



# Memorandum

September 18, 1985

Chief, Laboratory of Tumor Cell Biology, DTP, DCT, NCI

Subject Response to Dr. Fischinger's memo of September 10, 1985 on "HTLV-III Patents"

To Associate Director, NCI

- I. You ask: Since we found new viruses in November-December 1982, why didn't we mention this fact in our many ensuing publications over the next year? There were not many such publications. The premise is wrong. There were only two publications on this subject. As we have tried to make clear many times, there were no reagents to HTLV-III/LAV at that time because the virus could not be mass-produced by anyone then. We found HTLV-I or an HTLV-I-like virus a few times (two of 33 attempts). Since we had reagents for the virus, we could define it. We could not, however, be certain that the HTLV-I was not a minor variant (rather than HTLV-I itself) and hence could be the cause of AIDS. If, on the other hand, it turned out to be HTLV-I itself (as we subsequently learned from detailed molecular characterization) it was still important to note that this leukemia-causing virus was being spread by this same group. In fact, we did not believe that we should publish marginal data of a reverse transcriptase-containing particle in a few patients, uncharacterized and not yet linked to the cause of AIDS. Obviously, the Pasteur group's report of virus in one case in a man with lymph node enlargement and no evidence of AIDS was simply that--a case report--and without any detailed viral characterization and no viral-specific reagents. They, too, in the ensuing months detected this virus only sporadically. It was not until our November 1983 breakthrough on mass production of HTLV-III were any specific reagents made enabling us to link the virus to the cause of AIDS. Please remember, we isolated HTLV-I in 1978 but did not say a word about it until late 1980; i.e., until it was characterized and we had data in hand linking it to the cause of human T-cell leukemia. I have followed this approach to human biomedical studies since observing the lessons of past experiences from the opposite approach. What good would it do me or the field to slip in a few sentences that another retrovirus is occasionally detected (at that time it was only occasional) and that it is not HTLV-I or II; but we have no evidence that each time this new virus is detected it is one and the same virus, i.e., this could have been an HTLV-III in patient one, no virus detected in patient two, three, four, five, six . . . and when detected again in, say, patient seven it could have been an HTLV-IV, i.e., not the same as HTLV-III and only an opportunistic infection. Proper specific viral reagents were, in my mind, required to establish the identity of what was believed to be a new virus.

Regarding the flow of continuity of my thinking and our experiments: I proposed in early 1982 that the cause of AIDS was most likely a human T-lymphotropic retrovirus; i.e., an HTLV. I headed a group to find this by the fall of 1982. My idea was to look for any and all T4 lymphotropic retroviruses in these patients. The first idea was: could it be a variant of HTLV-I or HTLV-II? By March 1983 or thereabout we had one HTLV-II and a few HTLV-I isolates from AIDS cases. Alternately, these could have been minor variants of these viruses and, as such, could have been the cause of AIDS.

By this time we also had, as you know, a few retroviruses not reactive with HTLV-I or HTLV-II probes (later proven to be HTLV-III). We couldn't propagate these at the time so no specific reagents were available. Therefore, we stored these and we continued a two-prong simultaneous approach. First, to molecularly characterize the few HTLV-I/II viruses to see if they were variants that could be AIDS causative; and second, to continue to try to find a way to grow the new retroviruses. Remember, at this stage (March 1983 to the fall of 1983) the Pasteur group had one claim of one virus and they reported that it was significantly cross-reactive with HTLV-I (see their one and only paper in 1983 in Science). Remember also that Max Essex, Chairman of the Department of Cancer Cell Biology at the Harvard School of Public Health, also published in May 1983. In fact, his data was really the first claim to link a retrovirus to AIDS (serology of many cases) and he used HTLV-I as the antigen. No one knew then whether, in fact, the disease was caused by a retrovirus: whether it was a close variant of HTLV-I, a moderate variant, or a very different T4 lymphotropic retrovirus. So, to answer your question, of course we did not abandon the search for a non-HTLV-I agent between December 1982 and June 1983. In fact, the search was intensified. It was at that very time that I asked Zaki Salahuddin and Phil Markham in our group to join in this search and for them to be the ones pushing the search for the retroviruses not so related to HTLV-I. (See enclosed data sheets.) It should be noted that at no point did either Dr. Essex or I say that the prototype HTLV-I was likely to be the cause of AIDS. In fact, both groups emphatically stated that it was a variant strain of a human T-lymphotropic virus.

You ask about the thinking on the development of the HTLV-III ELISA testing. Obviously, this is a standard procedure and requires no special invention once the virus is mass produced. We did our first test in December 1983 with bona fide mass-produced HTLV-III. This was then sent to two contractors. Naturally, this is directly related to the test of other human retroviruses; i.e., HTLV-I or HTLV-II. Obviously, even if the name HTLV-III is not used it is still a human T4 tropic retrovirus. The ELISA for human retroviruses was developed in my laboratory in the 1979-1980 period by Dr. Marjorie Robert-Guroff, Dr. Larry Posner, and myself and published in the Journal of Experimental Medicine in 1981. A reprint is enclosed. Although this is not with HTLV-III, the technique is, of course, basically the same. We did not test sera by ELISA with non-mass-produced viruses because quite obviously the results would be crappy. The Pasteur group elected to do so; i.e., they used the few virus particles transiently released on the dying primary blood T-cells and that is why they got such inconclusive results.

Note that in November 1983 at the Seillac, France meeting on human tumor viruses Montagnier reported only 20% sera of AIDS reacted. Their patent of September 1983 reports the same. Naturally, Biotech did use our input and some of our technology in their patent of August 25, 1983, which to my understanding is a generic patent for human retroviruses.

- IV. You state some researchers claim that the first clear linkage of LAV (HTLV-III) to AIDS was accomplished by Dr. Chermann at the Park City, Utah meeting in February 1984 rather than by me (and my colleagues) in the May 1984 series of papers in Science.

Before responding to this, I urge you to send copies of my reply (or its essence) on this point to those researchers. The reply is as follows:

- a. Some researchers, and especially those who publish in non-reviewed places, e.g., MMWR, are of the impression that stating something is the same as publishing in a reviewed scientific journal. It is not. It is as if they have no experience in established scientific documentation.
- b. Even if stating something is acceptable as a claim of priority, how is fairness established when we go through the time and effort of writing the papers, submitting, getting the reviews, revising, resubmitting, and then waiting for the publication to come out? Obviously, what is published in early May didn't fall into our hands in early May.
- c. Even if the above were not the case, I challenge whether Chermann showed this. First of all, it is true his data improved a lot (probably with the help of Kalyanaraman who, as you know, was hired from our group by CDC and sent to Paris at that time). However, even so, by then Chermann reported only about 40% sera positivity in AIDS or less. My role at this meeting was chairman and overview lecturer. I gave no details and did not wish to do so because our papers were being prepared for publication, and our data was not yet in the hands of Dr. DeVita or Dr. Wyngaarden. I feared press commentaries and/or an MMWR type of release. I think I acted judiciously.
- d. Even if the above three points did not matter, the conclusion would still be erroneous since 1) I had already lectured at the Pasteur Institute in Paris in January 1984 and told a very extensive audience I was sure we had the cause of AIDS by numerous virus isolates plus wide seroepidemiology with 90 to 100% linkage to ARC and/or AIDS depending on which of many already completed studies we quoted. In a private meeting with Drs. Chermann and Montagnier I gave many of these details. 2) Also in January 1984 I called Jim Curran and requested a large panel of CDC sera (AIDS, ARC, normal, etc.) to be sent to me "blind" (all our testing was and still is done with coded sera). I told him then I was sure we had the cause of AIDS and that it might be the virus identified in the lymph node patient by the Montagnier group. Obviously, to tell Jim Curran this meant that we already had the data and simply wanted to convince him. Sera were sent some weeks later. At the beginning of March 1984 Jim Curran, my collaborator Dr. Sarngadharan, and myself met for lunch at La Miche

in Bethesda. The code was broken and verified our conviction. It was also at this time that I let NCI officials know: a) the etiology was conclusively solved and b) we had developed a real blood test for this virus. No one in the world ever made these claims before this time.

- Y. Your next point (#5) has to do with the relationship of the prototype HTLV-III (HTLV-III B) and LAV and the unfortunate innuendo. Our laboratory had multiple isolates from the beginning and we were the first to discover heterogeneity among isolates from analyses of the genomes of some of these (published first, in fact, in 1984 from data originated in December 1983). The Science paper we recently published (Wong-Staal, et al 1985) contained two pieces of information that are extremely relevant to the points raised by this scientist.
- a. Although the 18 isolates obtained in our laboratory and examined in that study were distinguishable from each other, two isolates (MN and SL) obtained from the same geographical area around the same time were very closely related differing in a single restriction enzyme site. We now have nucleotide sequence data that indicate MN and SL are as closely related (if not more so) as LAV and the prototype HTLV-III called HTLV-III B or clone BH10.
  - b. Scientists at the University of Paris have recently obtained evidence that at least three independent isolates they have made from Zairian patients are very closely related. (To date, one or no restriction site differences with use of multiple restriction endonucleases.) These data indicate that some Zairian HTLV-III/LAV isolates are as close or closer to each other than prototype HTLV-III (HTLV-III-B) and LAV.
  - c. Scientists at Chiron Laboratories have recently made special probes and assessed relatedness among various isolates of these retroviruses. Included in these analyses were characterization of many new isolates. The conclusion: there are several new isolates very similar to LAV and the first prototype HTLV-III (HTLV-III-B).
  - d. In two instances, we had observed more than one genotype in a single patient. Further analyses suggest that the second component is closely related to the first, probably reflecting heterogeneity generated in vivo. The two forms in LAV may be explained in a similar fashion. The H9/HTLV-III<sub>B</sub> cell line is more complex because virus from ten different patients had been put in, and it is hard to know which or how many viruses actually took. But if the different components (there are at least four, see Shaw, et al Science and more likely six) also represent polymorphic variants generated in vivo, they are obviously expected to be related to each other. If LAV and HTLV-III<sub>B</sub> are similar because they were derived at the same period of time from New York at a time only shortly after the virus entered the U.S., then all the polymorphic variants would also be expected to be highly related. We were surprised that this scientist can say the two secondary components in LAV and HTLV-III<sub>B</sub> are identical, based on a single polymorphic Hind III site. That accounts for only six nucleotides \*of the 10 Kb genome. It is in my opinion inaccurate to call something

\* = 10,000 nucleotides

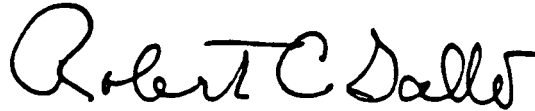
a "Hind III variant". In the same Science paper, we showed that there were only three SST I genotypes, even though all 18 viruses reported (over 25 now analysed) can be distinguished!

- e. There is also evidence that the heterogeneity that is seen by us may be an over-estimate of what can be propagated long-term. HTLV-III capable of being mass propagated in H9 cells or CEM appears to be limited to some strains. This is our data and independent agreement of L. Montagnier (personal communication) and of D. Zagury, University of Paris.
- f. We received an extraordinarily small amount (11,000 CPM reverse transcriptase) of Montagnier's virus September 24, 1983. Mika developed the clone H9 in early November 1983. Can anyone possibly imagine mass production of this amount of virus in five weeks? Further, can anyone possibly imagine 150 nucleotide changes, deletions, and additions coming into a genome in six weeks of culture!
- g. As an alternative interpretation of point 4: There are several proviruses in H9/HTLV-III B cell line. We stated in our paper we used samples from several patients. Montagnier used only one patient. When analyzing virus from a single patient at any one fixed time we found only one form. How did Montagnier get more than one form?
- h. Finally, we isolated, mass produced in H9 cells, patented and published on a major variant HTLV-III-RF (Haitian isolate), very different from LAV, at exactly the same time, making all this crap irrelevant. In addition, last month (August 1985) we published in the Proceedings of the U.S. National Academy of Science on one hundred and one different isolates of HTLV-III/LAV. This paper was submitted for publication six months ago. Now the number of isolates approaches 200. (See Enclosure 1)

I have also learned that a scientist states we were pushing the "AIDS Virus" as closely related to HTLV-I. This is, of course, a misunderstanding of the history or a misrepresentation of the facts.

1. In the May 1983 papers Montagnier and co-workers stated their virus was an HTLV. They showed a cross-reaction with HTLV-I. Clearly, the virus was a human T-lymphotropic virus, as they said. As I have already indicated, they were able to suggest it was probably a new virus because I sent them all the necessary HTLV-I and HTLV-II reagents. This is recognized in the acknowledgement in their paper.
2. We reported on a few cases of an HTLV-I-related virus via molecular hybridization. As indicated earlier in this and in other memorandums, we also had evidence of retroviruses other than HTLV-I or -II; but for numerous important reasons in the absence of their proper characterization, we did not publish these until we solved the problem of mass production of these viruses. Everyone in our ad hoc advisory group knew this and most recommended against our publishing on these at that time. What we published in 1983 we did not claim HTLV-I as the etiological agent. Doesn't the scientist know this? The point was they could be well characterized (HTLV-I reagents were available), they could have been minor variants of HTLV-I which caused AIDS, or they could have been opportunistic infections and this was important to note because they are spreading and they cause leukemia. That is how our paper reads.

3. NOTE: That once we characterized HTLV-III (by December 1983), we never claimed anywhere that it was closely related to HTLV-I. The only claim for this in 1984-1985 is, in fact, ironically from Montagnier and his co-workers in a July 1984 Science paper in collaboration with CDC. (See Enclosures 2 and 3.)



Robert C. Gallo, M.D.

Enclosures

- xc Dr. Chabner  
Dr. DeVita  
Dr. Fauci  
Dr. Wyngaarden

.