2 Long term colleagues and friends from Yale.

Today one is there actually speaking on the same topic which is by which he said degradable nanoparticles for skin cancer.

So it’s great to have them here today.

And one second left to find some notes right?

OK, so our first speaker is Mark Salzman.

He’s with Cela foundation professor, biomedical engineering and professor biomedical engineering and professor cellular molecular Physiology.

He focuses on trying to develop methods for disease prevention and to effectively deliver drugs to cells,
particularly to deliver chemotherapy to brain tumors.

He’s interested in controlled drug delivery to brain bow polymers, to supplement or stimulate immune function.

Still, interactions with polymers.

In tissue engineering and in fact he’s developed what is now the standard care for treating some brain tumors is very exciting.

He will be joined today by another colleague and friend, Mark Mike Gerardi, who’s a professor of dermatology.

He received his MD degree and his clinical training here.
His principal focus of research has been on the relationship between the immune system and cancer with clinical expertise in areas including cutaneous T cell lymphoma, squamous cell carcinoma in the and extracorporeal photochemotherapy. He’s credited with major contributions to understanding skin biology, immunology and skin cancer development, and has actually foot co-founder of two Yale startup companies to exploit some of these discoveries. So today they’re going to talk on a collaboration looking at Bio case of
nanoparticles with long interest of
Doctor Salzman to treat skin cancer
along interests of Doctor Gerardi.
And so I think it will be very exciting.
Talk about a new approach that’s an alternative to fit too.
Surgery so will start out with Mark.
Great thank you. Thank you Dan.
It’s a pleasure for me to be here and to speak in this forum again. I was hopeful that this month we’d be back to meeting in the usual way where I actually have to stand up to give a talk but we will do it this way and I look forward to talking to you today about this
work that Michael Gerardi and I have been collaborating on over the past several years.

First some financial disclosures the most important one here is the top one. Mike and I have our Co founders of a company called Stratified Biosciences which is licensed intellectual property to the technologies that we'll be talking about today and we receive some research funding from them. Next so I'll start with just a general introduction to both health care products that are
collaborations of physicians and engineers and then to some biomaterials and then to the particular technology that we've used in this project. And so you know, many of the products that make modern healthcare effective are innovations that came from collaborations between physicians and engineers and the first one I show here is is hemodialysis for treating end stage kidney disease. This is a medical device with a specially designed material. This is.
Responsible for his most important function.

In this case, it’s a polymer hollow fiber that allows separation of waste products from blood.

The second is shown here on the right is drug eluting stent.

This is one that’s made all of polymers.

Stents have made remarkable progress for treating. A vascular disease.

This is again a medical device with a special function.

Here there’s a coating on this stent that slowly releases drug to prevent restenosis of vessels.
And last is an orthopedic product. Another medical device.

This one formed of two different kinds of materials. It’s an artificial hip affirmative metal strong material so it can support your weight. But there’s a polymer involved and you can see that as the white replacement for the acetabular cup, which provides lubrication between the two components of the artificial hip and other medical device with who that uses a material that’s specially designed. And responsible for its most important
function which is replacement of mobility in the hip.

Now the and these products that were the collaborative works of teams of physicians and engineers have had a huge impact on health care, and you can see some evidence for that here.

We’re going to talk about using degradable polymers as a basis of drug delivery. A product of ethicon’s called vicryl sutures made of a copolymer of lactide and glycolide, have a long history of use in medicine.
and it’s a material that has mechanical strength, so you can use it as a suture as you see on the bottom here. You can also use it in orthopedic applications by forming this polymer into a bone screw. And it remains mechanically strong for some period, typically weeks or months, and then it slowly degrades down to safe components. Lactic acid and glycolic acid. We and others have done over the last twenty years or so is is. Figure out how to make these degradable.
polymers into tiny particles, and that’s shown in this scanning electron micrograph here. These are spherical particles that are about 100 nanometers in diameter, so that’s about the same diameter as a virus, but they’re made of all synthetic components in this case. This picture is of pure plga. Nanoparticles, but you can load them with agents like chemotherapy agents or or others, and make them into pharmacologically active particles. Next, and they have some features.
which make them interesting.

One is that if made of the right materials like Plga, which I just showed you, they’re non toxic.

If you add them to into cell cultures or you inject them into animals and in fact you can deliver very high doses of these into animals and people without any significant side effects.

If the particles are loaded with drugs, then if they’re engineered in the right way, the drugs are slowly released into an aqueous medium, but also released into the body if they’re deployed that way.
Sometimes, and when it’s shown in the bottom left panel here, this is when we added different concentrations of camp to Thiessen loaded nanoparticles to cells in culture that the loaded particles are actually more effective at killing these tumor cells than the drug is when it’s delivered on its own. And so there’s some. There’s some property of the particles which makes the drugs more active and as a result you can inject these particles into
tumors and is shown in the bottom.

Right diagram here, in this case, injected into a tumor in the flank of a rat. You can arrest the growth of the tumor with a single injection of nanoparticles, these features of nanoparticles seem to be related to the fact that the particles themselves. Can be highly loaded with drugs and they’re much smaller than tumor cells that we’re using to treat them in these examples, so the particles get internalized into tumor cells as shown in this confocal image.
Here you can see the green nanoparticles are inside of these tumor cells in culture, and they surround the nucleus and they’re releasing their active ingredients very close to the target of action from many anti cancer drugs. The technology that we’ve developed for this collaborative project is shown schematically here. It involves a block copolymer, so there’s so there’s two polymers that are covalently coupled together. One is lactic acid and that’s shown as the blue in this diagram,
and the second is hyperbranched polyglycerol, which is shown as the green with red pendant branches coming off of the surface of the nanoparticle, so the core is this degradable. Poly lactic acid polymer that can be loaded with drugs or active ingredients and that’s shown by the white dots here. And because it’s a block copolymer that’s assembled in a particular way, you have this degradable core surrounded by a green sort of corona of Hyperbranched polyglycerol. And it’s that hyperbranched polyglycerol, which gives the nanoparticles certain surface properties which
we’ve wanted to exploit.

And one of the interesting things about Hyperbranched polyglycerol is that in its native state it has a lot of hydroxyls at the end of the branched polymer chain, so this would be a non adhesive nanoparticle that has hydroxyl rich surface, and so it doesn’t adhere very well to proteins or to cells has a property of stealth. But I’ll show you in just a few moments, but you can.
Convert this particle into a different form by a brief exposure to sodium per iodate, which converts the vicinal dials on the surface of the nanoparticle into aldehydes, and it then becomes a very adhesive particle adhesive.

Because the aldehydes that are now covering the surface of the nanoparticle can react with amines in proteins or means on a cell surface and they’ll form a shift base covalent attachment which allows the nanoparticle to adhere to the cell. Or a matrix of very strongly.
So this shows two of the typical properties of our non adhesive nanoparticles, NPS or bio hesive nanoparticles BMPS. The non adhesive particles because they have very little interaction with biological cells and tissues, will circulate for a long time if you inject them intravenously. They avoid uptake in most organs and that results in long circulation. You can see here the blue dots show a circulation half type. Time of about 10 hours compared to a conventional nanoparticle, which has a half life of.
much less than an hour.
And so that gives you the opportunity to deliver nanoparticles to highly dispersed regions of the body.
On the other hand, the bio adhesive nanoparticles are BMPS because they’ll adhere to a tissue surface, can be made into very local drug delivery systems, and we show you this here in the diagram on the right which shows BNP adhesion to the outside surface of skin. So in this example, the red fluorescent nanoparticles were just added in solution on top of the skin on the side of the stratum cornea,
and you can see that even after extensive washing those particles not only for mcconn formal coating on the on, but they are abundant on the surface as well and very difficult to wash off. So we want to talk about using these kinds of materials in two different but related applications. One for prevention of skin cancer, and in this case we’d like to convert the nanoparticles into a sunscreen by incorporating FDA approved UV absorbing agents into the nanoparticles. And we think that will
have several advantages.

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Safety, because the adhesive nanoparticles don’t enter the skin,

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and so they’ll keep these chemicals outside of your body.

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But they’ll still provide long lasting protection because of the adhesion and presumably increased efficacy.

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And then secondly want to talk about using these same materials to treat tumors, and we’re going to give some examples of different tumors in animal models, but our focus here is on treating skin cancer and the advantages of the approach here is that you can load chemotherapy agents that are slowly
00:12:35.940 --> 00:12:37.870 released from the nanoparticles because

00:12:37.870 --> 00:12:39.230 of their bioadhesive properties,

00:12:39.230 --> 00:12:41.309 they are get retained in the tumor

00:12:41.309 --> 00:12:43.150 microenvironment, and they said that.

00:12:43.150 --> 00:12:46.600 That bio adhesion also facilitates

00:12:46.600 --> 00:12:49.302 uptake into tumor cells,

00:12:49.302 --> 00:12:51.729 that that reduces systemic toxicity.

00:12:54.420 --> 00:12:55.875 I think my friend and colleague

00:12:55.875 --> 00:12:57.730 is going to take over from here.

00:12:58.380 --> 00:12:59.868 Yes, thank you Mark.

00:12:59.868 --> 00:13:02.478 So sunscreens are something we use all

00:13:02.478 --> 00:13:04.910 the time and may take it for granted

00:13:04.910 --> 00:13:07.116 what we’re putting on our skins.

00:13:07.120 --> 00:13:09.650 In particular, these multi benzene

00:13:09.650 --> 00:13:10.970
ring structures that form what are called the chemical types of actives within sunscreens and as such being so hydrophobic they penetrate into and through the skin right into the bloodstream and deposit in your fat. There are concerns about off target effects, in particular estrogen and progesterone receptors, and another major effect is as they absorb this UV energy and and help protect against UV exposure, they are prone to give off reactive oxygen species and that is a major focus of something we’re trying to prevent with this technology.
On the other hand, we can use some of the physical sunscreens, in particular zinc oxide and titanium dioxide. They have limited penetration really through the skin. They will kind of work their way through hair follicles and through broken areas of skin. Even micro breaks a major concern about their use in general, as their aesthetic appearance, but they are major producers of Ross. If they do get into cells, even though they’re less likely to. They’re not just physical blockers,
00:14:17.990 --> 00:14:19.978 they clearly will generate Ross as well.
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00:14:19.980 --> 00:14:22.844 And here you can see why they don’t have
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00:14:22.844 --> 00:14:26.499 some of the appeal of a views otherwise.
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00:14:26.500 --> 00:14:30.180 So this is a confocal we made of the skin.
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00:14:30.180 --> 00:14:30.684 You know,
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00:14:30.684 --> 00:14:31.944 we’re studying some of the
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00:14:31.944 --> 00:14:32.970 relationship of cells here,
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00:14:32.970 --> 00:14:34.586 but I want to point to one thing
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00:14:34.586 --> 00:14:36.213 this is towards the top of the
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00:14:36.213 --> 00:14:37.771 skin and you see longer hansel’s.
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00:14:37.771 --> 00:14:39.335 These dendritic cells are
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00:14:39.335 --> 00:14:40.508 populate the epidermis,
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00:14:40.510 --> 00:14:43.720 extend their dendrites really right up
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00:14:43.720 --> 00:14:46.978 through these claudin tight junctions to
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00:14:46.978 --> 00:14:50.080 really be samplers of the environment.
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00:14:50.080 --> 00:14:50.640 And people.
Think of, you know, skin as an impenetrable barrier with its stratum, cornea, minutes, lipid. A protective component but in point of fact it is very interactive with the environment. In many ways oops circulate that for you a little bit and you can see how they can bring potential agents down into the deeper layers of the epidermis, they can bring potential agents down into the dermis, and they will actually navigate from there through the dermis, into lymphatics and lymph nodes too. So another kind of spark on the
controversy of sunscreen usage came about a year and a half ago when FDA was studying the plasma concentrations within folks that frequently applied these sunscreens and noted that they achieved these levels of concentration that are known to have a special designation by the FDA as requiring toxicology studies, which. Of course, have never really been done by the sunscreen industry, but are taking place now after that study. So the bioadhesive nanoparticle technology really allows for us to
NOTE Confidence: 0.9095454748
00:16:07.821 --> 00:16:09.948 develop nonpenetrating sunscreen
NOTE Confidence: 0.9095454748
00:16:09.948 --> 00:16:12.626 and avoid some of these concerns
NOTE Confidence: 0.9095454748
00:16:12.626 --> 00:16:14.196 about these agents getting in.
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00:16:14.200 --> 00:16:15.460 In particular,
NOTE Confidence: 0.9095454748
00:16:15.460 --> 00:16:17.980 these hydrophobic chemical agents.
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00:16:17.980 --> 00:16:19.926 If you apply just on the surface,
NOTE Confidence: 0.9095454748
00:16:19.930 --> 00:16:21.150 it doesn’t just sit there.
NOTE Confidence: 0.9095454748
00:16:21.150 --> 00:16:22.991 There are a lot of film formers
NOTE Confidence: 0.9095454748
00:16:22.991 --> 00:16:23.780 and technologies that
NOTE Confidence: 0.920084252666667
00:16:23.830 --> 00:16:25.120 the industry tries to use,
NOTE Confidence: 0.920084252666667
00:16:25.120 --> 00:16:26.849 but they work only to some degree,
NOTE Confidence: 0.920084252666667
00:16:26.850 --> 00:16:29.298 as the FDA showed.
NOTE Confidence: 0.920084252666667
00:16:29.300 --> 00:16:31.760 But if we’re able to
NOTE Confidence: 0.920084252666667
00:16:31.760 --> 00:16:33.582 encapsulate those within BMP’s,
NOTE Confidence: 0.920084252666667
00:16:33.582 --> 00:16:35.976 we can keep these agents on the
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surface bound to the stratum corny AM.
Otherwise, if they penetrate within after photo exposure, you'll see very high levels of Ros generation directly attributable to those sunscreen agents that are supposed to be protecting.
Here's what it looks like when we use fluorescent loaded BMP nanoparticles on the skin surface, and you can almost form a confluent. Blanket as a as the sun might see it.
So this affords several major advantages. One of them is this durability after application.
This is a covalent bond.
It’s a shift based bonding that takes place with the aldehydes on the on, the bioadhesive nanoparticles and in particular affords it a waterproofing protection, water resistance and so that can be tested in these animals that can be tested on other surfaces. And it can be applied to industry standards. Like to wash off these agents and see how protective they are. Current sunscreen formulations require reapplication every two hours. You don’t see anything that lasts longer than that.
but we can see these sticking around for much longer than a couple hours.

The other thing that clearly helpful by using BMP to incorporate these agents within is that we don’t see penetration of the active sunscreen agents to the point that with free sunscreen we might generate endproducts of Ross damage. For example gamma H2X or recruited proteins to sites of DNA damage due to Ross, but if the agent is incorporated within the BMP’s, we don’t see that damage. After UV exposure, we’ve already applied these to human skin. We don’t see we. We see a nice physical appearance to him.
We see the capacity for them to protect against what’s called minimal or THEMA doses, and we can do SPF testing for example with them and see their performance. But if we really want to kind of vigorously studies and. According to industry standards, we use materials such as vitro skin. This is a proprietary material that has the means within it, which is actually quite good for us to look at and study this bio adhesion. This is evil Ben Zona, very active in the UV spectrum.
Agent incorporated into NMPS.

So you see how that looks on a pre wash and you see after it’s exposed to washing for three hours in a water bath.

What happens to the PHOTOPROTECTIVE Spectra?

And you can see that just deteriorates immediately and in contrast to Eva Benzon incorporated within BMP’s, which maintain quite nicely there.

The photoprotective capacity across the full spectrum of the performance of evil Benzon.

We’ve done it with other agents, including Juvenil A to see that continued protection even clearly after three hours.
And longer.

We’ve taken this to the next level of using poor sign skin and really trying to vigorously wash that off, wrapping up the revolution per minute and the time constraints and then using HPLC in a very quantitative way to see how much evil benzon we were able to keep it here to the skin. Here it is at 150 RPM for 20 minutes. This is the industry standard for waterproof measurements and MPs will come off at a 60% lost. The BMP’s will adhere quite nicely. Stayed here through all of that.
at greater than 95% retained and then we start to Rev it up too.

Way past industry standards 450 RPM's three hours and see that you know we get the same relationship and the the full adherence of BMP's upwards of about 80%.

After three hours at that level. We were quite surprised to actually see that BMP's that gave us another advantage, and that is the capacity to prevent photodegradation of a quality called photostability. This is very important in sunscreen formulation, able benzon in particular as being really the main UV, a protector active agent.
It’s a major concern 'cause it’s so susceptible to photodegradation. You could see that here after. An industry standard dose of UV. What happens to the performance of evil Benzon? So you imagine you put it on. You get exposed to ultraviolet light and it just degrades. So if you incorporate it within BMP’s. And we’re not completely sure of exactly how this is happening, but obviously within the PLA there’s a protective millou that help prevent some of that degradation.
from the Eva benzol quite nicely.

So Octocrylene is a nice partner for able benzol because it’s a UV absorber, so it complements it in that way, but also because it itself is a photo degradation stabilizer for able benzol.

We can see a rate of degradation photodegradation, but if we come incorporated with octocrylene we were hoping to maintain a photostability at very high levels of UV.

Exposure upwards of three hours and we were able to do that by Co, incorporating those agents and and
found an optimal ratio for those also, but we were very surprised to see if we incorporated them separately that we still had that capacity for protection against photodegradation. Again, not something we completely understand as relationship between particles where agents are individually incorporated. So if you look at zinc oxide, so-called physical blocker as we described before, you’re going to see a lot of reflectance that helps in its
performance and protection against UV. 

But it also gives it some of this shiny, sometimes even purplish whitish hue to people skin. Whereas if you just use 3. Able benzon and octocrylene. You don’t really get much of any reflectance but within bpce for whatever reason able benzo not crawling do provide some reflective or extra protection from UV exposure, probably because of the state that they’re in. Something that we might refer to as kind of a hydrophobic crystal if you will.
If you can imagine as opposed to being in a more of an oily millou or emulsion. Empty BMP’s don’t do that, so this is really about the actives within the PLA. And then we can do some in vitro SPF measurements using some industry standard spectrophotometry and see that we can gain a level of performance that would be predicted to be above the active ingredients. In addition, we can see that we can sprinkle in some of the physical blockers here, in this case titanium dioxide at 1% or 5% and get levels of SPF.
00:24:51.615 --> 00:24:54.642 protection with that combination that.
NOTE Confidence: 0.838786426363636
00:24:54.642 --> 00:24:57.114 Kind of speaks to where we're
NOTE Confidence: 0.838786426363636
00:24:57.114 --> 00:25:00.000 heading with a prototype for this,
NOTE Confidence: 0.838786426363636
00:25:00.000 --> 00:25:02.704 use as a as a novel sunscreen formulation.
NOTE Confidence: 0.900935326
00:25:05.720 --> 00:25:09.825 I want to just come use this slide to talk
NOTE Confidence: 0.900935326
00:25:09.825 --> 00:25:11.940 about our other major collaborator here.
NOTE Confidence: 0.900935326
00:25:11.940 --> 00:25:15.853 Douglas Brash, who is a really a
NOTE Confidence: 0.900935326
00:25:15.853 --> 00:25:20.290 pioneer in understanding triplet state.
NOTE Confidence: 0.900935326
00:25:20.290 --> 00:25:22.996 Species that get generated after UV
NOTE Confidence: 0.900935326
00:25:22.996 --> 00:25:25.910 exposure and how they do damage DNA
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00:25:25.910 --> 00:25:30.229 even well after the lights are out.
NOTE Confidence: 0.900935326
00:25:30.230 --> 00:25:32.566 We are also working with a with the
NOTE Confidence: 0.900935326
00:25:32.566 --> 00:25:34.567 Center for molecular discovery here at
NOTE Confidence: 0.900935326
00:25:34.567 --> 00:25:37.290 Yale to screen a bunch of compounds.
NOTE Confidence: 0.900935326
00:25:37.290 --> 00:25:40.522 In this case a about 1000 natural found
NOTE Confidence: 0.900935326
00:25:40.522 --> 00:25:43.468 in nature compounds and and looking
for their capacity to be photostable UV absorbers and then looking at their capacity. To not be so toxic to the skin and then not generate Ros after UV exposure and using this series of steps, we’ve really come down to a handful of major candidates that we’re really excited about moving forward. For example, if we deem them safer than current agents outside of the particles. If there needs to be protection, we can put them inside the particles. So this is something that we think
that we can will be very complementary to what we’re working on.

Mark going to change gears for the rest of the talk slightly and talk about using these BMP’s for therapeutic drug delivery. So this slide just sort of reminds you of the potential for the particles that are converted into the bioadhesive state. BMP’s to interact with the proteins or any any. Amine containing group by because the aldehyde that’s on the surface of the BMP will form a shift base which leads to this covalent attachment,
and so we think there’s potential advantages for particles that work by this mechanism to deliver therapeutics locally.

And in addition, because the core of the particle is Poly, lactic acid and pretty hydrophobic polymer that’s really compatible with drugs that have low solubility’s. In aqueous media, so you can load them highly inside the particles and that allows you to have controlled release overtime at

45
the site of action and hopefully limit systemic exposure to the toxic compounds.

So here's one example of using these. Biodiesel nanoparticles to treat tumors in animals, and this is a collaboration with Alessandro Santin in OB GYN. And here, what we did was deliver the particles intraperitoneally.

So these in the panel be shown here shows you the retention of either NNPS which are on the left or bppe Switcher on the right you see if you inject them intraperitoneally and animals. After five minutes they distributed widely throughout the intraperitoneal space.
After four hours, the concentration of NPS and not easy particles dropped substantially, while the BMP concentration and distribution remains pretty much the same after one day. Still a lot of BMP's in the IP space where most of the NPS are gone and we even see persistence in the IP space for up to five days. So this kind of data convinced us that maybe you could treat my peritoneal carcinomatosis with these kinds of nanoparticles by injecting them IP and exploiting.
the mechanism where the bioadhesive nanoparticles would associate with the tumor cells or tumor nodules that are distributed throughout the peritoneum. We tested this with a drug called a path alone B. You can see that when it’s loaded in the nanoparticles and panel see here, relatively slowly overtime, although most of it comes out over the first 12 hours and then it sort of leaks out after that. This is an in vitro release, very difficult to measure. The corresponding release once
it’s deployed in the animal, but you see the most impressive result up in panel a. These are animals that got intraperitoneal injections of a of a uterine serous carcinoma cell line that doctor Ellis Dr Stanton had developed. If you don’t treat them, it’s difficult to find a dose that doesn’t cause early toxicity and still provide some increase in survival. You can see that by the black line here.
but if you put the EB inside the biodiesel nanoparticles, we see no toxicity and a dramatic improvement in survival.

A similar example, but now we’re treating locally in the brain. Here we’re infusing the nanoparticles by convection enhanced delivery into the brain of animals that have intracranial tumors. This is work by Yazi Wang in my laboratory in collaboration with Raymond Hall at the University of Connecticut. And here we put into the nanoparticles and anti mirror. Actually two anti mirrors,
These are two micro RNA’s that have been highly associated with gliomas, so we do in the animals.

One infusion we introduce the tumor as you can see on the timeline at the top, at day zero, at day six.

At the tumor is growing, we infuse the nano particles that contain these anti mirrors and then one day later we given IP dose of Tim’s Olamide and so the hope is that the anti mirror activity will sensitize the tumor cells to Tim’s Olamide and so it will be active.
At low doses and you can see the result down here, which is pretty dramatic animals without any treatment dead by 50 days. If you just treat them with the bio adhesive nanoparticles with the anti mirrors, you see some prolongation in survival. That’s the green line if you just treat them with TMZ, you see some prolongation and survival. That’s the red line.

If you treat them with both we see 100% survival out to 120 days here, which is pretty remarkable. Next and you can deliver other agents to other tissues as well,
so this is an example of delivering to mucosal surface. These were nanoparticles that were delivered intravaginally in mice, either NPS or BMP’S. You see the same sort of effect on sustained retention of the BMP’s in the up to 24 hours, and these these particles were delivering. Antiretroviral drugs to the reproductive tract. And you can see if you take that issue and you dissociate it and look for cells that express CD 45 or cells that express epithelial
markers that with the bioadhesive nanoparticles the majority of the cells are have nanoparticles within them and nanoparticles that contain the active drug.

So. You know the burden of human skin cancer is most striking when we consider volumes, numbers of cases per year at 5.5 million in EU. S. Uhm? You know more cases of skin cancer than all other cancers combined and this. Though most of them in particular basil cell and squamous cell a little bit Melanoma. Much more can result in death Accumulatively it’s about 15,000 per year in EU. S and it’s just a burden
on the health care system. Tremendous burden on treating all of these cases of skin cancer multiple on a lot of patients in particular transplant patients. Fair skinned individuals, multiple scars that can run into. Each other and cause other complications from destructive and surgical procedures. So there’s really an unmet need for non-surgical options for patients. Those that may not be great surgical candidates, or those who would like something a little more simpler and less cost dependent.
So a minimally invasive local alternative would be ideal for patients who might have superficial or minimally invasive lesions, so numerous simple ones they may have locally advanced cancers where you want to come in with something local and that could be used in in conjunction. For example with a systemic agent or combination, that could be an immunotherapeutic. Or there may be some that you really have really deep ones and you want to minimize the side effects of providing a systemic.
Chemotherapeutic agent and how you might deliver it locally and in those cases it could be a targeted therapy.

It could be a chemotherapeutic agent. The point is you're going to maintain high concentrations of the actives where you put the particles.

So here’s a model that I’ve worked with. Uhm. For many years of keratinocyte tumor squamous cell carcinoma, it’s a set up quite simply by transplantable injection and it grows over a course of about four weeks and forms a nice big nodular blue ball of cells.
It’s very aggressive.

But if we treat it with BMPS with camp to thicken incorporated as the chemotherapeutic active agent, we can get complete clinical and histologic resolution and those pathologists in the audience can appreciate the tumor destruction and amorphis changes that we see here after resolution.

So I’m trying to understand. Process here and so that we can maybe potentially leverage some of that. We can look at how the particles, for example, die Incorporated BMPS. Might interact with the tumor cells.
and Mark alluded to some of the interactions with other tumor cells, but we were studying here in skin cancer squamous cell carcinoma. The NPS barely will stick to the cells and barely getting side. But you can just see this tremendous adhesion to cell surface, which of course that is a protein rich environment and that further facilitates and triggers. And we’ve broken down the mechanism a little bit of micro Pinot cytosis a passive internalization that occurs.
to bring these particles and their payloads right within the tumor cells. And we can really get very quantitative with this interaction, and we can use dyes that are bound covalently to the PLA. Or we can do in ones that are loosely within the appeal doesn’t matter, they will readily get incorporated with into the tumor cells taken up. Very readily over the course of three days. Relative to BMP’s that don’t have that bio adherent surface component to him. We can also create kind of a an in vitro tumor matrix where we put use Poly L lysine as a tumor rich environment and
adhered adjacent to cells and show that our BMP’s are the ones that are going to provide a kill because they will bind not just to cell surface but just to this tumor matrix and MP’s don’t do that. So we don’t see that tumor kill and we don’t see it with CPT. These were our with a washout. From the tumor matrix. But the BMP’s in here, there and then are readily available to the tumor cells to kill, so we think there’s two. Mechanisms that work together there one where the BMP’s with their payloads
or binding to the tumor rich matrix

We can move to in vivo established tumors, inject our bpce with with Die

We can measure that over days and we can do that by harvesting the tumors, pulverising them and extracting and doing HPLC quantification on the drug levels.

And you can see here this is intralipid with the capital seeking chemotherapeutic agent.
We just don’t detect it after day zero if it’s in any piece, there is a little bit of detection today too, but that pales in comparison to what BMP’s due to keeping drug present. Again, there may be released. Maybe particles that contain deposit more slowly release. They may do that in the Peri tumoral area of the tumor matrix. They may do that within tumor cells themselves. And then we can look at the therapeutic efficacy of using for example,
00:39:07.840 --> 00:39:10.460 camp to thicken incorporated within
NOTE Confidence: 0.940593850769231
00:39:10.460 --> 00:39:13.796 BMP’S to treat establish screen or
NOTE Confidence: 0.940593850769231
00:39:13.796 --> 00:39:17.152 cell carcinomas injected here at day
NOTE Confidence: 0.940593850769231
00:39:17.152 --> 00:39:20.588 four we can measure tumor size and and
NOTE Confidence: 0.940593850769231
00:39:20.588 --> 00:39:23.520 and see what we do to tomb of growth.
NOTE Confidence: 0.940593850769231
00:39:23.520 --> 00:39:26.192 We can also harvest at the end and
NOTE Confidence: 0.940593850769231
00:39:26.192 --> 00:39:28.154 do histological analysis for the
NOTE Confidence: 0.940593850769231
00:39:28.154 --> 00:39:30.199 presence of any residual tumors.
NOTE Confidence: 0.940593850769231
00:39:30.200 --> 00:39:33.548 We do get an inflammation with the BNP CPT.
NOTE Confidence: 0.940593850769231
00:39:33.550 --> 00:39:34.970 As you might expect,
NOTE Confidence: 0.940593850769231
00:39:34.970 --> 00:39:38.057 we do with both arms of the CPT alone.
NOTE Confidence: 0.97936355
00:39:41.030 --> 00:39:43.664 So that. You know,
NOTE Confidence: 0.97936355
00:39:43.664 --> 00:39:45.896 clinical tumor measurements are are not
NOTE Confidence: 0.97936355
00:39:45.896 --> 00:39:48.057 as definitive as the histologic ones,
NOTE Confidence: 0.97936355
00:39:48.060 --> 00:39:51.848 but both the clinical tumor growth
NOTE Confidence: 0.97936355
00:39:51.848 --> 00:39:54.768 curves were showed protection with
btes relative to CPT alone at the same
dose of drug and in histologically we saw a 62% tumor free rate with the BNP skeds that was impressive in a parallel experiment at at four weeks out. So we were really interested in whether this localized treatment could be combined with immunotherapeutic strategies, for example with a localized. We are designing experiments for checkpoint inhibitors which might be on the minds of several people. We’re working with Marcus Bosenberg on what that might look like, for example with a localized.
Invasive Melanoma or metastatic nodule of Melanoma, but in this case this is our BMP screen PDV squamous cell carcinoma again and we looked at again the capacity for BMP’s to incorporate CPT but be combined with a local immunotherapeutic agent in this case. Kcs people familiar with know this this is a TLR. Nine login, so we’re kind of creating a Kill and thrill strategy, where we’re not just killing tumor cells, but help trying to harness local immunity to help clean up residual ones. Maybe some of that tumor debris,
tumor antigen rich material, and immunostimulation might create an in vivo. Vaccination effect when we compare it to just intralipid CPT with that same immunostimulatory agent we just do not get the level of protection we can get by pushing the system hard on the tumor side. This might be a little bit more easy to see, and when we look at individual tumor growths and you see the shutting down of a lot of those tumors that were treated with combination.
just to finish up.

Just remind you of the two classes of nanoparticles.

We’ve worked here really the same when they’re synthesized and converted from NPS into BMP’s.

We can load agents into the PLA polylactic acid shell, and then we manipulate the hyperbranched polyglycerol in the order to either make stealthy particles and NPS, or adhesive particles BMPS.

So Polly PLA is made from L. Lactide is a monomer that costs about $5000 per 10 kilograms. It’s been going up over time.
because of worldwide demand, for lactide based polymers. There are some alternates that have been used quite a lot in medicine like caprolactone or Penta deco lactone loser, shown here. They’re cheaper, but not. But but maybe by a factor of two, but we focused on ethylene brassil 8, which is also a lactone. But it it, but it’s much cheaper 10 times cheaper than L lactide, which makes a big difference in terms of manufacturing costs.
Another advantage of ethylene brassil is that it’s produced in large quantities. It’s been used a lot in the fragrance industry, so it’s been put on lots of people skin and its properties are known. It’s a sustainable product ‘cause it’s produced from Castor oil. It’s not made from petroleum like those other polymers you could make them with similar mechanical properties to play. So can you make them into bio adhesive? Nanoparticles,
the answer is yes, and post auction all put up Pythia and graduate student Alex Johnson, which have shown that in the next slide that there’s these are particles that were made variety, particles that were made variety, different conditions, which shown in the graph here, but you can see some of the particles, but by scandal around micrographs. In this scanning electron micrograph, we’re encouraged that there this is something that we can accomplish, not just with the material we’ve shown here. We certainly have proof of principle that
that material works in a variety of settings, but one can innovate on the material side as well, and potentially make things that are that are better.

Alright, I'll summarize our joint efforts and skin cancer prevention and treatment. We've worked on formulating a prototype for our sunscreen that shows this bio adhesion advantage, photostability advantage anti permeation advantage and SPF optimization advantages. We're working now on preclinical modeling. For that, this is the MC1RE mouse, so it has the same defect as fair skin red
hared people with freckles to look at both acute and chronic kind of modeling. With that to really try to optimize our performance prior to moving to the clinical spectrum. All in addition, we’re also looking at protecting specifically against both squamous cell carcinoma and Melanoma mutations. Over chronic exposure protocols. With that as part of the sport and then you heard about some further BMP bio engineering improvements that we’re working on. In addition,
you heard about our efforts on localized therapy for skin cancer
as a nonsurgical alternative, the advantage of matrix bio adhesion,
tumor cell binding and uptake advantages, and how this translates into
drug retention advantages, efficient drug delivery and tumor elimination when delivered locally
decrease systemic toxicity levels which we had didn’t show here
compatibility with immunotherapy. Which I to me is very
very exciting for the potential to use a localized therapy in combination
with a systemic immunotherapy
or localized immunotherapy.

And that is. Are ping pong tag team talk for the day? Obviously a lot of people working in in both our labs, in particular, Julie Lewis, Sholud Komar, and Amanda Zoo contributed extensively to data you saw on the skin cancer side and mark highlighted people in his lab, but in particular he wants to been the tremendous link between our two labs to bring up the. By many engineering component to skin cancer and skin cancer prevention.
modeling and Doug crashes are also

our partner and developing other

strategies on skin cancer prevention.

Who's also been very much involved

in how we try to make these

formulations that might ultimately

also prevent some of the oxidative

damage that we talked about.

Marcus Bosenberg and Harriet Kluger

in particular as part of the.

Or have been tremendously supportive

of our work and Ruth Taliban runs

a core here that has provided us

with numerous human skin samples

and they were very appreciative,
especially Antonella as part of that.
And of course, funding sources include the cancer spore, but other grants from NCI, NIAMS, and IEHS.