many of the cells dormant in the human body?

[00:45:27] Well, that's right, but they not cancerous. Dormant cancerous cells is still a malignant cell and it has a different biology, different pathways that are driving that dormancy. And awakening than non cancerous cells. So the difference between dormant

cells in the brain that are not cancerous would be that they probably cannot wake up and start proliferating.

[00:45:52] Whereas cancer cells, they can, and dormancy, although us, as difficult as it is to comprehend dormancy is a hallmark of STEM cells. STEM cells, are the ones that are. That we teach are the bad guys because they proliferate and they cause the tumor to grow. But at the same time, these STEM cells that can super rapidly proliferate and cause too much to grow at the same time, this STEM cell will transition to a dormant state.

[00:46:23] So that I don't think. I don't know. I mean, once we get there, we will know once we develop drugs that can be used in patients. This can be also, not only then, but right now the biology I know is different. I hope that answers that question.

[00:46:42] **Patrick Sexton:** Yeah. I guess extending from that, given they have a different biology.

[00:46:47] So presumably, you know the you could have strategies for selectively activating the cell cycle in the cells and then combine that with your deep polymerization. Has there been much thought in the field about how you might do that?

[00:47:01] **Lenka Munoz:** Yes. Yes, there are. So there are few targets that have been identified and you can do that.

[00:47:08] And we are actually, hopefully soon we'll be able to publish that paper where we can reactivate these dormant cells with inhibitors against a certain kinase. And then when we treat them with microtubule targeting agents, they respond better. So we are getting much better efficacy. The curve goes down, there will be no a drug tolerant persister cells once we combine those.

[00:47:36] So that is, I liked that approach because it's different. But like I said, clinically, when I presented, and there are clinicians, they always look at mandates and I'm crazy because I want to make the cells to proliferate even faster than they are. So that would translate. That approach will be difficult, but that's doable.

[00:47:59] **Patrick Sexton:** Yeah. Yes, yes. I'm sure there's always going to be a difficult one to get across. So, a question here relating, from the Harry. Hi. I see 50 values may be related to enhanced autophagy of compounds. Mmm. And can you evaluate differences in molecular action in dormant cells versus, non-dominant cells with respect to this or, or is there, no proliferation first, if there is no proliferation first?

[00:48:26] **Lenka Munoz:** So, whether I can evaluate the mechanism of action in dormant versus non dormant cells?

[00:48:34] **Patrick Sexton:** Yeah, I guess.

[00:48:36] **Lenka Munoz:** Well, from the, from the curves, when we do the dose response curves, you don't really get the mechanism. You can just get an idea of whether you are a cytostatic, so you're maintaining dormant cells dormant or cytotoxics, so you're killing them.

[00:48:54] The mechanistic investigations of dormancy is mostly driven by epigenetic because it's reversible. And lots of, focus in the field is on histone methylation. So it has been shown by several nice papers is that, the dominance is driven by increased methylation of the histones or increase the methylation.

[00:49:17] And it also depends which type of cell model the paper is using. So all this is, reviewed in the review that I had on last slide, and we are looking at this as well. So we did the histone proteomics of dormant cells, and it looks very different to the proliferating cells. So all we have to do now, it sounds easier said than done, is to figure out which of the epigenetic enzymes are responsible for the changes in the histone, proteomics, metalation, and which of those sites is relevant to dormancy.

[00:49:56] So it's doable. But it's a long mechanistic research, I would say

[00:50:03] **Patrick Sexton:** Yes. Perhaps a time for one last question. This one's from Evelyn. She asks, I see no reason why I, microtubule targeting agent cannot just be used on a longterm basis and that that would eventually eliminate the proliferation of dormant cells.

[00:50:19] So why can't we just do this?

[00:50:21] **Lenka Munoz:** Yeah, it would be good if it worked. And now, because dormant cells do not proliferate, and microtubule targeting agents, I mean, it's, there is a bit of a controversy in the field, but they usually target just the proliferating cells. The cell has to go through the mitosis and divide in order to be hit and killed by microtubule targeting agents.

[00:50:45] So what do we actually notice is the longer we treat with MTAs microtubule targeting agents, the more surviving dormant cells we get because some cells just reactivate their programs and they switch to dormancy. So that I don't think that will work because like I said, the biology of these dormant cells is very different to the proliferating cells and MTA targets.

[00:51:11] MTA target only the proliferating cells.

[00:51:17] **Patrick Sexton:** Great. Well, thanks everybody for your questions. Apologies to those that we didn't get to. Mmm. And thank you Lenka for your words of wisdom. If there's one important lesson that our listeners should learn, from your presentation today, what would that be?

[00:51:33] **Lenka Munoz:** I don't know if it's from my presentation, but here in Australia, it's already Valentine's day.

[00:51:38] So I would like to say is to spread the love of science.

[00:51:48] Thank you for watching this presentation. ACS Webinars is provided as a service by the American Chemical Society as your professional source for live weekly discussions and presentations that connect you with subject matter experts and global thought leaders concerning today's relevant professional issues in the chemical sciences, management and business.

