- VOLUME A -

IN THE UNITED STATES DISTRICT COURT

IN AND FOR THE DISTRICT OF DELAWARE

- - -

THE JOHNS HOPKINS UNIVERSITY, A Maryland Corporation, BAXTER HEALTHCARE CORPORATION, A Delaware Corporation, and BECTON DICKINSON AND COMPANY, A New Jersey Corporation,	:	CIVIL ACTION
Plaintiffs	:	
v.	:	
CELLPRO, A Delaware Corporation,	:	
Defendant	:	NO. 94-105 (RRM)

- - -

Wilmington, Delaware
Tuesday, March 4, 1997
9:05 o'clock, a.m.

- - -

BEFORE: RODERICK R. McKELVIE, U.S.D.C.J.

- - -

APPEARANCES:

POTTER, ANDERSON & CORROON
BY: WILLIAM J. MARSDEN, JR., ESQ.

Counsel for Plaintiffs

Official Court Reporters

doctors and scientists interested in doing if they could put out these stem cells?

A. Well, several things. As I'd said, one thing would be to, on the fundamental level, to study these cells. Every time we learn something fundamentally about a cell type, we learn something practical about better diagnosis treatment or prevention for example how leukemia occurs. But another very quick application of this would be to be able to, if you could flag these cells, you could probably flag some types of leukemias. So this would have an application probably in leukemia diagnostics.

A lot of the other antibodies that had already been made to T cells and B cells, for example, had such an application. And then the third thing is what you've heard about a lot already, that these are the cells which you need to transplant, which you do a bone marrow transplant. These are the active cells in the marrow. So this could enable a transplant of purified stem cells instead of just bone marrow.

Q. And in addition to bone marrow transplant, were there other possible clinical uses of purified stem cells?

A. Sure. One use would be for purifying the cells from the blood, not just from the bone marrow or from other tissues, and once you've heard about classical bone marrow transplants where you give very high doses of

chemotherapy and radiation therapy to kill the cancer and, as a consequence of that, wiping out the immune system and the bone marrow factory systems, the hematopoiesis system of the individual, and then rescuing that individual from the toxicity by giving the bone marrow or the purified stem cells nowadays but there are other applications in what we call gene therapy.

For example, sickle cell anemia is a pretty common disease and that disease has a defect only in the red blood cells on that picture on the extreme right.

If you gave somebody corrected cells, stem cells had that corrected by gene therapy that you could take that patient and convert that patient theoretically into a normal individual if you could get the stem cells, the fixed stem cells by gene therapy to function in that individual.

Q. Thank you.

And could you tell us the time period of the -- the time period in which you were doing the work that led up to the CD34 antigen discovery?

A. Well, I started that work in around 1981. A couple of years after starting my lab, as I said before I started off working on the granulocytes and moved to the stem cells when I got the courage to approach this needle in the haystack problem. And in the couple of years

subsequent to that, we did the studies which really characterized the expression and the importance and other details of this molecule that was affectionately called My-10 and later a group of scientists called CD34.

Q. Could you tell us something about the tests that you did that led to the conclusion that you had found what you hoped that you would find?

THE WITNESS: Your Honor, may I go to the chart again?

THE COURT: All right.

THE WITNESS: Well, how would we know we had a stem cell if when we got it we didn't know what it looked like? We had kind of a circular problem, so we made -- we made all the hypotheses and reasonable guesses we could. The first thing was if we made such an antibody, it shouldn't bind to any or very many cells in the blood, shouldn't bind to all these types of cells.

So one of the first things we did was does this antibody stain any cells in the blood? And we looked very carefully and it didn't stain any beyond the level of background stain in the blood. That got us interested.

We then said, Well if it's an antibody which binds selectively to stem cells, not to everything else, then it should only bind to a small percent of cells in

the marrow. Remember I said that these cells are about 1 percent of the marrow. Well, when we tested 50 marrows from normal donors, we found that 1.5 percent of the cells were positive with this antibody, were CD34-positive. So then we took those cells and we not only flagged them, but we hooked them and looked at them under the microscope.

And remember I said earlier that you could tell crudely whether a cell was immature or mature under the microscope. Among these babies, infants and toddler in the bone marrow, you could make out the earlier cells. And these were -- this was an enrichment of the earlier cells in this 1 percent.

Then it got really exciting when we did what we called colony forming assays. Those are tests for these stem and progenitor cells and what we found was that the, that the antibody purified in that small 1 percent, all the cells that were capable of forming later red blood cells, all the cells that were capable of forming white blood cells with granulocyte type and all the blood cells that would be pictured up here that were capable of forming multiple types of colonies, so they were less than -- so despite the fact this was only 1 percent of the marrow, roughly, it had all of the colony forming power in the marrow. So if we do this line horizontally, we would think maybe this antibody would

bind to all these cells.

There were no colony assays for those, but there was another way we could test called a TBF test which showed these cells at this early stage were CD-34-positive.

Q. Now, Dr. Civin, I just want to interrupt. Could you tell with the jury what you mean by CD34-positive?

A. What I meant -- what I mean is that, when we used CD34 to purify the cells. That these cells were the cells that were selected, were purified were hooked, and then reeled in by any one of a number of techniques with the CD34 antibody, they were not in the CD34-negative negative area, so once we had this hook we could use a tool, one tool we used among many available was a flow eye Tom meter or FACS machine -- a FACS machine different from the one that you send a page on, but a laser machine where we could tell cells that were florescent and it's easy to put various tags on antibodies when you connect florescent tags.

So the cell, if stained with the antibody, our CD34-positive stain, would shine green fluorescence. Shine a light on it, it shows green. And that shows the green cells from the unlabeled CD34-negative and later found mature cells. So we do that and found these populations there.

The other thing we did was we tested each of these populations with the fluorescence activated sort of FACS machine and showed these mature cells did not even label. Not only were they not hooked, but the most sensitive tests showed they did not even label with the other cells, with the CD34 antibodies. So at this point we were kind of at the "Eureka" point where the light bulb goes off and here we said, It looks like we have an antibody that binds to all of these progenitor cells and possibly to the stem cells.

And, in fact, what we found was the earlier progenitor cells we could test, the greater the level of expression. And this argument led us to conclude that it was very likely and then proved that at least that the pluripotent stem cell was CD34-positive as well, these mature cells. That was a point that we felt that it was time to write a paper and apply for a patent.

Q. Now you've made several references to antibodies and monoclonal antibodies. Could you give us some sense as to what the technique is that you are referring to when you talk about using monoclonal antibodies?

A. Sure. Well, an antibody is part of our body's defense system. Our lymphocytes over there, the B lymphocytes make B with help with the T lymphocytes. For example, when we get an immunization or an inoculation

and worked on that project.

Q. Would you turn to Exhibit 24?

A. Yes, I have it.

Q. In Footnote 1, on the first page, you see once again there, there is a reference to two NIH grants?

A. Yes.

Q. And one of them is this grant to Dr. Strauss, who was a post-doc in your lab; correct?

A. It wasn't a grant to Dr. Strauss. It was a grant to Albert Owens, who was the principal investigator and used part of that grant to support the training of Dr. Louis Strauss.

Q. When you first came to Hopkins, did you receive any NIH grant support, not directly, but through someone else as Dr. Strauss did here?

MR. WARE: Objection. 401.

THE COURT: Overruled.

THE WITNESS: I think this is the CA06973 that provided some support for me and my laboratory. That grant, part of its purpose is to foster Cancer Center development.

So part of its purpose at that time, I believe, was to help start up young investigators. I don't remember how much and what percent of the start-up that provided. But it was a minor percent.

BY MR. BLOOMBERG:

Q. Would that have been available approximately when you joined Hopkins?

A. I don't know if it was used during that -- during, when I first came to Hopkins.

Q. But it would have been available to you?

A. It would have been available to Dr. Owens.

Q. Just as Dr. Strauss was able to use the grant moneys from Dr. Owens?

A. There are currently 70 faculty and I don't know how many post-doctoral Fellows at our Cancer Center. The principal investigator of the grant will decide which young investigators and which post-doctoral Fellows will be supported.

I don't recall right now the years that I was supported. But I did receive some start-up funding from that. I had forgotten about that.

Q. When you say from that, you are referring to from that NIH grant?

A. From the NIH grant, yes.

MR. BLOOMBERG: No further questions, your Honor.

THE COURT: Redirect.