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R V ANDRE CHAD PARENZEE

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RESUMING 10.08 A.M.

MS MCDONALD RECALLS

+DAVID LLEWELYN GORDON ON FORMER OATH

+FURTHER EXAMINATION BY MS MCDONALD

Q. Since you were last in court, you have provided a 2-page letter, or report, addressed to Mr Borick, in response for a request that you do that.

A. That's correct.

Q. What I would like you to do is talk us through that document, explaining in simple lay terms what it means.

A. The question here was: was HIV proteins part of normal human proteins? If that was the case, you would be able to search the human genetic database and protein database and you would obviously find those proteins in it. What we have done here is to utilise a program called BLAST, which means Basic Local Alignment Search Tool. What this does is you submit a query sequence, and this can be a DNA sequence which is made up of basically four nucleotides A, C, G or T and that is basically the genetic code. Every three of those nucleotides make up a specific protein, so you can search nucleotide databases and also search protein databases, or if you have the nucleotide, you can convert the nucleotide database to a protein. You can do all sorts of comparisons with this database. Perhaps I'll give a brief analogy. It is like; if you had someone's fingerprint and you want to see if the fingerprint was the same as another that had been detected somewhere, so you would take someone's fingerprint and search it against hundreds of thousands

of other fingerprints until you find the correct one. There would be some fingerprints probably that would have a bit of similarity - they might have a whirl or a turn in the fingerprint at one particular point, but they may still be quite different. What we have done here is to utilise the database from the National Center for Biotechnology information which is a standard gene bank database. From that you can take out either a partial or a full-length HIV sequence and that is your search query, and then you blast it against all the genetic sequences that are out there. You could blast it against human sequence, various animal sequences or just absolutely everything that is out there. It is quite a complicated program. It will not only pick up identical sequences and say 'we have found that piece of sequence in the human database', it will also detect sequences in which there is a deletion. So there might be a bit of a sequence there but a deletion and then another bit of sequence and it will find those. It is basically a very sophisticated way of finding really anything in the genetic database that has similarity to the query sequence that you're putting in. What we first did was to take out to first access, the entire HIV sequence -

Q. I might get you to pause there, have you brought to court today something to assist in explaining the entire HIV sequence that you used and focused in on a particular area.

A. Yes, I think that is about to be tendered.

MS MCDONALD: I tender that.

MR BORICK: I have seen that, I have no objection.

A. This database has 9,181 nucleotides in the sequence.

EXHIBIT #P92 ENTIRE HIV SEQUENCE TENDERED BY MS MCDONALD. ADMITTED.

A. In the first instance, we took the region of that sequence that encodes what is called the gag gene of the HIV virus and that includes - the reason we did that is

because that includes the p24 protein, along with some other proteins as well. That was one of the contentions, that the p24 protein was a protein that was part of normal human tissue. We then BLASTed that against a human genome database. This genetic sequence includes the entire human genome, which has now been well described, to see if there is any identity or similarity between the HIV sequence and the sequence in the normal human genome. It has actually been BLASTed against a number of genomes and partial genomes.

Q. Can you refer to 7,067.

A. Yes, partially. The result of that was that there were no significant areas of similarity found.

Q. Pausing there, referring back to the full genome that has just been tendered, P92, you said in your report that the particular area you focused in on was from nucleotide 336-1838.

A. Yes, that encodes the gag - that is the gag gene which encodes the p24 protein.

Q. If we look at the whole of the genome, there is a line commencing 301 and you have put a little marking underneath the letters 'ATG'; do you see that.

A. Yes. That is basically where the protein - what is called the protein translation begins at that site. Some of the genetic sequence upstream of that is involved in regulation of the replication, so it is basically the beginning of the protein, where the protein begins.

Q. About nucleotide 336.

A. Yes, that is about right. There was no similarity found between that consequence and the human genome database. The next thing we did was to BLAST against what is called the human translated database. Translation just means the protein sequence that is derived from a particular DNA sequence. What we found in that situation was that there were a number of proteins that had some similarity to the gag proteins and the most significant was a particular protein, which I don't know

much about, called Human Prokineticin Receptor 2. What this program does, is it will give you the results in order of significance, or in order of similarity. This was the one that came up as the most similar protein to the gag gene proteins. That had a region of similarity in a portion of the protein. The gag protein sequence we BLASTed was about 500 proteins long and there was a region of 156 proteins - so you start off with a big protein like that (INDICATES), there's a region of 156 proteins and within that sub-region of the protein, there was a 35% similarity, or 35% identity, in that particular stretch. That means, when you were looking at the identity to the entire protein, it is going to be less than that because the identity - it is probably around about 11% or so, total identity of the entire protein. This is not particularly unexpected but this is still a very low level of identity. My conclusion from that is that HIV proteins are not part of the normal human genetic make-up. Basically the same thing with the full HIV genome, rather than just the partial one, but basically the results are exactly the same; that the DNA level, there is no similarity detected and if you look at the whole comparison, there was some relationship to some proteins called zinc finger proteins. The overall similarity was fairly similar to what we found with the partial one.

CONTINUED

Basically, the bottom line is there are small regions within proteins that have some similarity to HIV proteins, but they are clearly distinct and the maximum amount of identity is about 30% or so in small regions of the protein.

Q. Yesterday, it was suggested by my learned friend, Mr Borick, that, in your report, when you've used the word 'indicates' - I'll just take you to the last paragraph - 'This analysis indicates that there are no HIV proteins present in normal human tissue', it was suggested that the use of the word 'indicates' by you meant that that was less than certain.

A. I wouldn't interpret it as that. It is conclusive evidence.

+FURTHER CROSS-EXAMINATION BY MR BORICK

Q. Some of my questions might be a bit awkward because I'm finding this topic very difficult, but I just want to make sure I understand as best I can. In relation to your letter which was sent to me and then sent on to the others - we call it P85 - as I understand it, the work you did was in two stages. In the initial process, you were looking for one viral protein, p24.

A. It is actually a bit larger than that. It is actually what is called the gag gene. The gag gene encodes for a number of proteins, but they include p24.

Q. About 1,500 bases were used, that is the nucleotides 336 to 1838, which represents about 16% of the whole; is that correct.

A. The whole genome is about 9,100.

Q. We were working on 9,500, but roughly 16 to 20% of the whole.

A. Yes.

Q. Is it correct to say that you were only looking with regard to one viral protein, p24, that is what you were really looking for.

A. In the original letter we have compared the gag gene. The gag gene encodes for a number of proteins, including p24, but also some other ones; p16, p6, I think. There

was a focus of p24 in previous discussions.

Q. Your finding was that 'There is no significant similarity in a comparison of the 1,500 bases with the human genome sequences'.

A. That's correct.

Q. This shows that there is, in human genomes, no nucleic acid sequence that is the same as the viral sequence in that particular range, 336 to 1838.

A. Yes.

Q. This would mean that human genomes could not code for exactly the same protein as that found in the virus.

A. That's right.

Q. That was the first stage.

A. Yes.

Q. The second stage was comparison of the 'viral' protein amino acid sequence with human material.

A. Yes.

Q. Your finding was 'There is a number of human proteins that have some hemology (similarity of sequence) with some parts of the viral protein. The most significant similarity was about 11%'.

A. That's right.

Q. Does this mean that, whilst there is no protein in normal human tissue which is exactly the same as the viral protein p24, there are some human proteins which have some degree of similarity with parts of the viral protein.

A. They have some similarity in terms of their protein sequence. That amount of similarity is very, very low. If you are looking at comparing closely related proteins, they may have similarities of 95 or 100%. So, in fact, having 35% similarity over a very small region of the protein is an enormous difference. The similarity between humans and primates is about 99%. We are clearly distinct from primates.

Q. Doesn't your finding show that there is at least some potential for cross-reaction with some human proteins with some antibodies to viral p24.

A. The analysis is not really looking at that, it doesn't really support that or refute that. It is not really looking at that issue.

HIS HONOUR

Q. It is an apples and oranges question.

A. As I said, similarity between two proteins of 11% is actually a very, very small amount of similarity. It would depend very much on how that particular protein is folded and the structure of the protein. The bottom line is that is an enormous difference between a protein.

XXN

Q. The question is - I've heard your answer: is there some potential for some cross-reaction with some human proteins with some antibodies to viral p24. That is my question.

A. I can't determine that from this analysis. This analysis is not looking at protein folding and protein structure and the crystal structure of protein.

Q. I want to refer you to some evidence which Professor French gave on this topic. I will read it to you - it is not long - then I would like you to comment on it. It is at p.808.

HIS HONOUR: You will have to read it because I haven't got my evidence with me. I haven't got my computer at the moment. If you want me to put it up, I will.

MR BORICK: It won't take me long.

XXN

Q. Professor French said, at line 21 on p.808, 'What we detect when we detect antibodies to p24 in the serum is what we call polyclonal antibodies and this is a mixture of antibodies that reacts with lots of different regions on the protein'. I jump a few lines to 34. 'The serum of people with HIV reacts with many different parts of the protein, not with just a small sequence or small part of immuno acids. It is not surprising that you see cross-reactivity of antibodies to parts of p24 with

other proteins. It is not surprising at all'. Going to p.809, I asked him some questions, then I said 'Breaking it down' -

OBJECTION: MS MCDONALD OBJECTS

MS MCDONALD: My learned friend has just missed out a question and answer that is fundamental to this part of Professor French's evidence which refers to the totality of the antibody response.

XXN

Q. I said 'What you are looking for is the totality, the combined effect. A. The totality of the antibody response. When we're talking about monoclonal antibodies and antibodies against small peptides, it is irrelevant to the discussion because that is not what we detect in the Western blot. We detect polyclonal antibodies to the whole protein. Q. Breaking it down for us, what is it you are detecting when you are looking for those - A. P24 antibodies? Q. Yes. A. We are looking for a mixture of antibodies to different parts of the - so, we talk about an antibody to p24 protein but that is a misrepresentation of the situation because it isn't one type of antibody, it is in fact many types of antibodies - a mixture of antibodies that is reacting with different parts of the protein, but only the protein. Each individual part is what we call an immunology epitope and each antigen on a protein can have a number of different epitopes. You are not detecting one type of antibody, you are detecting a mixture of antibodies. Arguments about the non-specificity of monoclonal antibodies are irrelevant because a monoclonal antibody reacts with only one of those hundreds of epitopes. Q. That is why the test is so specific. A. Yes.' First of all, could you comment on Professor French's description. Is there anything you disagree with, any point you want to make about it.

A. I think the point I make is antibodies recognise a shape. A protein is folded into a shape. The antibodies don't recognise necessarily the immuno acid

sequence. Basically, at the end of the day, the protein is folded into a complex shape, and there might be a little bit of the protein sticking out, or making a big loop, and that is what he really means when talking about epitopes. So, there are parts of the protein that are particularly recognised or important in a region to which antibodies develop. So, in nature, each protein has a unique shape. The antibody response will be directed against particular shapes or particular regions of the protein. It won't be solely recognised against, perhaps, one fold in the protein. There are lots of other folds or lots of other regions in the shape of the protein that determine whether that particular region of the protein raises an antibody or not. It will also be relative. There will be some regions in which the antibody binds strongly, other regions where the antibody binds weakly. If there is a very strong reaction, say against the envelope protein of the HIV virus, there will be a very strong antibody reaction with that particular shape. Now, there will be other proteins that might have shapes that are a little bit like that and there will be, or there is, a potential for a small amount of cross-reaction, if it sees a shape that looks similar. We know that can get cross-reactions between antibodies, particularly what is called polyclonal antibodies. The difference with the cross-reactions is that the reaction is usually much weaker, so it is an incidental sort of accidental cross-reaction. The immune system is really designed so that there is very little cross-reaction, or the cross-reactions that occur are very weak, because if the immune system couldn't do that, then we would get into all sorts of problems because our immune system would keep making antibodies against normal human tissues. I'm not quite sure the point you are making. This whole thing, I guess, relates to issues we discussed previously; that you can get weak reactions in the antibody tests.

HIS HONOUR

Q. That is a different question to one relating to genetic sequence.

A. Yes, it is totally different.

Q. We are talking about apples when we are talking about antibody tests and oranges when we are talking about the sequence of the genome.

A. I think so. I can't see any relationship between that issue and the analysis of the genome.

XXN

Q. When Professor French said 'So we talk about an antibody to p24 protein, but that is a misrepresentation of the situation because it isn't one type of antibody, it is in fact many types of antibodies, a mixture of antibodies that is reacting with different parts of the protein', do you agree with what Professor French has said there.

A. I don't have the full transcript and the contents. Essentially it is the same thing I was trying to say; if you have a protein, it has a particular shape and you develop antibodies against a number of regions in that protein in most cases. The effect of that on the protein will sometimes vary. There are some antibodies that will, for example, block the actual virus infection, and then there are other antibodies that will bind to a region close by, but they may not actually be able to block infection. So, we know that all proteins have a number of regions, or epitopes, to which antibodies develop to greater or lesser degrees.

Q. When Professor French says 'It is not surprising that you see cross-reactivity of antibodies to parts of p24 with other proteins, it is not surprising at all', you would agree with him.

A. The immune system is designed to be as specific as it possibly can, it doesn't want too much cross-reaction, but if you have all the proteins in the body and all the different shapes that all those different proteins make, then it certainly is possible that an antibody against a

particular shape will have some weak cross-reaction with a shape that might be quite similar in a particular region of that protein.

Q. I just want to be clear. When Professor French said 'It is not surprising that you see cross-reactivity of antibodies to parts of p24 with other proteins, it is not surprising at all', do you agree with him.

A. It is not surprising there are weak cross-reactions with other proteins.

NO RE-EXAMINATION

NO FURTHER QUESTIONS

WITNESS RELEASED

+THE WITNESS WITHDREW