Edward A. Berger Oral History Interview
August 14, 2017

This is an interview with Dr. Edward Alan Berger about his research identifying the second receptors necessary for human immunodeficiency virus (HIV) entry into T cells and macrophages. The interview is being conducted on 14 August 2017 at the National Institutes of Health (NIH). The interviewer is Dr. Victoria Harden. Founding Director, Emerita, of the Office of NIH History and Stetten Museum.

Harden: Dr. Berger, to begin, I want to ask you to state your name, that you are aware that this interview is being recorded, and that you've given permission for it to be recorded.

Berger: My name is Edward Berger. Edward Alan Berger. I understand this interview is being recorded. I give my full permission for the recording.

Harden: I'd like to start the interview with just a bit about your family background and education. You were born on March 25th, 1948, in the Bronx in New York. Would you talk a little about your family--what your parents did, for example--and your early education? I am especially interested in anything that influenced your decision to become a research scientist.

Berger: I was born in the Bronx. I had one sister [Nancy Berger] and my parents [Max and Rosalind Berger]. We lived in an apartment in the Bronx. It was a great place to live. Many of my friends were just right there out in the street, lived in my building, lived across the street. I had a very active childhood. I was very involved in school, but I was also very involved in having a good time with my friends. Playing ball in the street. Singing doo-wop songs. Doing lots of things that kids like to do.

In contrast to the way today kids have to be driven everywhere to participate in whatever activity they want to do, my mother would just say, “Go out and be home by 6:00 pm.” I'd go out onto the street, and literally it was a street. We played off the curb, curb ball, off the street. We'd have to wait for the cars to finish going by. There were bases painted in the street. There was also a marble field where we would shoot marbles, and one kid would just sit by the curb with a marble, and the shooter would stand maybe fifteen feet away, right in the middle of the street, and roll the marble and try to hit the other marble. I had a lot of fun with my friends. I enjoyed my friends. They enjoyed me. I would say I was a well liked kid.

I went to elementary school at PS 109 which was maybe a fifteen minute walk from my house. I walked to school everyday for the first six years, then walked home, usually with a bunch of my buddies.

Harden: What did your parents do for a living?

Berger: My mother was a teacher in the New York City public school system.

Harden: What did she teach?

Berger: She taught a number of things. But when I was school age, my mother taught in the junior high school that I eventually went to. At that point she was teaching mainly typing, but also math. After I finished elementary school at PS 109, grades one through six, I went to junior high school at Junior High School 82, which was even closer. Maybe a five, ten minute walk away. My mother was a teacher there, but I didn't really pay attention to that. Most of the teachers didn't pay attention to that. I did junior high school there.

Junior high school in New York City is seventh, eighth, and ninth. They have a program called Special Progress or SP, where you do seventh, eighth, and ninth in two years. I was in that program, and pretty much all my friends were in that program--all except for one, who was in an unfortunate situation, which I can tell you about if you wanted to hear it. Basically, you didn't have to be a super genius to make the SPs, but you had to be good in school and you had to convince your teachers that you were going to make use of the opportunity.

Harden: What did your father do?

Berger: My father had his own business. He sold fabrics. He sold mattress ticking. He had these long bolts of fabric in the back of our station wagon. He would go to mattress manufacturers and make direct sales with them. He would come home, and he would keep his books. He had this ledger where he would hand write all the information for the books.

Harden: You then went to high school at the Science and Technology High School.

Berger: The Bronx High School of Science.

Harden: Yes. Tell me about that.

Berger: The Bronx High School of Science, I think at the time it was the number one rated high school in the country. You had to take a special test to get in there, and the test was available citywide. It was the same test that was given for the Bronx High School of Science, and also for Stuyvesant High School and Brooklyn Tech High School. I took that test, and I guess I did well enough, and I was accepted. A number of my friends were accepted as well. That's how I started my high school, beginning at grade ten. Tenth, eleventh, and twelfth years in high school. I was a little nervous about going to that school. It seemed like it was a pretty high bar. But I got over that in the first couple of days. I enjoyed it there--being at the school, taking the classes, and hanging out with my friends. It was a very good experience. I enjoyed it. I had to take public transportation to

get there. For most of that time I think it was a bus, and then a train, to get to school. Maybe it took forty-five minutes to get there, and then a ten minute walk from the train station to the school. But it was my routine, and I did it for three years.

Harden: Were there teachers, either at the Bronx High School of Science, or at the City College of New York, that inspired you to study chemistry?

Berger: Let me actually go back a little bit further than that. I've got one memory from back in junior high school of a teacher, his name was Dr. Daniel. I remember, he would sort of joke around and jive, tease in a friendly way, with the kids. He used to say to me, "Edward. Now Edward, I want you to go forward. Not backward." He would get up on the desk and sort of act this out in a very comical but somehow engaging way, that somehow I still have that memory.

I also remember him talking about Abraham Lincoln, what he had to do to get to school everyday. For some reason that image sticks with me. I'll acknowledge something to you that I don't know if I've ever acknowledged with anybody before. At times I find myself telling myself as if I were still in that class with Dr. Daniel. I don't know if it's a sign of some pathology I have, or just an interesting memory that just pops into my head now and then. I mean, you're talking about things that made an impression?

Harden: That's right.

Berger: That made an impression. I can't say that he guided me towards any particular career, that he guided me towards science versus something else. He just said, "Edward, go forward."

Then in high school, I had a chemistry teacher in a special chemistry program, in which I did very well. His name was Dr. Harwell. At Bronx High School of Science, there were a number of PhD teachers. A number of them were quite good. I remember taking a class there, and I remember having one experience where there was a nationwide test for all the people, the kids, who were taking this program in high schools across the country. It was a two-part test. I remember studying very hard for it. By the way, I never considered myself a genius, even though I had apparently a really high IQ. My mother knew, but she would never tell me. But anyway, I never considered myself a genius, particularly compared to some of the other kids at Science who seemed to me to be geniuses, and I think some of them have proven themselves to be geniuses as they've progressed in their careers.

But any case, I was comfortable at Science. I remember taking this class, and I came out of that first part of the test thinking, "I think I did really well." We were talking about it amongst ourselves in the hallway, and then I guess the next session, the teacher announced the scores. It turned out, I got 100 percent. I was the only one in the class who got 100 percent. I was delighted. Then we had the second part of the test. I took that and I came out of that thinking there's a shot I might have gotten 100% on that. I remember

making a bet with one of my good friends. I won't say his name, but he was a friend/rival. He lived on my block. We had gone through school and the same classes all the way through grades one through six. I'm not sure about junior high school, but then we were in some of the same classes at Bronx Science.

I remember making a bet with him. I really didn't think the chances were very high, but when the results came in, I got 100 on that too. I remember standing up going, “Pay up,” in the class. That was one of the glory days of my high school career. I was never a big shot in high school. There were the jocks; there were the political people in high school. I was never part of that, nor did I aspire to be. I had my group of friends. Again, we sang doo-wop in the cafeteria. I really remember that very, very well. I finished Bronx Science feeling like I had a good time there.

Harden: Tell me about your studies at the City College of New York.

Berger: Let me make a general statement about much of my early academic life. I never had a real solid idea of what I wanted to do. I was not one of these kids who knew from early on, “I'm going to be a scientist.” You heard about kids like that back then, and you certainly hear about them now. I was not one of those. I really didn't know what I wanted to do. When I got to college, I was good in the sciences and math. I actually started out as a math major. Then I realized I think within the first year that it was more than just arithmetic and trigonometry and geometry. There was a level of understanding and interest that I didn't really have to pursue a major in math.

I wanted to get into biology, but the chemistry department had a better reputation than the biology department. It was a better department. I switched my major to chemistry. I did pretty well for the most part, although I do remember one class in physical chemistry where I didn't do very well. The teacher questioned whether I really belonged there. I think that it, on the one hand, upset me and maybe hurt my confidence some, but I think it also triggered me a little bit to prove that I could do better. I ended up doing very well in that class. I did fine throughout my whole college career.

Harden: Was there any particular professor who influenced you there?

Berger: In my last year there, I took biochemistry. My teacher there, Dr. Russell [Dr. Charlotte Russell], had enough of an impact on me that I chose to then go for a PhD in biochemistry. I can't say there was any particular memory or moment that I can put my finger on that was a turning point for me, but I decided to go for a PhD in biochemistry. Let me just acknowledge that in doing so I really didn't know what a career in biochemistry was. I really didn't know. That became clear to me as soon as I arrived at Cornell. To put another aspect of my school perspective on this, City College was a commuter school. Basically all through my college years, I lived at home. That was fine

with me. I never pushed my parents to go out of town. They felt that City College was a great school and it was.

Obviously the costs of my attending there were far, far, far lower than attending a private school, either within New York City or outside of New York City. I had no problem with going to City College. A lot of my friends were there. In a lot of ways, it was a glorified extension of high school. I had the same friends. It was expanded maybe from a four block radius to maybe a four mile radius of friends. Of course, I met students from other parts of the city, but my nucleus of friendship was still within the Jewish kids that I had mostly hung around with through my high school years and, again, into my college years. It wasn't until I went to Cornell in Ithaca, New York, that I had much more of an exposure to people who were not like me.

That's where I discovered that in order for people to understand anything I was saying, I had to speak slower and I had to try to articulate more, compared to my fast talking Bronx accent, most of which I've lost, but occasionally I'll have people say to me, “Hey, what part of New York are you from?” I love when that happens, but I also have people say to me, “You're from New York? You don't sound like it at all.” In any case, I started at Cornell. That was my first time away from home, and I guess maybe for the first week I might have been a little bit nervous about being away from home. I lived in a college dorm and I made friends very quickly there, some of whom are pretty good friends to this day.

I began at Cornell in the biochemistry department, biochemistry and molecular biology. I really didn't have a perspective on what that meant either in terms of what I had to do to get my degree or what it meant once I got my degree in terms of moving on. I was always good in school. The easiest thing was to just do the next thing. That's what I did. My career focus really evolved all along the way during my training in high school, college, graduate school and even beyond then.

Harden: Okay, but you did your PhD work with Leon Heppel [Dr. Leon Heppel], correct?

Berger: Correct.

Harden: Did he tell stories about NIH? Did you learn anything-

Berger: Dr. Heppel--at Cornell, we were not on first name basis with the professor. Dr. Efraim Racker was the chair of the department and the most prominent figure, although Bob Holley [Dr. Robert W. Holley] won the Nobel prize during the first year I was there and then he left. He was not there for most of the time I was there. Dr. Heppel was an eccentric man, fidgety, nervous and very, very focused on science in a way that I didn't see myself being. I can tell some amusing stories about that if you want to hear. Dr. Heppel really watched over everybody. One day apparently he had a discussion with his son, who, I believe, was at Yale as an undergraduate. Apparently he told his father, “Dad,

all you know is science. You don't know anything about culture." Apparently, Dr. Heppel took that to heart. He decided he was going to learn something about music and art, so he had the shop build a light box. He put the light box in the lab and every week he would get a print from the Ithaca Public Library of a piece of art. He would put that print in the light box. He loved to tease, and whoever came into the room, whether it was somebody he worked with everyday in the lab or whether it was a visiting seminar speaker, would get quizzed on who painted that. What period is it from? What does it represent? I didn't know squat about art. I didn't know anything. I was learning some stuff as I was there, but really I didn't know anything. One day I came in to the lab and he said, "Okay, Ed, there's a new print. Can you tell me who painted that?" I looked at it and didn't have the vaguest idea. I said, "Look, Dr. Heppel, give me a break. I can't guess who painted that. Let me guess who didn't paint it." He said, "Okay, I'll give you two guesses who didn't paint it." The previous week he had been viewing a Vermeer painting, "Woman Weighing Gold." It was very striking. I really remember that painting even to this day. This painting, the new one in the light box, was clearly not a Vermeer. I said, "It's not a Vermeer." He said, "Okay, that's one. Give me one more. Who didn't paint it?" I just picked the first name that came to my mind. I just said, "It's not Picasso."

He went crazy. The painting was on the cover of any Picasso text book or book about Picasso's blue period. He took that painting out and he dragged me around all over the department telling everybody the story. I remember him taking me to Dr. Vincent du Vigneaud, who was a Nobel laureate, and telling him this story. It was embarrassing on the one hand, but I guess I had fun with it. I remember it and I love telling the story. Obviously whatever negative impact it had on me was very short lived.

Harden: It appears to me that during your time at Cornell is when you got into the study of cell membranes and how enzymes transport across the cell membrane. What got you interested in this process, which seems to have continued through your entire career?

Berger: Gee, what it is that first got me interested is that? It's hard for me to know, but I know in some of my rotations I had done some work related to membranes. Something got me interested on the inside versus the outside. When I joined Dr. Heppel's group, he had been developing some novel systems to study transport systems in bacteria. That seemed like a really interesting spot for me. I chose to work in his lab. I had a particular project that he basically assigned to me. It was a pretty good project, but I think I was slow getting started. Somewhere into the project Dr. Heppel confronted me with a paper that I should have known about. Back then, of course, you didn't have the web to do searches. You had to go to the library and look them up in this huge volume. It wasn't nearly as easy to do.

This was a paper that was directly relevant to the work I was doing. I was studying transport of the amino acid cysteine. It was a paper that was directly related to that. I was a bit ashamed that I didn't know that. That stimulated me to pay more attention and to

take the work more seriously. I came up with evidence that there appeared to be two different transport systems for this amino acid cysteine. By using inhibitors that block one but not the other, I was able to characterize the cysteine specific and the cysteine general amino acid transport systems. Those were the names that we gave to distinguish these two systems.

I have another amusing story about that that shows where I was in this giant field of science. In doing these studies of cysteine transport, we used inhibitors, which I bought from one of the [chemical supply] companies. I had a collection of them. Some of them inhibited and some of them didn't. Dr. Heppel said to me, “Let's go visit Dr. Vincent du Vigneaud over in the chemistry department, who is a world authority on sulfur containing amino acids. He probably can give us some insight and maybe offer some more compounds.” We walked over there and there I was in Dr. du Vigneaud's office. There was the Nobel prize right there in the office. I felt a little bit intimidated. I began explaining as Dr. Heppel said, “Why don't you explain to Dr. du Vigneaud what you're doing?”

I started explaining and I mentioned some of these compounds. He was listening and nodding his head. Then I mentioned the compound lanthionine which had some effects in the systems I was studying. He got this puzzled look on his face. I said, “Lanthionine, that's like cysteine except instead it only has a single sulfur instead of a disulfide.”

“What?” Dr. du Vigneaud paused and took the cigar out of his mouth. I don't know if he really did, but that's the image I have, and he leaned back. He said, "Yes, son, named and first synthesized that compound back in 19whatever. “Ba ba ba boom.” Heppel burst out laughing. I dove under the chair. Again, that was another instance where Heppel went around telling everybody about this experience. Again, this was something which was probably a little bit embarrassing at the time, but I find it really amusing and fun to talk about now and enjoy telling it. It's a memorable moment in my PhD career, but it got me really much more seriously into the work. I published a couple of papers with Dr. Heppel as co-author. I think it was just the two of us. Then let me tell you something else. I'm going to be fully honest about my career as it developed. I had other interests. I liked playing ball; I was playing guitar; I was playing in a band that I had started, The East Hill Fork and Spoon Family Jug Band. We played in coffee houses around the Cornell campus and the Ithaca area. We had our following. I was not a great musician. I would say we had one guy in the band who was really good. He was also a biologist.

Harden: What instrument did you play?

Berger: I played guitar. I have a neat photo on my phone, which I could show you later, from the very beginning of that. We started it in my apartment in my second year at Cornell. The point I was making is that I had other interests and science was not my whole life. I was different from a lot of the people who end up becoming serious academic scientists later

in their careers. I wondered whether that was the direction I wanted to go. In any case, I was not as fully involved as Dr. Heppel would have liked. Then in the graduate program, you have to take your A exams, which are exams where I have to invent a research project with a very defined question and experimental strategy to address it.

I remember one of the ones that I picked was Elizabeth Neufeld's discoveries of enzymes that are missing in certain genetic conditions and lead to early childhood death and the discovery that these enzymes from normal cells can be released into the medium. You could purify them. I did a proposal based on that. I also did a second proposal and I took the exam. I did very well. I was very happy with myself. I came back to the lab and I said to Dr. Heppel, “I'm taking a few days off the weekend to go camping.” He didn't like that. He said, “You've been out of the lab for a long period now.” I said, “Well, I was studying for my A exams.” He didn't seem to accept that as an alibi. He challenged me on my commitment. We had a real argument. He said, “You signed up for a PhD to have an academic career in science.” I said, “I never signed off on that. I'm still deciding what my career is going to be.” We had a real confrontation, which I don't think many people ever did with him. But he forced me into it. I remember it was in his tiny little office. I was backed up against the wall and I fought back.

What ended up happening, I think, was that Dr. Heppel was put off by me or frightened by me, or maybe he thought that I'd do better if he stayed off my back. This was in the last year of completing my thesis. He didn't get on my case looking at my notebooks every week. He left me on my own.

I applied for a postdoc position at a number of places, including with Dr. Paul Berg at Stanford who had just done the first molecular cloning. Dr. Berg wrote back saying, “I'd be very interested in you possibly joining my lab, but I can't take you in three months. My lab is full. Maybe sometime down the road I could.” I decided I would stay on at Cornell an extra year after completing the work that was the focus of my PhD thesis. Dr. Heppel was leaving me alone. That was the first opportunity that I really had to think on my own. That was a breakthrough moment in my life. I started thinking, and I came up with a question that wasn't being asked by any of the people in my lab.

It was a novel question. It was related to the question, “How does the cell get the energy to drive active transport across a membrane where amino acids that might be in dilute concentrations in the medium are taken into the cell in high concentrations, so the amino acids are going against the flow, and energy is required to drive that process?”

There had been some studies, in particular from a very prominent researcher in the transport field, Ron Kaback [Dr. H. Ronald Kaback], on a mechanism involving direct coupling to the electron transport system as the driving force for the transport system he was looking at. I started thinking, “Well, let me ask that question for the cysteine transport systems, then other transport systems that I was looking at.”

I developed a novel approach to study this, where I would basically starve the cells, deprive them of energy overnight, so that they could only carry out active transport based on whatever energy supplying ingredients I added. I started doing that work. I don't remember how the idea evolved, but I came up with very discrete evidence that the class of transport systems that Dr. Kaback had studied were in our hands--in my hands—yes, they were driven by electron transport systems.

But the other systems that fell into a different category that the Heppel lab was studying were different. They didn't play by that rule. They appeared to be driven directly by ATP [Adenosine triphosphate], whether it was directly metabolized in the cell, or whether it was made via the electron transport system.

I had two different mechanisms, and I devised a series of experiments to test that idea using inhibitors. This was my own thinking. I don't remember how much I shared it with the other postdocs in the lab. I had one postdoc who had the bench right across from me. His name was Barry Rosen [Dr. Barry P. Rosen]. He's done extremely well. He's been chairman of departments, initially at Wayne State, and now he's at Florida International University. I talked to Barry a fair amount, but in the end I came up with this model of two different mechanisms of energy coupling, and Barry didn't believe it.

I took it into Dr. Heppel, and he looked at it. It was all new to him. He didn't really quite understand it enough to be able to evaluate it, so he took it up to Dr. Racker, whose main focus in research was energy metabolism. He looked at it and he said, “This is really good.” Dr. Heppel, to his credit, said to me, “You got it. This is your paper.” I published in *PNAS* [*Proceedings of the National Academy of Sciences*], which was a very prominent journal. My submission was supported by Dr. Heppel, who was a member of the National Academy [of Sciences]. It remains the only new data research paper that I am the sole author on. It was the first paper describing what today are called ABC transporters [ATP-binding cassette transporters]. That's a big field today. That paper still gets cited today.

I was very proud, and I was grateful to Dr. Heppel. There was a period where I didn't really appreciate him. But certainly now I recognize--and I think I recognized back then--that he gave me the opportunity for a sole-author paper. Then I finished at Cornell. That was basically an extra year, sort of a postdoc year at Cornell. Afterwards, I went for my postdoc at Stanford. There we followed up with an additional paper, Berger and Heppel, extending this different mechanism to more than just the two systems that I reported in the first paper, to a whole class of systems.

That was the most important outcome of my work at Cornell, even though that work was not really part of my PhD thesis. I remember feeling good enough about my relationship with Dr. Heppel that I wanted to give him a gift upon leaving the lab. I remembered that he had been charged by his son of being a mono-focused scientist who had no interest in

culture. He had expanded his interests into art and music, so I got an idea. I hired a caricaturist to do a caricature of Leon Heppel being the conductor, standing at the bandstand with his baton, and conducting all of the lab workers in the lab. I really enjoyed giving it to him. I visited him years later, and it's still hanging on his wall.

Harden: In 1973 you moved to Stanford then as a postdoc for three years, in the department of genetics, biochemistry, and neuro-biology. You worked with Dr. Eric M. Shooter on nerve growth factor. As you doubtless know, the Stanford department of biochemistry was founded in 1959 by Dr. Arthur Kornberg, another NIH luminary, before he was lured away to academia.

Berger: Dr. Kornberg was very close with Leon Heppel.

Harden: He was, yes. But would you tell me about your experience at Stanford, and comment on how it expanded your research?

Berger: First of all, I loved that period. That was absolutely a wonderful three year period in my life. In the lab, outside the lab, everything about it. The people I worked with. The people I met. The Bay Area, California, environment, which I loved. Everything was terrific.

Eric Shooter's laboratory was formerly in the department of genetics. That was right next door, but on the other side of a set of double doors from the biochemistry department. Each of them was a small department, but each was populated with major luminaries in research during the early '70s. Arthur Kornberg, Paul Berg, Dave Hogness [Dr. David S. Hogness], were all in the biochemistry department, and they were at the beginning stages of molecular cloning. Eric was working on nerve growth factor. In our department, we had Len Herzenberg [Dr. Leonard A. Herzenberg], inventor of the fluorescence-activated cell sorting, and Josh Lederberg [Dr. Joshua Lederberg], one of the giants of early genetics.

We were part of the biochemistry department because Eric had a joint appointment. So that meant that every year when the biochemistry department had its retreat at Asilomar [Asilomar Conference Grounds], I and other members of my group would go down to the Asilomar conferences. I was part of the biochemistry department, but I never felt that, as a member of the Shooter lab and on the other side of those double doors, that they ever saw our group at real members of the elitist Stanford biochemistry department.

One of the things I remember when I first came to Stanford is that any new postdoc had to give a presentation on their previous work in the Stanford weekly seminars, in the lunchtime seminars. I gave a talk on this work that I told you about, the energy coupling mechanisms. I was giving it very clearly, and I remember that at some point, Paul Berg or Arthur Kornberg asked a question, and the other one stood up and answered it. The first one responded to that answer and the second one responded. And it was going back and forth until I finally said, “Stop.”

Harden: My seminar!

Berger: I said, "Let me explain it." And I gave a very clear explanation and that resolved everything. I felt like this ginger kid coming into this room full of the luminaries of science. Here I really held my own. I think Paul Berg wrote back to Leon just telling him what a great job I did. Paul wrote back or Arthur wrote back. I can't remember which or maybe it was both. I think it was Arthur actually.

So my years at Stanford were a time I really loved. During that period, I worked on nerve growth factor because I had been interested in questions about how the head works, how the mind works. Nerve growth factor had the combination that it was based on the nervous system but it was very biochemically oriented, so I felt that it was a part of the approach of science that I was comfortable with. The work there went well. But at the end, I decided that it was just too darn complicated for me. At that time, there weren't the good *in vitro* systems that could be used to study in detail something like differentiation. It was just too complicated.

I had heard a talk maybe a couple of times from Sam Barondes [Dr. Samuel Barondes] who at that time was at UC San Diego [University of California, San Diego]. He talked about a system called *Dictyostelium*, cellular slime mold. It was a very interesting system because you start out with a bunch of cells that are identical, and when there's plenty of food around they just keep dividing as single cells. But then when you take the food away, when they are put on an agar dish with no food, they will aggregate. They'll form these aggregates, and within that aggregate they'll differentiate, and you end up with something called a fruiting body that has two types of cells. It had stalk cells that were dead but which gave rise to a long stalk that suspended a pouch of spores, which, of course, could germinate under the right conditions and give rise to the process all over again.

So here you had a system in which, when you have plenty of food around, you can grow in liquid culture, and all the cells are the same. You put them on an agar dish with no food, and they become two cells. How simple can you get? I figured that's the kind of cell differentiation development system that I wanted to get into, so I made a plan to make a switch at the end of my three years at Stanford to get into this system. I'd never used the system so I arranged to do an extra year of postdoctoral work at UC San Diego. I chose to join a collaboration among three scientists who were prominent in their field, Rick Firtel [Dr. Richard A. Firtel] at UCSD, Bill Loomis [Dr. William Loomis] at UCSD, and Richard Lerner [Dr. Richard A. Lerner] at Scripps Clinic and Research Foundation.

Richard Lerner went on to make Scripps one of the most prominent research institutions in the world. There was an article just a few days ago about the fact that the current Scripps Research Institute—as it is now called--is rated the number one research institute

in the world. Rich Lerner is no longer the director but he really evolved that institution into the range of areas that helped make it what it is today.

In any case, I moved to USCD in 1976 and spent one year there as part of this collaboration. I actually spent all the time working in Richard Lerner's group. I learned enough that I was able to bring that system with me to the job I had lined up before leaving Stanford. It was as a principal investigator at the Worcester Foundation for Experimental Biology in Worcester, Massachusetts.

Harden: I thought they might have just hired you away from Scripps.

Berger: No, they hired me while I was finishing at Stanford and they gave me the year to go to Scripps to learn the *Dictyostelium* system. I brought that system with me and I set it up from scratch at the Worcester Foundation. I was feeling very stimulated and enthused. The next ten years, I'll tell you, was a very, very difficult period in my life both research and my extra research life.

I came from Stanford in Palo Alto, California, which in some ways was the height of living and working, and then I spent a year in La Jolla, which to many other people is the height of living. I actually didn't like southern California very much. It was--I don't know if I can get political here--but I felt that their attitude was, "Hey, we got it all here and we don't want to give it away." I never felt that way at Stanford. Stanford was very, very open. This was just my perspective. It was what I saw. I don't know how much of what I'm telling you now really reflects reality and what other people think about it, but that was my feeling about it. So I was happy to get out of La Jolla.

I moved to Worcester, Mass, on December 1st. I remember how gray it was. It just kind of hit me. I went to the Worcester Foundation, which was a fine institution. It was a small to intermediate sized independent research institute that made a big name for itself in the development of the first oral contraceptive. It had established departments including the cell biology group that I was a part of. They had some really good people in it. I was very happy with that choice. I actually was looking forward to moving back to the East coast. I like the East coast. But Worcester has a reputation for being left out. I don't know if I'm saying things that are inappropriate for something that's going to be viewed by other people, but Worcester seems to have had a reputation of always having missed it. When they built the Mass Turnpike it went several miles away from Worcester. It didn't go through Worcester. Worcester was always kind of out of it.

Basically, I felt out of it--not so much scientifically, because I felt the Worcester Foundation was very much in the mainstream, but certainly socially. I was very unhappy socially living out there. It felt very narrow-minded. I didn't have to be in the big city; after all, I lived in Ithaca for five years and I loved it. I lived in Palo Alto, which is a really big city. But living in Worcester was a whole other thing. My whole social life was

basically going into Boston. I had a lot of friends in Boston. I got very much into swing dancing there. That was a big part of my social life in Boston.

The funny story I was going to tell you illustrates what it was that made me so unhappy in Worcester. I got a call from a reporter from the Worcester newspaper saying that she was doing a story about being single in Worcester. She knew about me and she wanted to interview me. I said, “Fine.” So we did an interview. I thought that was fun. The article came out in the weekend section, and on the front page of the weekend section, the headline was something like, "Alone." It had a picture of a disheveled man sitting on a park bench. Inside there were three interviews. Mine was one of them but the other two were people who were basically like the cover story. Then there was me. I looked at that and I said, “Oh my god. Why am I living here?” Afterwards, I started getting phone calls from women who were very interested. I went out on a bunch of dates. But basically that was one of the nails in the coffin about living in Worcester.

Eventually, I moved much closer to Boston, in Newton, Massachusetts. I was about ten miles from Boston and had maybe a twenty-eight-mile commute to the Worcester Foundation. Very easy commute. It was not highly trafficked. I was right at the intersection of two major highways, the Mass Turnpike and Mass route 128. 128 is analogous to the Beltway--it goes around Boston. I sort of say that 128 went through my living room and the Mass Pike went through my kitchen. I could just hop down to my car and—boom!--I was on the highway. It took me exactly the same amount of time every day.

When you're starting your lab as a PI [principal investigator], as a tenure-track PI living that far away, it is not a good thing. I had to make a choice about what was most important to me, and I made that choice. The ten years at Worcester didn't really work out for me. I certainly do not blame it on the Worcester Foundation at all. It was based on some choices that I made. I decided that I needed to get into a more expansive field and ideally something related to health.

One of the things that happened during my ten years at the Worcester Foundation was the explosion of recombinant DNA, which wasn't being applied in the slime mold system. There were some technical problems. You couldn't transfect them. The field was being propelled by this revolution, which, ironically, when I was at Stanford, I was there on day one. My Stanford roommate was a graduate student with Dave Hogness, who cloned the first structural gene. I mean we're talking about the early days.

I'll tell you another story. While I was at Stanford, I had a visit from John Donaldson [Dr. John Donaldson], who was a graduate student a couple years ahead of me at Cornell. He was involved in Ray Wu's lab [Dr. Ray Wu] sequencing the sticky ends Lambda phage. I don't remember the precise number of bases but that was like the world's record.

It was like a dozen bases. John Donaldson went on to postdoc with Fred Sanger [Dr. Frederick Sanger] who of course won the Nobel Prize for DNA sequencing.

John came back while I was at Stanford, my early days at Stanford. He spent just a few weeks there as a visiting scientist after having spent a postdoc at Sanger's lab and he held the global record for the number of nucleotides sequenced. I don't remember the exact number but it was a hundred something. I smile about where that stands relative to today's technology. I tell that story to current postdocs and students that work with me these days. They have this shocked look on their face as if the world was born with being able to mega clone. But I got off the track here.

Harden: I want to get you from Massachusetts to Bethesda. What drew you here? You must have had many opportunities.

Berger: I needed to get into a field where I could really learn recombinant DNA technology and ideally apply it to systems that are of public health interest. I looked around, and I thought NIH was a good place to do that. NIH had openings for visiting scientists. I interviewed with a number of people including Bernie Moss [Dr. Bernard Moss]. Bernie had just started doing some work with HIV. He was well known for his work on vaccinia virus and pox virus--his explosive work on neurology using pox viruses included the technology of using vaccinia virus as an expression vector. His lab was developing novel technologies for expressing proteins using the vaccinia system and a particular system using bacteriophage T7 to drive expression from a T7 promoter. Tom Fuerst [Dr. Thomas Fuerst], a postdoc in Bernie's group, was the lead person on that project. So when I came to Bernie’s lab NIH in 1987, 30 years ago, I began working basically with Tom.

Harden: Would you give me a sense of what it was like in 1987 in Bernie Moss's lab. Were you in building five?

Berger: I was in building five. That's correct. For one year, and then I moved to building four.

Harden: This was the time when acquired immunodeficiency syndrome [AIDS or later HIV/AIDS], was such a focus of the work in the intermural program, especially in NIAID [National Institute of Allergy and Infectious Diseases]. Do you have any memories or sensibilities of how things were and what people were saying and thinking and talking about?

Berger: When I came to Bernie's lab, it was just alive with just excitement. Hardly anybody was working on HIV, they were working on various aspects of vaccinia virus, including expression technology but also understanding the molecular biology of vaccina virus, how to promote this work, how does gene expression work. It was a very stimulating environment. The postdocs there were great, it was very interactive, the people were fun. I got along really great with Tom. He became a good friend.

Bernie's group had published an important paper on HIV where they expressed the envelope protein using vaccina expression technology. They showed that if they expressed it on cells, and mixed it with cells that didn't have the envelope protein but had the receptor, the major receptor CD4, which had been identified at that point, then the cells would fuse, and they would form these giant cells, or syncytia.

That was an aspect that I thought was a really interesting project to work on. Bernie's lab was not really focused on HIV, but Mal Martin's lab [Dr. Malcolm Martin], in the same building, was. He had weekly research meetings, about the work that was being done in his group. He was glad to have me join those meetings and that's really where I learned about the most current work--all the names of the different cell lines and the viruses and the people and who was doing what.

So now that gave me a tremendous boost that was absolutely critical for me to put the work that I was doing in Bernie's group--and the work was in Bernie's group--in the much broader perspective of HIV research and HIV disease and what was going on experimentally and clinically. When I went to the first international AIDS meeting, I had a perspective that was greatly facilitated by the rich interactions I had being a part of Mal's weekly meetings and also the interactions I had with people in his group, coupled with the work in Bernie's group.

Harden: Would you to go back now and talk about your work with Tom Fuerst.

Berger: The very first project that I had when I joined LVD [Laboratory of Viral Diseases, NIAID], we knew from the work of several other groups that preceded my joining LVD, that CD4 was the primary receptor. It's a membrane protein, a type 1 membrane protein. It has a single membrane spanning domain.

During my first few days when I arrived in Bethesda--I arrived in Bethesda on July 1st, 1987, but I didn't actually start work until July 6th-- Bernie invited me to his home for dinner. I met his wife Toby and I remember that we talked about a project to see if I could make a soluble version of CD4, by just making a construct that had the extra cellular region but didn't have the transmembrane region. Maybe it would be secreted and it would bind to the HIV envelope protein and maybe even neutralize the virus.

That was the goal of the project, and I began working with Tom Fuerst using the T7 expression system. There were other expression systems that could have been used, even other vaccina expression systems, but the T7 was the hot one going on in the Moss lab at that time. And it turned out that using that particular system gave me ideas for what became important developments that I made over the next few years.

In any case, we made this soluble CD4 construct. The extracellular domain of CD4 had been described as having four immunoglobulin-like domains. Domains one, two, three and four. I made constructs expressing domain one, domain two, etc., and different

combinations of those domains. I found that a if a construct with all four domains was secreted, we could purify it. A construct that had only the first two domains also was secreted. We did a bunch of experiments, and we showed that they bound to or were common with gp120 and they could also neutralize the fusion process.

I was really excited by that. The work moved incredibly fast. I came, I had started working in July of 1987, and I believe it was Christmas
Day 1987, that I personally drove the paper, the first submission, to the *PNAS* office, and it got published and it came out the following year. But I also remember that in January 1988, an issue of *Nature* came out that had four back to back to back papers on soluble CD4, showing that it binds to gp120, and it can block HIV.

I started looking at those papers, knowing that we had shown that the construct that had all four domains worked, but a construct that had only the first two domains also worked, which meant that we could say the binding site was localized. So anyway, I read the first Nature paper, and the data were really good. These were from major academic groups, and in many cases, collaborations with big pharmaceutical groups. The first paper had only used the construct with all four domains. The next paper also utilized only the four domain long construct, as did the third paper. But then, in the last paper, they showed both the four domain construct and the construct with only the first two domains also worked.

Harden: You also worked with Ira Pastan's lab [Dr. Ira Pastan, Laboratory of Molecular Biology, National Cancer Institute (NCI)] during this time. Could you tell me about that collaboration?

Berger: That collaboration began a few months after we succeeded in making the soluble CD4. I knew of Ira Pastan's work in making immunotoxins, and his were based on derivatives of *Pseudomonas* Exotoxin A, which is a single chain protein, and Ira's group had defined or helped define regions within that protein that were involved in specific functions. Most importantly, the amino-terminal domain was involved in binding to cells. And once the toxin got inside a cell, the C-terminal domain was an enzyme that carried out a reaction to shut down protein synthesis and kill the cell.

These toxins were very potent. There were estimates that in toxins of this type, these ribosome-inactivating protein toxins, a single molecule is enough to kill the cell. So the Pastan group was applying this to cancer by making constructs of this single chain *Pseudomonas* Exotoxin A, in which they removed the first domain involved in cell binding and replaced it with something that would allow it to bind to a cell that they want to kill, namely a cancer cell.

Basically, most of that work involved attaching antibody-based regions to the rest of the *Pseudomonas* Exotoxin A protein. I had the idea that we could link CD4 to that protein. I

approached Ira with it, and I believe he said that he had that idea too. And he was very eager to do it. So we began this collaboration, and we published a really nice paper, very soon thereafter, describing CD4 *Pseudomonas* Exotoxin A hybrid protein that was potent at killing HIV infected cells. And we got that published in *Nature.* That paper made a very big impact. We began a collaboration with Upjohn to help bring this to development. I was just amazed that, in less than a year after I started at NIAID, I had papers published and one of the things that I made was already on a track towards being tested in clinical trials.

Something else I should mention here—it deals with research politics, but I'll say it, because it happened and it's the truth. All of the work that I had been doing with respect to showing that CD4 *Pseudomonas* Exotoxin A hybrid protein blocked the function of the HIV envelope protein was based on work in Bernie's lab, where it blocked fusion between a cell expressing envelope and a cell expressing CD4. We were not doing any experiments with real HIV, and Bernie didn't have an HIV lab.

We decided that to move the immunotoxin work forward, we had to do experiments with real HIV. We approached a number of HIV labs at NIH, and I very distinctly remember that we talked with Sam Broder's lab [Dr. Samuel Broder, Clinical Oncology Program, Division of Cancer Treatment, NCI], who was making major contributions that are very well recognized--the first reverse transcriptase [RT] inhibitors that proved valuable in treating HIV infection. The Broder lab said that they were willing to work on the immunotoxin. I then took a vacation to California, to King's Canyon National Park. The issue of how my research would work with the Broder lab had not been explicitly worked out, and I remember receiving a call from Bernie—the phone booth, I think, was in a tree. Bernie had had a discussion with Sam Broder, and Sam said they would be happy to do the work, but they would consider it their project. I said to Bernie, "No way. I'm not giving this up, we'll have to find another lab."

When I came back--I don't remember the details of how it worked out--but Norman Salzman [Dr. Norman P. Salzman]**,** who was running an HIV group at Georgetown University Medical Center, gave me access to his lab. And so for several months, I went down to Georgetown, did experiments there, and learned how to work with live HIV. I worked with Kathleen Clouse [Dr. Kathleen Clouse], who subsequently moved to FDA [U.S. Food and Drug Administration] and is still at FDA. We've been in touch on and off over the past thirty years and completed experiments that led to a number of papers. Eventually, we got to do HIV work on the main campus at NIH in an HIV laboratory that Mal Martin had set up, but it became a shared lab with Mal's group and Bernie's group.

Harden: We're still talking about the late 1980s when, had you become infected with HIV in your lab work, it was still a death sentence.

Berger: That's right.

Harden: What kind of concerns did you have?

Berger: NIH had a volunteer program in which you gave a blood sample at the beginning of any HIV research, to get a baseline sample, presumably HIV negative. Periodically, after working with infectious HIV in the laboratory, you would have additional blood samples taken. There was a formal name for that program, but I don't recall it. I gave blood samples, and we were all just very careful, we worked in a BSL 2/3 lab [BioSafety Level], a BSL 2 lab with BSL 3 practices, and the regulations were adhered to rigorously, so I never felt really uncomfortable with it. We never used hypodermic needles—sharps--when we were working with the virus. I think that in other places where people do HIV work, they are not as restrictive, but at NIH, it was confined in the appropriate way to the HIV lab. So I never worried about it.

Harden: Let's launch into the work that leads to your discovery of the second receptor. From what I have seen, looking at your work, I think the vaccinia T7 hybrid expression system that your postdocs, Ofer Nussbaum [Dr. Ofer Nussbaum] and Chris Broder [Dr. Christopher C. Broder] demonstrated, was the one thing that set you up to move forward.

Berger: Yes, that was really important. Working in Bernie's lab was an extraordinarily stimulating and productive environment. I'd been using the T7 system just to express envelope protein. And actually there's a curious advantage of the vaccinia expression system for expressing the envelope like a protein. Because it turns out that HIV, like other lentiviruses, has a somewhat more complicated life cycle compared to gamma retroviruses, and that wasn't really appreciated at the time I first started working on HIV.

Basically, when the HIV RNA is transcribed in the nucleus, it's processed to yield sub-genomic RNAs, which then go into the cytoplasm and encode for some key regulatory proteins like Tat and Rev. The unspliced transcript does not get out of the nucleus, but in order to make Env, the envelope protein, you need the unspliced transcript. But it is stuck in the nucleus. And it's only when the protein Rev accumulates in the cell and binds to the Rev-responsive element that the unspliced RNA make it into the cytoplasm, where Env can be transcribed, translated.

People were having problems making Env until this whole system was worked out. But vaccinia is a cytoplasm virus, which of course, Bernie knew from the very beginning. It turned out that this characteristic provided a huge advantage because the whole vaccinia transcription machinery is already taking place in the cytoplasm from the beginning. So there's no issue about getting it out of the nucleus into the cytoplasm. You could make Env using the vaccinia system by just throwing in the gene. You don't have to worry about Rev and the Rev response at all. In contrast, other people who were using the more HIV-based system or even other standard nuclear expression systems, were having problems.

That gave me a big advantage. The other advantage was related to the T7 expression aspect, where, the system that the Moss Lab had developed, primarily through Tom Fuerst's work, involved having the phage T7 RNA polymerase produced, often times just with a plasmid that encodes it. And then you have a target gene that's got a T7 promoter linked to the gene that you want to produce. So you put those two things in the same cell, and the T7 polymerase jumps on the promoter and makes lots of protein. So that was how I was using it to make envelope and to make the CD4.

At some point, we're using a fusion system to express Env on one cell and express CD4 on another cell. When the cells fused, you can quantitate by looking in the microscope and measuring the number of giant multi-nuclear cells. That's very inefficient. You have to look and count, and it's subjective, and it's very tedious. I thought, “Why not express the T7 polymerase in one cell and have a reporter gene in the other cell? One of the reporters that the Moss Lab was using for other work was beta-galactosidase.

The idea was to express Env on one cell and put the T7 polymerase in that cell, and express CD4 on the other cell, and put the reporter gene with the T7 promoter in that cell, and you'd get variable--only background levels--of beta-galactosidase. But then, if the cells fused, the T7 polymerase and the reporter would be in the same cytoplasm, and you'd get robust production of beta galacosidase. So you had a very robust, very sensitive, very quantitative and very easy and very reproducible assay. When you ran duplicates, they produced results that were very close. And so we published a paper describing this system. Ofer Nussbaum was the first author. Chris Broder was co-author, and I was senior author.

We went on to show that there was a critical tropism feature that had been described originally in 1986, in Richard Axel's group at Colombia University [Dr. Richard Axel]. They showed that, if you express the gene for CD4 on a cell that didn't have it, then that cell could now fuse with HIV. Which is what you'd might expect if CD4 was the essential receptor and all that was required. But it turned out, their technique only worked when they expressed that human CD4 on a human cell line. But if you express it on a mouse cell, a commonly used cell like a NIH3T3 cell, the CD4 was there, it was on the surface, and it could bind to p120. But the virus didn't infect the cell. Axel’s group was not doing fusion, so all they could say was that they got no HIV infection of these mouse cells. And they did experiments that showed that the block to infection was at some early stage.

That was where the problem stood in 1986. I started at NIAID in '87. With the vaccinia-based system, in particular with the very quantitative reporter system, we were able to recapitulate the Axel group’s work. Importantly, we showed that cell fusion occurs if you express the CD4 on lots of different types of human cells, but not if you express the same human CD4 on a mouse cell. So the block was at the level of the very first step, that was in fusion. That was an important distinction that we were able to make. And then we did some collaborative studies with Robert Blumenthal's lab [Dr. Robert Bluemthal] at NCI,

where we asked the question, "Why doesn't the mouse cell that's expressing human CD4 serve as a good fusion target?" And there were two simplistic reasons: either the mouse cell has an inhibitor that prevents it from fusing--some kind of restriction factor--or the human cell has something else that's required that the mouse cell doesn't have.

We did a simplistic experiment of making hybrids. The vaccinia system lent itself to some tricks about doing that. We could just make a hybrid that didn't have to propagate. I think we used measles virus envelope and measles receptor on two different cells to make the hybrids. Our thinking was that if we made a hybrid between a mouse cell expressing CD4 which, either had an inhibitor or didn't, and we got a hybrid between that cell and a human cell, which had the accessory factor, if that was what was missing, then we could see whether that hybrid would fuse with the cell expressing envelope. We did that experiment. And the answer we got was that it fused; the hybrids fused.

So if the mechanism involved in an inhibitor from the mouse cell, that inhibitor would still be there in the hybrid, and the cells wouldn't fuse. Whereas if the human cell had an additional component, and accessory co-factor, then the hybrid would also have that co-factor and the hybrids would fuse. So we concluded that human cells have a co-factor that was required and that was an explanation for why CD4 worked when it was expressed on a human cell, but not when it was expressed on a mouse cell.

And we called that a "co-factor" because we had no idea what it was. Maybe it was another cell's surface protein that the envelope protein points to--in other words, a co-receptor. Or maybe it was something else. Maybe it was an enzyme that modified the CD4 so that it would have features that allowed it to function as an entry receptor, whereas, if CD4 were not modified, it couldn’t function in that way. So we just called the required factor a "co-factor." We didn't call it a co-receptor. I was asked, even in the first paper where we finally identified this co-factor, why we called it a "co-factor" and not a "co-receptor," and that's the reason, because, in the earliest experiments, we didn't know that what we were looking for was a co-receptor that actually also binds to the envelope protein of HIV.

Harden: At this point, you have defined that there is something in a human cell that is a second factor involved in permitting HIV to infect a cell. One other avenue that I believe you explored in taking the next step, but which didn’t quite work out was a collaboration with Phil Murphy [Dr. Philip M. Murphy] in order to use his micro-injection technique. Would you tell me more about this?

Berger: Yes. That was very interesting because of the way it ultimately turned out. We needed someway to figure out what was it in the human cell that was allowing the CD4 to work as an entry receptor. We thought about different ways of doing it, and one approach was to see if we could get RNA from the human cell, inject it into the non-human cell, and get that non-human cell expressing CD4 to now fuse with the cell expressing the Env protein.

The question was how to do that, and there were a number of systems for doing molecular injection of RNA. One of the easier ones was being done by Phil Murphy, who was over in Building 10 and who also was a PI in NIAID. We did not have any scientific interests that were overlapping ecause he worked in a totally unrelated field. He worked on something called chemokine receptors. Which, at that time, we had no interest in.

But, he had a microinjection system that he used to inject RNAs in *Xenopus* oocytes and look at function of the proteins expressed. So, Yu Feng [Dr. Yu Feng], a postdoc in my group who had taken lead on identifying the cofactor for which we were looking, and I talked with Phil, who was extremely helpful. But in the end, we decided there were certain technical aspects of that system that were not compatible with the question we were trying to answer. The cells we wanted to use didn't like the same kinds of conditions, that sort of thing.

We turned in another direction. We decided to try to apply some kind of screening of a library of cells. I had the idea that if we had a plasmid library that was derived from RNAs from a human cell, then that cDNA library should include amongst the many different sequences cDNA that encodes this missing factor. That was the approach we took, and we decided to go with the library from HeLa cells just because we had been working a lot with HeLa cells, and we knew that if you put CD4 on them they fused very, very well with cells expressing HIV Env protein. We thought that since they fused well, presumably they had decent amounts of the factor that we were looking for.

Harden: Correct me here if I'm wrong, this library ... the cDNA library from HeLa cells that you wanted to use, was commercially available through the NIH AIDS Research and Reference Reagent Program. Right? It had been funded this library by that program?

Berger: You may have done more researching about such details than I have! But yes, one main reason for picking HeLa as the cell for our research is that a cDNA library of HeLa cells already existed. Its source is probably indicated in the paper.

Harden: So now it is May 1995, and from my reading, everything started to happen really fast and it all quickly came together. Tell me about how you used this library to narrow things down to identify the second receptor.

Berger: The idea was to take the library that had thousands of different cDNAs, and divide it up into fractions. The hope was that one of the tubes would have the cDNA that we're looking for in it, and some of the other tubes wouldn't. Or maybe one tube would have a lot more of the cDNAs than another tube. So we took NIH 3T3 cells--mouse 3T3 cells expressing human CD4 and the T7 promotor/*Lac Z* reporter gene--and we transfected them with cDNA from each of those samples from the original pool of HeLa cells. What Yu Feng found was that some of the fractions gave a higher signal than others. It was still

not a very high signal compared to background because the cDNAs in any one of those tubes are mostly cDNAs for things that we're not interested in.

But by doing that, he could say, "Okay, tube number seven and tube number 12 are getting a high signal, so let's expand the cDNA from those tubes, and then divide that and do it again." So, we went through several cycles of that. Eventually, we had a tube that was giving a very robust signal, so we just played it out. We had single plasmid colonies, and we picked individual ones and found the bunch of them that worked, and some that didn't work. And we took the ones that worked and expanded those, and we found that each of them had the same insert and we had DNA sequencing performed. We found the sequence of the DNA sequence of that insert and when we translated that into protein, we knew the protein it coded for. By comparing that protein sequence with proteins in the database, it looked most like a receptor for a protein called IL-8 [interleukin 8], which happens to be a chemokine.

It turned out that this DNA had actually been cloned by three different labs, but nobody had any idea of any function that the gene coded for. It was just a sequence in the database. We cited those earlier papers in the paper that we published. The protein encoded by the DNA that we isolated had homology with a known chemokine receptor, which, ironically turned out to be Phil's field.

When we got the results, I was visiting Barry Rosen, my old colleague who sat next to me at Cornell and was a post doc in Leon Heppel's lab when I was a graduate student. Barry Rosen was at Wayne State University, and he had a place in Wintergreen, where he and I were playing golf in July 1995. I called the lab, and they told me they got the sequence back. So, I said to Barry, "Sorry, no golf today. I'm driving back." When I got back, we started doing the critical experiments to prove that this DNA that we isolated was the missing cofactor responsible for human cells, but not mouse cells being able to support fusion. We did both what we call gain-of-function experiments where we took the cell that we can express CD4 on, but it doesn't fuse, and we put this gene in by transfection and now it works to support entry in a fusion assay and to support infection with real HIV virus. Then we did loss-of-function experiments where you take a cell that expresses the protein and is permissive for fusion and for infection and try to block this particular molecule. We did it by making antibodies against synthetic peptides from the predicted amino acid sequence. And we showed in blocking assays that those antibodies blocked the fusion assay and the infection assay.

Now there is a complication of this area of research that we haven't gotten into yet. That is that it was starting to become clear in the early nineties that there were two versions of HIV-1—there are different HIVs, also, but we are dealing only with HIV-1. These different versions of HIV-1 could be isolates from the same patient, maybe from different stages of that person's life with HIV. They were distinguished based on what types of cells they would grow in, that they would replicate in a cell culture. One class of isolates

were known as macrophage-tropic and the other as T-cell-line-tropic. T-cell-line-tropic viruses would grow in a human cell line that had CD4. That included CD4-positive T-cell-lines, like Jurkat, or H9, or even a HeLa cell if you put CD4 on it. The T-cell-line-tropic virus would grow very well in our fusion assay. We had a paper showing that. envelope proteins from T-cell-line-tropic viruses would fuse very well with those cell-lines expressing CD4. But the other types of HIV-1 isolates, which we call macrophage-tropic, those isolates have the opposite preference. They infect macrophages well, but they are very poor at infecting the T-cell-lines.

Another confusing aspect of this is that both types of isolates can infect primary T cells. That's why I don't call them T-tropic or M-tropic. I call them T-cell-line tropic versus macrophage tropic.

We had a paper in which we showed that envelopes from the T-cell-line tropic viruses fused with cell lines expressing CD4 but not the macrophages expressing CD4, whereas envelopes from the macrophage-tropic isolates fused with macrophages expressing CD4 but not with T-cell lines expressing CD4. This indicated that the tropism difference was reflected in the fusion specificity of the corresponding envelope glycoproteins. And we had another paper in which we did an analogous kind of cell hybrid experiment where we made hybrids between a macrophage and a T-cell line to ask the question, “Is the reason why this envelope fuses with the T-cell lines but not the macrophages because the T-cell line has something that's needed, that the macrophage doesn't have? Or does the macrophage have a restrictive factor?” To answer that, we made hybrids. And again, the result came out that the hybrids fused, which supported the idea that the tropism was based on a necessary additional component that was cell-type specific. So we were now thinking that there might be different co-factors that bind with the HIV-1 envelope protein that we call macrophage-tropic and a different co-factor that binds with the HIV-1 envelope protein that we call T-cell-line tropic.

Our original work, remember we got it from a HeLa library, because that was the easiest thing to work with. We could have just as easily gone after the macrophage-tropic but there weren't existing libraries. It would have been a lot more work, and there was a good technical and scientific reason to choose to go after the T-cell-line-tropic factor.

Harden: But once you had identified it there and you said it had similarities to IL-8.

Berger: The receptor for IL-8.

Harden: Yes the receptor. And you named your receptor FUSIN.

Berger: Right, because the only function that we had for it was that it supported fusion mediated by the HIV-1 envelope protein.

Harden: I want at this point to get a picture of you with the automobile license plate you had made with "FUSIN" on it and that you are donating to the Stetten Museum. [Photograph is taken].

Berger: Okay, so at the end of July 1995, when I made the phone call from Wintergreen, I actually knew the sequence. I knew that the people in the lab had gotten the sequence back and that it was homologous to some other proteins in the database. Over the next several weeks, we began gearing up to do all these gain-of-function and blocking-of-function experiments that I just told you about. By February of 1996 I felt ready to present this in public. Now at that time, I did something that I probably would not do this way today, should I come across anything that significant again in the future. But I came from the old school where you just told people what you had found. At a meeting, I went overboard. I actually showed the protein sequence of this gene that we had cloned.

Harden: This was at the Keystone.

Berger: Yes, that was at the Keystone meeting in Santa Fe in February. I didn't appreciate how explosive it would be. I gave the talk, and I showed the sequence. Within the next few days and weeks I started getting phone calls from reporters. Of course, we knew that there was still the other factor that enabled fusion in macrophages, and we had started looking for that. I said in the "Finding Fusin" article that I was enamored with the library screening approach. A new postdoctoral fellow was taking the lead on that project, and I encouraged him to make a library from primary macrophages. That meant making our own library, which isn't that hard to do, but it's extra work, and there are a lot of subtleties that have to be taken care of.

I now need to bring in another story, which was remarkably coincidental. Initially, it was totally unrelated to the work we were doing. That was effort to identify the so called CD8 suppressive factor. This was a phenomenon that Jay Levy [Dr. Jay Levy] at the University of California, San Francisco, had described, I believe in the late eighties, that CD8 T-cells grown in culture released something into the medium that, when you then add it to an HIV-1 infection assay, it blocked infection of the virus. Back in those days the only viruses that people were working on were the original, lab-adapted viruses, many of which came from the original isolates of HIV. They grew very well in T-cell lines. I mean, that's how a lot of work got done before the discovery of these macrophage-tropic viruses. Anyway, Jay Levy's group had been trying to identify the factor, released by CD8 cells that suppressed HIV infection, and other groups were as well. A report came out in December of 1995 from Gallo's laboratory [Dr. Robert C. Gallo] in NCI. Fiorenza Cocchi [Dr. Fiorenza Cocchi] was first author and Paolo Lusso [Dr. Paolo Lusso] was the senior author; Bob Gallo was a coauthor. This paper attributed the suppressive activity in HIV infection, released by CD8 cells, to three proteins that they purified. The happened to be three different chemokines, RANTES, MIP-1α, and MIP-1β. They showed that these proteins suppressed HIV infection, but interestingly,

they didn't make the point that the viruses suppressed fell into the category of M-tropic HIV-1 viruses. I think they had one figure showing an original lab-adapted, T-cell-tropic cell line infected with HIV-1, and the proteins they had discovered did not suppress HIV infection very well in that cell culture. That was in December 1995. We already knew about fusin from our totally, unrelated, independent work. We also knew that fusin looked like it might be a chemokine receptor and in their paper, we were being informed that these three chemokines block fusion in M-tropic cell cultures. They also showed data that the block occurred in an early step in the replication cycle. I don't think they ever mentioned entry, and I'm pretty positive they didn't say that these proteins act by binding to a receptor.

In any case, we knew that fusin looked like a chemokine receptor. Another thing, that I happen to find out about this time was that Phil Murphy and his group independently cloned the chemokine receptor blocked by RANTES, MIP-1α, and MIP-1β. Phil gave a couple of talks NIH, saying that they had cloned the chemokine receptor that had exactly the same chemokine specificity for the chemokines described in the Cocchi et al. paper. He had no idea how they worked and he didn't know about our work. Phil was not an HIV guy.

Anyway, the postdocs in my lab, including Ghalib Alkhatib [Dr. Ghalib Alkhatib], were saying, “Phil's got this cDNA, maybe that's the one, let's just test it,” but I held on for too long wanting to do it by the clean way of not making any assumptions. I thought we just needed to start with a cDNA library and see what we could find. Eventually, I listened to the people in my group who continued to say, “Get in touch with Phil,” so I called Phil, and I sent him an email. Phil gave us the clone, we did our experiments and we showed the same gain-of-function and blocking-of-function experiments, but by this time we were not alone in the game.

Starting with my not-so-brilliant revealing of the sequence of fusin, showing that it looks like a chemokine receptor, everyone began thinking about a chemokine receptor as the necessary cofactor. When the Cocchi paper came, everybody began thinking that maybe it would be a chemokine receptor that binds to those particular chemokines. Then there was another remarkable coincidence. A Belgian scientist in the chemokine field, Mark Parmentier [Dr. Mark Parmentier] independently cloned a new chemokine receptor.

Then, somehow, the HIV people who were hot after getting this, were able to get the Parmentier clone for that chemokine receptor. Of course, we were collaborating with Phil. Some of these people had invited Phil into a collaboration before they got the Parmentier clone, but Phil had already started collaborating with me. Anyway, there was this race going on, and I remember being at a meeting--it was a Keystone meeting--but it was held in Hilton Head, South Carolina. I don't remember the exact month, but I remember calling the lab and talking to Ghalib, and he had the data. He had the data showing that if you put Phil Murphy’s chemokine receptor gene into a cell that had CD4,

it now supported fusion with a macrophage-tropic HIV-1 envelope. I know we had it before everybody else, but there was a big controversy at the time. There were a lot of battles about it.

I think I sent you a bunch of emails including from people who were in the competition, saying, "Ed, we all knew you had it first."

Harden: Yes.

Berger: Our paper didn't come out first. In fact, in our original paper, we didn't have infection data. We only had fusion data and *Science* wanted to hold it up until we did the infection experiments. I can understand their position, but we knew that all those other papers were coming out in two weeks, so we did a very quick infection experiment. I think we said in the paper that it supported infection, but we don't show any data. I think that's the case.

Any case, there were five papers from five different groups, all of which were hotly competing with each other.

Harden: During that fall, the Parmentier group also showed that the delta 32 mutation--

Berger: Okay, okay. Hold on, hold on. Delta 32, we'll get to that, but let me give you a timeline. The first paper describing the first identified second receptor, which was T-cell-line tropic and which we called fusin, came out on May 10th, 1996.

Harden: Okay.

Berger: In June, the following month, the five papers came out in *Nature*, *Cell*, and our paper was in *Science*. Our first paper, the fusin paper, was published first, and then came the five papers about the macrophage-tropic second receptor, which was called CKR5 at that time. Eventually, the chemokine receptor people--they had their own nomenclature system--renamed both the receptors as chemokines. Now fusin is known as CXCR4. At the time we identified it, it looked like it might be a chemokine receptor, but we didn't have any data on that. Two other groups heard about it, and they had the sequence, so they could express the protein and they could look for a chemokine. Two other groups independently cloned a chemokine called stromal-derived-factor-1, SDF-1, that binds to CXCR4, and so now, fusin officially qualified as a chemokine receptor, so they renamed it CXCR4. That name, appropriately took over and is the name that is currently used. So, the name fusin lasted for a while in the HIV field, but eventually we all began using CXCR4 and that's what we use today. You don't see fusin much at all, but I see it every day because my license plate says fusin.

Harden: I'm just amazed at how fast all these things happened in the fall. I believe the last big one that happened that fall was identification of the CCR5 delta 32 mutation as the mechanism that prevented individuals with it to resist HIV infection.

Berger: We need to talk a little about how that happened. To be honest, I wasn't even thinking about these so-called co-factors because we still didn't know yet that they actually bind to the envelope protein. I was not thinking about this work in the context of resistance to the HIV infection. But the Aaron Diamond group in New York [Aaron Diamond AIDS Research Center], was working on this. I don't remember exactly which investigators, I know Rick Koup [Dr. Richard Koup] was involved, and probably John Moore [Dr. John Moore], David Ho [Dr. David Ho], maybe Ned Landow [Dr. Nathaniel Landau]. I'm not sure if all of those people were involved in studying gay men who had volunteered to be studied because they were engaging in repeated high-risk sex but were not infected. I think they had data suggesting that cells from those men were refractory to infection. I don't recall if they made a distinction between the so called macrophage-tropic and the T-cell tropic. But if they saw them being resistant, they must have been looking at macrophage-tropic isolates because they are the cells with the mutated CCR5 receptor.

So in any case, they were studying those people and then they were one of the groups, actually there were two papers that included investigators from Aaron Diamond that were saying these men who were highly exposed were not getting infected. I was in a meeting an international conference in virology in Jerusalem and this was, I believe, August of 1997. I had already given my talk, and it was my first and still my only visit to Israel. I was spending time with some of my scientific colleagues there when somebody handed me a newspaper, an Israeli newspaper. I had them translate it for me, and basically it described two reports that had just come out from different groups describing a mutant allele of CCR5 that has a 32 base pair deletion right in the middle of the open reading frame. And because 32 is not divisible by three, means it causes a frame shift in the gene, and so it codes for non-sensing protein that isn't even expressed.

And this allele, amazingly, is present at a pretty high frequency, particularly in the Caucasian population. Also amazingly, there are a lot of people who are homozygous, which you wouldn't necessarily predict. You might think “If this is a chemokine receptor, it might be essential for life, and so somebody who doesn't have it isn't going to live.” But it turns out, these people seem to be perfectly healthy.

And so, the folks from the Aaron Diamond who were studying these gay men look at the CCR5 gene and found that they were homozygous for the delta 32 mutation. Independently, a group involving Bob Doms [Dr. Robert W. Doms] at the University of Pennsylvania independently got data that the homozygosity for CCR5 Delta 32 is associated with resistance to the HIV infection. I don't recall whether Parmentier was a part of that. I don't want to misstate, I just don't recall. And we all knew from studies of HIV transmission that the viruses that first take hold in a newly infected person are macrophage tropic. They have that tropism. They infect macrophages but they don't infect human T-cell lines expressing CD4.

Now you can say “Okay, that's because these viruses are using CCR5.” And so, the discoveries that chemokine receptors were co-receptors were able to explain in molecular terms what distinguishes the macrophage-tropic viruses from T-cell line-tropic viruses. The T-cell-line tropic use CXCR4 but not CCR5, and the macrophage tropic use the opposite. There are some isolates that can use them both. They’re called dual-tropic.

The viruses that initially take hold in a newly-infected person are invariably macrophage tropic or CCR5 specific. And that's an amazing thing because this happens even if the donor is harboring isolates that are either type. So presumably the viruses that are in the fluid that gets introduced into a newly-infected person can have both types of isolates. But the ones that take hold as the person becomes HIV-positive are invariably specific for CCR5. And we still don't have a good understanding of that.

And then a person who was infected by HIV before antiretroviral drugs in the highly potent combination cocktails were available could live with HIV for years and not even know that they were infected and seem perfectly healthy. The isolates you get from such a person are specific for CCR5. It is only when the viral load starts increasing and the immune system starts declining and the CD4 T-cells are declining that are able to isolate that viruses that use CXCR4.

Harden: That was a wonderful chart you had in your talk showing that, and I know that we're jumping ahead in time a little bit because it was 1998 when you published a new classification system for HIV-1.

Berger: Well it's a nomenclature system. The ones that use CCR5, we call them R5, and ones that use CXCR4, we call them X4, and the ones that can use both, we call them R5X4. We don't use mac-tropic or T-cell line-tropic anymore. We use R5, X4, or R5X4.

Harden: But let's go back now to the end of 1996.

Berger: Okay so there's the Aaron Diamond group that were studying the men who have sex with men, high-risk exposures, identifying homozygosity for delta 32, whereas the University of Pennsylvania group showed it. And Phil Murphy's group, without hearing about any of that at all, was analyzing the CCR5 gene. They were working with Peter Zimmerman [Dr. Peter A. Zimmerman], and they found a mutant CCR5 receptor that had this delta 32 mutation. They began asking the same question, “Could it be that homozygosity is associated with resistance to infection?” The HIV establishment had set up various cohorts of individuals based on various parameters from whom they were able to obtain various kinds of samples, including blood samples. And those samples were supposed to be made available to the entire research community. There was the MACS [Multicenter AIDS Cohort Study] cohort, which had samples from people who were HIV positive, people who were HIV negative, and people who were highly exposed but were HIV negative.

So Phil Murphy and Zimmerman and their colleagues went to the person who had control over those samples. And that was Stephen O'Brien [Dr. Stephen O'Brien] in NCI. I don't want to go into any details, a) because I don't remember them and b) I don't want to say anything wrong. But it became a very, very, unpleasant interaction basically because those samples were not made available to Phil Murphy and his colleagues at that time. And there's a discussion of that in a September 27, 1996, *Science* issue that has an article by John Cohen and a sub-article on the same pages of that article describing the situation. Phil Murphy is quoted, and several other people are quoted in there about the fact that they were not given access to the samples.

So Phil Murphy's paper--I call it Phil Murphy's paper even though we are co-authors on it--because eventually he did the studies and got cells from people who were delta32 homozygous. We showed that they were not susceptible to the macrophage-tropic isolates. But Phil was the leader in that idea in the paper. That paper came out well after the two August papers and O'Brien's September paper, so our paper, which didn't come out until early 1997, is typically not cited.

Harden: At the end of 1996, when all this happened so fast and was such a big deal that your paper was named Breakthrough of the Year by *Science* .

Berger: To be fair, they didn't actually name our paper Breakthrough of the Year. They named the discovery of chemokine receptors as the Breakthrough of the Year. And there was another whole discussion or argument, including letters from top people here at NIH, Harold Varmus [Dr. Harold Varmus] and Bill Paul [Dr. William Paul], directed to the person who wrote that article, Michael Balter, about whether he gave appropriate credit to the work that started the whole thing. They give credit to me for starting the whole coreceptor discovery. They don't necessarily give credit to me for being the first to identify CCR5. Now I can't say that I can prove we were the first, but I believe we were. And then there are patent things related to this.

Harden: We'll get to the patents in just a minute.

Berger: I don't want to discuss that in great detail because first of all, I might get things wrong and secondly, the Technology Transfer Office doesn't really like us talking about behind the behind-the-scenes stuff that goes on in the context of patents.

Harden: I was struck by your colleague John Moore's comments, which must have been very sweet when he said, that his lab and others were racing to build on your work, but they were simply picking over the carcass that you had already killed for them, that you were T. Rex.

Berger: I guess this was right around when Jurassic Park was coming out, so everybody was sort of riffing on it. They referred to somebody as a raptor, Velociraptor. People were making jokes about it.

Harden: And there's the wonderful picture, which you may want to hold up for the video camera.

Berger: In our lab, of course, we were really having fun goofing on this, so I made this picture using Adobe Photoshop and we called it Cofactor Park. We have a T. Rex up there and other dinosaurs. And that, of course, emanated from John Moore's gracious referral to me as the T. Rex.

Harden: And all of this, is shown on the steps of Building One at NIH.

Berger: John More sent me a package with some things in it, including a plastic T. Rex, and I still have that.

Harden: Of course.

Berger: I have a little altar related to the coreceptor discoveries.

Harden: One day you might wish to donate that as well to the Stetten Museum. Put it in your will. I'm quite serious about that.

I had just a few more things I want to ask you about. There was the unpleasantness over the patenting of genes, and this is a whole different discussion but I thought I'd ask if you want to make any comments on it. This is the fact that before the cofactor papers were published--in June 1995--Human Genome Sciences had applied for a patent on a polypeptide sequence that turned out to be CCR5, even though they didn't know what it was at the time. That became a huge issue. The scientific community, as you have noted a number of times, is primarily interested in priority in terms of scientific publication. The Patent Office, in contrast, is interested in priority of patent applications.

Berger: Right, right. Priority from the scientist’s perspective is not always equivalent to priority with respect to the Patent Office.

Harden: Absolutely.

Berger: And there was this whole issue about whether, if you have just cloned a gene, do you have ownership of that?

Harden: That's what I'm getting at. The priority in the scientific community, you're thinking about an intellectual process that you have completed. The priority for the patent office did not require that you knew what this thing did. That seemed unfair.

Berger: Right. There was a meeting between Tony Blair [U.K. Prime Minister Tony Blair] and Bill Clinton [President Bill Clinton] about this. I'm not sure exactly what was said, but it was something to the effect that ownership should be associated with knowing something about what it is you're getting ownership of.

Harden: But the commercial firms argued it differently.

Berger: The commercial firms argued it differently, that you encourage people to file for something, and then they can figure out what it does.

Harden: Yes. I can understand their thinking as well. If you have a gene and you have no idea what it does, having ownership of that gene means that a company can license it or that company can try to discover what that gene does, maybe incur a heavy financial investment but with the hope of recouping that investment. If they don't have any protection from the patent, then they might be much less inclined to devote the money and the time and effort to do that research ... or to do those clinical trials, which are extremely costly.

Has this argument caused any fallout in terms of future basic research in this area?

Berger: There were a lot of ruffled feathers and decisions and outcomes about who got what with respect to royalties. But I don't know of any real impairment of science that happened because of this. Now it could be that somebody else listening to the same thing might say, "Whoa, wait, what about this?" And I'm not denying that. We were involved in a lot of that patent stuff, but it didn't get in the way of our being able to do the science.

Harden: There was great hope that the second receptor discoveries would lead to new therapies and preventative efforts, but all I can find is that in 2007, Pfizer introduced the first new class of drugs that would inhibit CCR5. Can you expand on this for me?

Berger: It turns out that the chemokine receptors are seven-membrane spanners. They have an external N-terminus and then they have these loops traversing the membrane from the outside and then again from the inside. They have three extra cellular loops and an extra cellular N-terminus. So these are seven-spanners, and they all belong to the family of G protein-coupled receptors, which refer to a complex molecular signaling machinery involving a class of intracellular proteins called G proteins that are coupled to these receptors, and when these receptors are triggered by whatever triggers them, then these G proteins start a signal cascade.

The members of the G protein-coupled receptor family--it's the biggest superfamily of receptors in the genome--encode for extremely diverse things. I mean, rhodopsin is a G protein-coupled receptor, and it "binds to" light. In other words, it's activated by light. Receptors for chemokines, receptors for glycoprotein hormones, receptors for all kinds of things are within this giant class of G protein-coupled receptors, and the reason I'm bringing it up in the context of the question you ask is that it turns out that G protein receptors have proven to be really good targets for drug development in general.

I don't know what the numbers are today, but we used to say that if you go into your local pharmacy and just look on the shelf, maybe 30% of the drugs on that shelf work because

they bind to a specific G protein-coupled receptor. Maybe one, maybe more than one. So, now that you have identified chemokine receptors that are essential entry receptors for HIV, maybe you could make a drug that bound and blocked a chemokine receptor. In that way, you would be able to block HIV infection.

People also tried to make knockout mice. A 4 homozygous knockout is embryonically lethal. CCR5 knockout mice are viable. And just like CCR5 knockout people, i.e., delta 32 homozygous people, CCR5 knockout mice are normal. Now it turns out, and actually Phil Murphy did some important work on this, that under certain conditions, people lacking CCR5 are at a disadvantage. For example, if a person becomes infected with West Nile virus and doesn't have CCR5, they become more susceptible to the CNS [central nervous system] complications of West Nile virus. So I wouldn't say that a delta 32 homozygous person is totally ‘normal. That person does have some health vulnerabilities, but they don't show up in most people.

And there have been a handful, maybe a couple of dozen, of cases of people who are delta 32 homozygous and who got infected with viruses that use CXCR4, and unless they take anti-retroviral drugs really quickly, their prognosis is very poor. Several of them died.

I want to close with the very important search for an HIV cure. We have antiretroviral drug therapy, which has gotten better and better over the years since it was announced as one of the Breakthroughs of the Year. So the 1996 Breakthrough of the Year was the chemokine and the receptors, and also the combination antiretroviral therapy. Back in 1996, a person who was HIV positive had to take pills that target multiple parts of the HIV replication cycle, and in talks we would show a hand filled with pills. Nowadays, the drugs have improved and there have been collaborations between companies where there can be multiple drugs in the same pill, so there are single-pill, one-a-day versions and there are single-pill, less frequently than one-a-day versions. And it will continue to get better and better.

Antiretroviral therapy, for the people who respond, and a large percentage of people respond, as long as they keep taking those drugs, is very effective. There were side effects, and the degree to which individuals suffer from those side effects varied. Some people do totally well. And those drugs are being used not only to treat, but also to prevent infection in sexual partners. There's a huge movement and programs called pre-exposure prophylaxis [PrEP], where people who are HIV-negative, but are considered a high risk because of the activities in which they are engaging, can take these drugs to prevent HIV infection. That's a whole other field.

Nevertheless, there's a huge impetus and a huge investment in the search for a cure for HIV infection. And by “cure,” we use that word in two different contexts. One would be a true sterilizing cure where somehow, all infectious HIV, whether it be virus, cells,

nucleic acid, is gone. Whether you could ever prove that all HIV had been eliminated from a person's body, but this cure would mean that a person would be completely off antiretroviral drugs and no trace of the virus could be found. There has been one person who has been cured.

Harden: The Berlin patient.

Berger: He used to be known as the Berlin patient. His name is Timothy Ray Brown. He revealed himself publicly, and he's now very much out there in terms of the HIV positive community and the HIV research community. He comes to lots of meetings. He's going to be at a meeting that I'm heading to tomorrow in Seattle, an HIV cure meeting. His case has been a great inspiration for cure research, and his case is believed to be a sterilizing cure, although you can't prove that he has no infectious HIV, and if I were he, I would get tested on a regular basis, because it's still possible that there is infectious HIV in his body and that at some point it'll get reignited. But he's considered the only real cure.

The other kind of cure that many researchers, including our lab, are trying for is sometimes called a functional cure, which basically means a long-term suppression, where somehow you treat the person so that they can stop taking antiretroviral drugs and the virus doesn't come back. The person's immune system remains functional and the person remains healthy even though you know that there's infectious virus there and you can actually detect it.

Timothy Ray Brown was an American living in Berlin who developed leukemia. He was HIV positive, and to make a long story short, after failing the standard therapies for his leukemia, he was given a bone marrow transplant and his bone marrow surgeon, Dr. Gero Hütter, thought that maybe, of the potential donors for Brown's surgery, there might be one with the CCR5 delta 32 allele, since about one in one hundred Caucasians is homozygous for this allele. It turned out one donor did have this mutation, and he was the source of stem cells that were used in Timothy Ray Brown's bone marrow transplant. He's now cured, and there's a tremendous amount of research trying to resolve what enabled the cure.

Was it the fact that he got this intense ablation of his immune system before receiving the transplant, which wiped out all the HIV? There had been reports of individuals who had gone through this procedure and gotten a bone marrow transplant, but in their cases, they got normal CCR5 transplant. Nevertheless, they didn't see any virus come back, so they thought, "Maybe it's the ablation that did it." But eventually the virus came back in those people--they were the two Boston patients. So at least in those cases, the bone marrow ablation was not enough.

Maybe it's the fact that Brown got cells that are resistant to HIV infection, and I personally think that that's a huge part of it. They have tried to reproduce that with other

leukemia patients, with lymphoma patients who needed a transplant and they had a delta 32 HLA-compatible donor, but none of those have worked yet, and it's not that they failed because the HIV came back. They failed either because the leukemia came back or because the person died from the high-risk bone marrow transplantation. It is a very high risk procedure, with something like a 30% death rate just from the bone marrow transplant. If the patients died from their cancer or from the procedure, it was impossible to tell if their HIV had been eliminated for the long term.

You're not going to do bone marrow ablation in the average HIV positive person who's doing fine on antiretrovirals. You're not going to put him through a bone marrow ablation and transplant and give him a high risk of dying from that, just to give him delta 32 homozygous cells. Nevertheless, there is research aimed at knocking out CCR5 by editing a person's cells and giving those edited cells back to the patient in the hopes of giving him an HIV-proof immune system. This involves taking a person's own cells, treating them with gene editing technologies, including CRISPR-Cas and other methods. Zinc-finger nucleases have been going on for quite a while now. Of course, knocking out CCR5 doesn't help if that person has viruses that can use CXCR4.

Harden: This has been a wonderful interview. Is there anything else you want to say before we stop today?

Berger: I'm very happy to be able to tell this story to you and to people who want to listen to it. I've given my version of it. I'm sure there are other versions of it. I'm giving the version that I've experienced and remembering it and telling it as well as I can. When I first got into the HIV field, coming from the *Dictyostelium* field, which was a small field, it was amazing to me, and I told you that I was scooped by four papers. Nevertheless, I got to know those people really well. We all became friends and we interacted quite a lot.

I came from a field that was small and extremely cutthroat to a field which, certainly back in the early days, there was a lot of openness and sharing and communication and friendships, and those have continued. Of course, there's a lot of competition, and there are lots of cases where people may feel that things were done unjustly, but by and large, I've found that people in the HIV field make up a very good research community that has a lot of respect for the science that we do and for the individuals who are conducting it.

Harden: Thank you so very much, Dr. Berger.