Interview with Dr. Kirn: The Three-way Battle between the Tumor, the Immune System and the Oncolytic Virus

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Berkeley Scientific Journal: So, to start off, how did you get involved with your field of research? Not only [viral therapies] but also oncology (the study and treatment of tumors).

Dr. Kirn: I was a Berkeley undergraduate in what was called physiology at the time. It would now be called MCB. I knew I loved biology research. I wanted to get involved with a career where I could make a big difference for a lot of people, not just a narrow difference. So, I went to medical school but I always knew I would do fundamental research that lead to new therapies. During medical training, I really was very, very interested in both infectious disease and oncology so this field was a way for me to combine those two.

BSJ: How did you choose the family of viruses you treat and use in your research?

K: That's a good question. There are hundreds of different kinds of viruses and thousands of different viral strains out there. There is sort of a library of these things. We had to carefully pick the kind of virus we wanted to use, based on the features we wanted the product to have. We wanted something that could treat cancer effectively. When cancer causes problems, it metastases in the body, so we needed something that could go through the blood stream and seek out and destroy cancer. So, we needed a virus to go through the bloodstream as a part of its biology. We also needed something that could infect cancer cells efficiently and then multiply and blow up the cancer cells and spread very quickly because it's kind of a race between

Figure 1. The *vaccinia* virus.

destroying the cancer cell in the body and the immune system response shutting down the virus. We looked at a lot of different viruses and I interviewed a lot of virologists to ask which virus species out there has these features that we wanted. That is how we picked the *vaccinia* virus. We felt it had the best biology to do naturally what we were trying to do.

BSJ: Fundamentally, what makes the oncolytic virus that you use capable of being used not just specifically for one person but universally?

K: Well, most of these viruses have to evolve if they're going to be bread in a population to be able to spread from one person to the next. They have to evolve to be able to deal with different genetic backgrounds.

(And in terms of targeting a variety of cancers) The reason these are so active is that these viruses are very good at sensing, and there are a number of different switches that are turned on in

cancer that activate the virus. Those are common features in lots of cancers [regardless of whether it] breast cancer, lung cancer or colon cancer. They all have many of the same switches gone so that means these viruses can get access to them and be effective [against] lots of different kinds of cancer instead of just one kind.

BSJ: How are oncolytic viruses able to lyse cancer tumors without any negative effects on normal cells?

K: Different viruses behave differently. Some will just get to the cell and hang out there for long periods of time. Others will multiply and burst the cells. We picked one that would multiply and burst the cell. *Vaccinia* naturally does that as a part of its life cycle. It replicates and spreads from person to person. How we make it selective for the cancer cells is [by looking at the] two levels.

Figure 2. Edward Jenner vaccinating for Smallpox.

Most viruses are inherently much better at replicating and multiplying in a cancer cell than they are in a normal cell. It's just a fact of nature. A lot of the cellular defenses that our body has evolved over thousands of years evolved to protect us against them. Those same defenses are defenses against cancer. So, when a cell loses its defenses against cancer as a cancer cell, it now has an Achilles heel! It's defenseless against viruses as well, and we just take advantage of that. That's why these viruses can inherently target cancer cells. Things like avoiding the immune response and avoiding the effects of interferon (proteins released by the host cell in reaction to the virus) are all features of cancer and they're also features of virus susceptibility.

That's where we are starting. We already have a therapeutic window, a therapeutic difference, with cancers being more sensitive than normal cells. And then we usually do some additional engineering to the virus to delete [some of its requirements for replication] in a normal cell that the cancer cell could provide (at the site). There are certain enzymes, for example, that the virus brings along that it needs in a normal cell. If you delete that, now the virus can't multiply in a normal cell. But cancer cells naturally express that enzyme as a part of the cancer phenotype, and so that leads to the selectivity. Part of it's just nature and part of it's engineering.

BSJ: So you engineer it so that, even if it enters a normal cell, it would not replicate?

K: Exactly! So, what we get is something that gives us a very wide window so we can know the doses that treat the cancer and not affect the normal tissue. When you get an antibiotic, it kills the bacteria. It doesn't affect you. It's what we call a very wide therapeutic window between the two tissues. But with chemotherapy, there's a very small window: with the dose that kills the tumor, it also makes the person sick. While ours has a very very wide window. It would be much safer, [with fewer] side effects than chemotherapy.

BSJ: So, are there consequences of injecting an excessive amount past that window?

K: With any therapy overdose, there will be side effects. With the virus, the biggest side effect we've seen is that it doesn't really directly destroy tissue, but it can cause your body to overreact to it. It releases all these proteins that are immune defenses called cytokines. They're hormones that go around the body. As a result of saying, "Oh my gosh, there's an infection," the body releases too much of that, and that could cause low blood pressure, high fever, chills, these sorts of things. That's kind of what we would worry about.

BSJ: Going back to what you were saying about replication, there's much lower replication in normal cells. As the tumor decreases, does the replication of the virus and the effectiveness decrease as well?

K: Yes. Not the effectiveness of the virus, but the amount it in the blood. When we first treat it, there's a big tumor and lots of cells, then we see a big burst and a lot of virus in the blood. The amount after you administer it, goes down in the blood, but after the burst, it goes back up in the blood, [then later] it trickles down. The more cancer cells you have, the more replication you'll get, but it still should be able to mop up the last tumor cells.

BSJ: We read about how oncolytic viruses hinder tumor

vasculature. Could you go over the mechanism of how that happens?

K: That was a very interesting finding! It's not something we knew would happen. We knew that the *vaccinia* naturally liked to grow in injured skin. Hundreds of millions of kids around the world [vaccinated with *vaccinia* to] eradicate small pox. The first virus used for vaccination was called cowpox, which became *vaccinia*, essentially. Latin for cow is vaca. The word vaccine comes from the original use of *vaccinia*.

Have you learned the story of *vaccinia*? It's actually amazing! There's a guy named Edward Jenner, in the 1800s on the English countryside, who founded a big hospital and practiced there. Small pox was really the worst thing that could happen to anybody. [Around] 50% were killed (once infected). It's like Ebola today, but much more infectious because it could spread much more rapidly. Probably, more people have died from smallpox than the plague, malaria and HIV all combined. You go back to mummies in Egypt and there's smallpox. And it wiped out most of the Native American Indians, unfortunately, because they didn't have immunity [because] it came from Europe. Anyways, he was practicing in the countryside, and he somehow found out from the milkmaids that they never got smallpox. He asked, "Why is that? That's weird." And so he went and watched them work, and he realized that sometimes the cows that they were milking would have these pox. Maybe, he thought, they were somehow protecting them. So he scraped a bunch of that stuff, and then he shot it into an orphan boy and sent him over to somebody's house with smallpox. It's horrible. There was no ethical review board back then! The kid didn't get smallpox! Oh my gosh, we can protect people from smallpox with these cowpox that are benign.

He proposed that, and a lot of people freaked out saying we can't just shoot – they didn't know it was a virus back then, you know – cow pus from a cow's skin and shove into people. It was crazy! Fortunately, he convinced enough people to do it, and eventually had a formal campaign in the 1900s and eradicated smallpox. Arguably, it was medicine's greatest success and maybe humanity's greatest success. The eradication of this horrific disease.

Anyways, that's the background, I can't even remember how we got started on this. What was your original question?

BSJ: Oh, the mechanism through which –

K: Oh, oh! So, yeah, this is interesting! We knew from the vaccination campaign that if you just shot it into the skin it wouldn't vaccinate and so it wouldn't replicate. It needed the activation of these growth factor pathways. So, what you had to do is, what they call scarifying the skin: you actually have to scratch the skin and disrupt it and injure it. And then, because all these growth factors were released, like epidermal growth factors, which is again very important in cancer. These growth factors would stimulate proliferation of these viruses and then lead to immunization. So, we knew that you needed to injure the skin to get *vaccinia* replicated because of these pathways.

So, we predicted [that] those pathways are turned on in cancers. Well, it turns out that those pathways are also turned on in activated blood vessel cells, endothelial cells. And, [it probably happens] when you're scarifying the skin and releasing all these growth factors. It's probably when the vaccination is growing and is healing skin cells but also endothelial cells. So part of the biology that was probably always there, we just didn't know about it. We discovered when we treated cancers that there were some patients, not all that significant a proportion, maybe half in some cases, that would have very, very sudden, massive necrosis (premature death) of their cancer.

And we thought, "God, that's weird, how could the virus spread that fast?" You know, five days later and this tumor is 50% destroyed. So, we theorized that it would have to be something that worked much more quickly and we theorized that it could just be cutting off the blood supply. Low and behold, when we looked in animal tumors and also human tumors, it was actually multiplying in the endothelial cells and destroying them and causing shutdown of the blood supply to the tumor.

So, then we asked the question: what is the mechanism? We theorized that the same downstream pathway that turns the virus on in an epithelial cell with epidermal growth factor (EGF) was doing the same thing in an endothelial cell but with something called VGF, which is the big growth factor for endothelial cells. The bottom line is that both those cells have different upstream activators, the growth factors, but downstream, they are the same. So [it does not matter for the virus,] it sees the same signal, it multiples and destroys the same.

BSJ: So once you're cutting off the blood supply to the tumor, that is: once it interferes with the tumor vasculature, how do you further deliver the virus?

K: Well, yeah, that's a good question! If you knock out the blood supply and you want to re-administer, how would it get in there. And what I think is, what we theorized is that there's plenty of parts of the tumor that are where the blood supply is still active and so you can deliver to those. And then [for any] residual cells, you have to hope that the body's immune system can mop up. So, this treatment

really works by the virus multiplying in cancer cells and blowing them apart and hitting the blood vessels and cutting off their blood supply.

But the third part that's just as important is having all this virus and this inflammation. The cancer should make the body's own immune system come in and attack and mop up additional cells.

This kind of therapy always works better if you have an intact immune system. So, if you took a tumor in a mouse, the same exact tumor, and you treated a mouse with no immune system versus [one with] an immune system, it would work much better in the one with the immune system. Even though the immune system is fighting the virus, it's more important [that] it helps wipe out the tumor.

BSJ: What is the timeline in which you want this to act? The virus attacking the cancer cell versus the activation of the immune system against the virus.

K: Yeah, it's a delicate balance. If you could engineer it perfectly you could say, "I want the virus to multiply maybe for four to six weeks." That should be enough to really populate the virus and let the immune system come in and wipe out everything else. But tumors are very good at protecting themselves from the immune system, that's one way that they become cancers. So, compared to a normal tissue in your body, a cancer oftentimes has more immunity. It's developed these barriers, these very elegant barriers, to keep the immune system out. So the virus can come in and can probably replicate for much longer because there's no immune system in there.

Whereas other tumors naturally already have some immune cells in there. They're going to be much more susceptible. So, how much [is it the] virus replication and how much is it the immune system is going to depend on the patient and the actual cancer. So it's kind of a delicate balance between the two.

BSJ: What are those things that the cancer cell does? Is it just [along the lines of] surface markers that make it not [recognizable] from the immune system?

K: Oh, the interplay between your body's immune system and viruses and your body's immune system and cancers is amazing! Our body is this battle that where everyone is constantly evolving so your body will develop something that targets something on the virus and the virus will mutate to get around that, and the body will produce a cytokine so the virus will produce something that neutralizes that cytokine.

So, *vaccinia* virus has – it's a big virus, got a lot of genes. Like 90 genes, which is a lot for a virus. It's got 20 dedicated to nothing but fighting the immune system. So it knocks out something called tumor necrosis factor and it knocks out interferon, which is a really important anti-viral – it has 4 proteins to fight interferon. So it's like this battleship and the immune system is trying to evolve and it's trying to evolve and usually what happens is that they come to some sort of happy meeting where they both co-exist. The best virus kind of coexists with its host and doesn't kill it, it kind of traffics around and like the flu, it mutates to get around. [But it still] kind of persists. Something like Ebola is actually a horrible virus, it won't last for very long because it kills its host. You don't want to kill your host because then it kills the reservoir, right? So, you know, this balance between the virus and the immune system is fascinating.

Anyways, tumors do the same thing because they got to evolve and they're abnormal and they have abnormal proteins and so the body's going to come in and knock them out unless they come up with a mechanism [by which] they come up with toxic molecules that will kill a T-cell if it comes into contact with it. They secrete cytokines that suppress the immune system. Normally, cytokines are produced by your lymphocytes but the cancer learns to produce it, too, and shuts down immune cells. So that's this complex battle. There are a lot of new therapies in cancer that are actually immune therapies that kind of block the cancer's ability to defend itself.

So at any rate, viruses are an important piece of this because we can use them to not only destroy the cells directly but also stimulate the immune system and recruit in the immune cells. The virus gets in there and produces and we engineer it to produce this cytokine that recruits in the immune system cells.

BSJ: And that's to then overwhelm the cancer…

K: That has to overwhelm the cancer's defenses. So it's this

three-way battle, it's very interesting. And when we biopsy these patients we see that, we look at the tissue and it looks like a three-way battle. It's like, you've got blown away cancer cells which are dead. You've got some alive, some are infected, but they haven't died yet. [Then there's the] immune cells coming in, some of those immune cells are dying. [So are even cancer cells and so are the viruses]. It's complicated.

BSJ: Another interesting aspect we read about was the microenvironment of the cancer itself and from what we understand, rather than it serving as a barrier to the cancer, you try to use it as a selective measure for the virus that it would recruit would specifically to the cancer. Could you explain a little more about that?

K: So a tumor mass is more than just cancer cells, right? There's gotta be an interplay between those other normal cells and some of them maybe producing interferon which dampens the virus' effects. So they could act as a barrier to viral spread …. It likes the cancer cells but if there's fibrosis, like a fibrotic tissue plane, because there's all this fibroblasts that produced all this collagen, it could physically block the spread. So we view these other cells as sort of barriers to the spread of the virus rather than something else…

BSJ: Rather than recruiting (the immune system)?

K: Rather than recruiting. We can target the cancer cells, but [as for] the fibroblasts, the other kind of matrix cells that are normal and can produce interferons and other antivirals, we view that more as a barrier that we have to get over.

BSJ: Are [the oncolytic viruses] mostly effective against solid tumors or do they also work well with liquid tumors like leukemia?

K: Yeah, you know, it's gonna depend on the virus you use. [With] *vaccinia*, we wanted to treat solid tumors because 90% of all cancer that causes death is solid tumors. So we liked *vaccinia* because it was really good at infecting cells that had originated as epithelial cells, which is 90% of cancers. There are some things called sarcomas, which are different from the leukemia lymphoma which comes from immune cells. Blood cells and white cells are relatively resistant to *vaccinia*. So, those would not be a good target for *vaccinia*.

Whereas there are other viruses that we've identified, like measles that naturally, when it infects the body, infects the immune cells. So, if you have a B-cell tumor such a myeloma or B-cell leukemia, those could be very effectively treated with measles virus. So, it depends on what kind of tissues, what kind of cells you want to target, for what kind of virus you want to use.

BSJ: What were your concerns, if any, about widely used, pre-existing vaccines overlapping with the chosen virus?

K: This is an important question. The concern would be if people had been vaccinated against that exact same virus. Then, they would be immune and so the virus wouldn't be very effective. That was a question with *vaccinia* because lots of people were vaccinated, but that stopped about 45 years ago after small pox was eradicated. So, that is a concern for people over the age of 45 that if they were vaccinated they would have residual immunity. We are studying that and found that most people by the time that they are out 40 years or more from their vaccination, their immunity is very low. And usually, when you have cancer your immune system is suppressed anyways. We looked at how recently patients had the pox vaccine to see if it affects the response and found that it didn't. If we were treating 10 year olds and everyone had been vaccinated when they were 3 for that virus, then it would be a problem. Like [in the case of] measles. However if someone was much older then it wouldn't matter.

BSJ: What alterations could be made to make the oncolytic virus more effective in order to create a more comprehensive cancer drug?

K: Well, we're doing two big things. One is: the virus naturally traffics through the blood to the tumor and avoids antibodies and kind of cloaks itself; so we think that we can increase that ability so it can be even more effective through IV. So that's one way. And the other way we can make it more effective is by thinking about *vaccinia* like an iPhone: each application you can put in your iPhone we can put in the virus. So each application could be a transgene payload, meaning a gene from elsewhere, which we can insert in the virus. [And when] the virus would infect the cancer cell it starts pumping out that protein and that protein could be immune stimulatory, for example. But there are all sorts of stuff we can do and *vaccinia* is a huge virus so we have lots of room to stick in all these applications because it also has lots of memory. Our current virus only has one. I think our next virus should have 5 or 6 and eventually 10. The maximum capacity of *vaccinia* is 25-50 KB, which is big.

BSJ: We interviewed Dr. Schaffer last semester regarding his work on advancing stem cell model systems, where does that collaboration with him (through 4D Molecular Therapeutics) come into your research?

K: Professor Schaffer has two major research areas. One is stem cells, he's director of the stem cell center. But he's

also got a major gene therapy program which is using viruses as vectors to shuttle genes into cells. The gene-therapy that he's doing that our company is doing together is to use the *AV (adenovirus)* that goes in very quietly and sneaks into the nucleus of the cell. And then it sits there and doesn't replicate. It doesn't destroy the cell, it's the just very, very quiet. The whole goal is to make it as quiet as possible: Not have the immune system notice it, not have the cell notice. But have genes expressed from it to replace missing genes in the patient's cells.

Figure 3. Dr. David Schaffer, professor of Chemical and Biomolecular Engineering at UC Berkeley collaborated with Dr. Kirn to create "4D Molecular Therapeutics."

Say, if you have hemophilia, you don't have the normal factor that helps de-clot blood in your liver cells. So, you put in this stealth virus into the liver cells, have it quietly sit there but pump out the missing gene protein and replace it. So, your body now makes it normally. So, there are kind of two ends of the spectrum. I've been spending the last 12-20 years coming up with viruses that blow stuff up in cancers but expressing genes at the same time. He's been doing the opposite, which is coming up with very stealthy viruses in normal cells and not damage them: not notify the immune response. [This is] the exact opposite. [To] just quietly produce normal gene products that are missing due to a genetic disease.

BSJ: What exactly are these stealth aspects? How do you identify them?

K: Dave Shaffer and other scientists have tried lots of different viruses and they've found ones that are best at avoiding the immune response and notify the immune system of its presence. [The one that was most stealthy] or quiet was *adeno-associated virus (AAV)*. So, they can engineer it in a way that it cannot multiply in the host. So, once it is in the cell, it sits there quietly but produces the gene product and thereby does not harm the cell. It's kind of like the polar ends here: he's using this very, very small and stealthy virus which just produces one gene product. And I've got this battleship that's producing almost a hundred different genes and blowing stuff up. So, two ends of the spectrum.

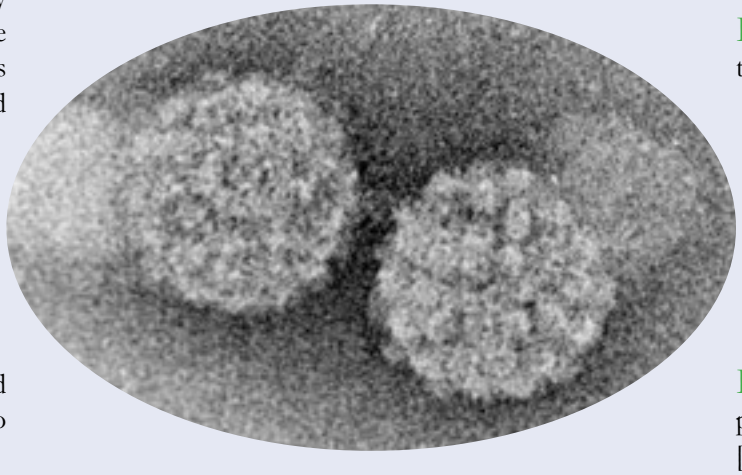

Figure 4. *Adeno-Associated Virus (AAV).*

So, it all depends on what you're treating.

If you're treating cancer, then you want something that says, "Hey, immune system! I'm here, come on and get me!" whereas if you want to quietly replace a gene in a child with a genetic disease and you don't want the immune system to see you and want to just sit there and produce that protein for years.

So, it's kind of a nice synergistic partnership that we [have by bringing] totally different viruses to the table for very different diseases.

With *vaccinia*, what we did is that we picked a virus with the biology we wanted and then we rationally engineered it to make it more selective. Dave Schaffer created a [different but very elegant] approach. What he said was, "Let's let nature decide." He took and created a hundred million different versions of his AV. They just randomly change every little coat protein. And then they took this soup of viruses and said, "I want to be able to get into a liver cell." So, he created it in the blood of a mouse and harvested the liver cells and saw that one out of a hundred viruses got in there. You grow it up and shoot it in again and find that one of a hundred get in. After you do that 5 or 6 different times, at the end you find a needle in the hay stack. You started with a hundred million and you've found the one that is amazingly good at getting into the liver cell. This is called directive evolution. It's working just like evolution: just creating diversity and finding what fits best in that niche, in that specific environment. Instead of rationally deciding, you let nature pick what's best! And then you can figure out, in reverse, what mutations were there and why it works better.

BSJ: So, are you now, [with the collaboration], trying to create viruses specific to cancers in different organs [using the directive evolution approach]?

K: Yeah! That's the next thing that we'll do!

BSJ: Along these lines, where do you see the field grow in the upcoming years? And what is the future of the medical trials that have been going on?

K: Clinical trials are in three phases: Phase I is safety, [dosage and dosage related] side effects, and how does the body clear it. Phase II is [about whether it works] in

patients and whether it benefits them and then Phase III is proving that it works better than whatever is standard. Standard could be nothing if there is no therapy or it could be some approved therapy. The oncolytic viruses are moving into Phase III with *vaccinia* next year. And there's another oncolytic viruses that's for local injection into skin tumors that a company called Amgen has. That'll hopefully get approved in the coming year as well.

And next for the field is exactly everything that we just talked about in terms of stealth and adding different therapeutic genes. And then long term with the directive evolution, we want [to be able to do that] with *vaccinia* on different cancers and get something that's even better against specific cancers.

BSJ: Regarding, "how the body clears it," as an important aspect of clinical trials… How does that work for oncolytic viruses? Is it that, as the cancer dies out, the virus [concentration] goes down?

K: Yeah, once the factory is gone. Then, over time it will be cleared and any residual viruses would be mopped up by different immune cells.

BSJ: So, if someone was treated with the same treatment twice with years in between. Would the immune system react better the second time and [the virus] wouldn't be as effective?

K: It's interesting because we could reuse it but it might be that the immune system recognizes the virus more quickly and clears it faster. It may not work as well. But we have done that in a few patients, a hand full, and they seem to have very dramatic responses. So, maybe the immune system revved up. It's revved up to the virus but that virus is in the tumor. So, it may recruit the immune cells into the tumor even faster. Again, it's a balance. It'll definitely clear the virus quicker but it may be more effective than before against the cancer.

BSJ: What would you do in cases of an immune system that isn't working as well? Or not at all in cases of immune compromised patients?

K: There, the oncolytic virus could just work primarily by its viral mechanism and not the immune [recruitment] mechanism. As long as the cancer is cancer specific, it should still be safe even in the cases of no immune system… it'll just have the viral effects.

BSJ: What about using these techniques with chemotherapy?

K: Yeah, we've done that but you just have to be very smart about it. For example, if you treat with a virus and let it go for a while and create all this inflammation. A lot of the inflammation is the cytokines or chemo-sensitized cells that make chemo work better. So, you can actually do and we've done it in patients. And we see that chemo works better after viral infection.

And there are drugs being developed now that will block the immune suppression in tumors. So, you're basically unleashing the immune response. If you use that with a virus whereby you use the virus first and then come in with the [unleashed immune response], that would be a very effective combination.

These powerful mechanisms can be used together except that you have to do it in an intelligent way. You have to think about which one first and think along those lines. There's another class of drugs that blocks the signaling in cancer, the on switches in cancer that make them grow. And we used that with the cancer, it actually blocks the virus because the virus needs the on switch to multiply. So, you can't use that specific one [in this chronology]. You can use it later by alternating them but not at the same time. Normally in cancer, we use combinations and we'll do that with oncolytic viruses but you have to be rational about it and figure out the best way to go about it.

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