

June 2, 1972

Dr. Lloyd J. Old
Sloan-Kettering Institute
410 East 68th Street
New York City, New York 10021

Dear Dr. Old:

Bob Nowinski called today to tell me of your kind offer to collaborate with us in studies of base sequence homologies between nucleic acids from human breast tumors and from Mu MTV. As Bob probably told you, we have been measuring rather precisely the amount of MuMTV-specific RNA in a variety of mouse tissues, in an effort to describe the degree of transcriptional regulation of the large amount of MuMTV DNA found in both GR and C57Bl strains (see enclosed preprint). The mouse studies indicated that we could anneal all of a single stranded DNA probe made by MuMTV polymerase to RNA from mammary tumors, virus-producing mammary glands, and lactating mammary glands from C57 mice. Annealing was demonstrated by assay with a single-strand-specific DNA nuclease and in CsSO₄ gradients. Incomplete annealing was obtained with spleen RNA. About .5% of RNA in the virus-producing tissues is MuMTV-specific, about .01% in the C57LNG is MuMTV-specific, and less than .001% in the spleens in viral-specific.

In view of the high sensitivity ^{and} stringency of our hybridization and assay procedures, we decided to try to confirm Spiegelman's results with human breast tissue. To our frank surprise, the first breast tumor sent to us by Nurul Sarkar contained 0.01% MuMTV-like RNA. We have been able to anneal 100% of our single-stranded DNA probe from MuMTV to this RNA as tested with both nuclease and CsSO₄ assays. In addition, we have studied the melting properties of this hybrid and find that it melts sharply with a T_m 4°C below the T_m for the presumably completely specific hybrid formed between the same probe and RNA from a virus-producing mouse tumor. This suggests the differences in base sequence between the probe and the RNA it is detecting are about 6%.

We have tested several other breast specimens; all normal breasts and benign tumors have been negative, but the 4 tumors we have subsequently tested have also been negative or questionable. The problem at this point is principally that the amount of tumor tissue in these specimens tends to be small; consequently we get little RNA (which may be contaminated by RNA from adjacent normal cells) and cannot push the level of detection to the limits the assay itself can bear. Therefore we asked Bob to approach you about supplying us

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with tumors (metastatic or primary). The suggestion that we examine your established human breast tumor lines is a very appealing one.

I will call you next week to discuss the specifics of the amounts of tissues or cells we would need. I am enclosing some recent papers from this lab to give you some details about the techniques involved. (I should mention that we are about to examine human tissues for MuMTV-like DNA and would want to do the same with any tissues you might provide us). In addition, I will be in New York the second week in August and perhaps could get together with you at that time.

Yours truly,

Harold E. Varmus, M.D.
Department of Microbiology

HEV:bb

$\frac{20 \times 10^6}{10^7} \rightarrow 50 \text{ } \delta - 100 \text{ } \delta$
 $10^7 = 50$
 $10^9 = 5 \text{ mg}$