IN THE UNITED STATES DISTRICT COURT
FOR THE WESTERN DISTRICT OF VIRGINIA
ROANOKE DIVISION

BARBARA S. CARTER, Administratrix of the estate of David Howard Carter,
Plaintiffs, Civil Action 88-0172-R
VS.
SHELL OIL COMPANY, et al.,
Defendants.

DREAMA N. RIDDLE,
Administratrix of the estate of Larry D. Riddle, et al.,
Plaintiffs,
Civil Action 88-0505-R
VS.
SHELL OIL COMPANY, et al.,
Defendants.

CATHERINE H. SLUDER, Administratrix of the estate of Edward F. Sluder, et al.,
Plaintiffs, Civil Action 88-0124-R
VS.
SHELL OIL COMPANY, et al.,
Defendants.

DEPOSITION OF RICHARD D. IRONS, M.D.

1290 Broadway Suite 700
Denver, Colorado 80203

August 15, 1990

APPEARANCES

THE LAW OFFICES OF HERSCHEL L. HOBSON, by HERSCHEL L. HOBSON, ESQ., Beaumont, Texas, appearing for the Plaintiffs.

AKIN, GUMP, STRAUSS, HAUER & FELD, by CLIFFORD J. ZATZ, ESQ., Washington, D.C., appearing for the Defendants.

Also Present: Daniel Thau Teitelbaum, M.D.

The deposition of RICHARD D. IRONS, M.D., produced, sworn and examined upon oath on the 15th day of August, 1990, at 10:21 a.m., at 1290 Broadway, Suite 700, City and County of Denver, State of Colorado, before me, Barbara Wishart, a Certified Shorthand Reporter and a Notary Public within and for the City and County of Denver, State of Colorado, pursuant to the Federal Rules of Civil Procedure, for the examination of the said RICHARD D. IRONS, M.D., a witness called for examination by the plaintiffs herein.

I N D E X

Witnesses: Page No.

RICHARD D. IRONS, M.D.

Exhibit No. Marked

RICHARD D. IRONS,
being first duly sworn to state the truth, the whole truth and nothing but the truth, testified on oath as follows:

EXAMINATION

BY MR. HOBSON:

Q. This will go under the rules. Dr. Irons, do you care to read and sign your deposition?

A. Yes.

Q. We'll make those arrangements afterwards.

Would you introduce yourself, please.

A. Richard D. Irons, I-r-o-n-s.

Q. How are you employed, please.

A. I am director of Molecular Toxicology and Environmental Health Sciences for the University of Colorado.

Q. Dr. Irons, I would like to hand you just a sheet of paper here, if I could, and I think you have a pen there with you. Could I ask you to sketch out for me, please, your view of how a human develops leukemia, the steps that the body goes through in the development of leukemia?

A. Leukemia is a whole constellation of

different diseases. To discuss every particular disease and what we know about it is not possible within the context of a single sheet of paper.
Q. Okay. Let's take a leukemic disease, then, that is of concern in this case. Tell me what you understand the disease process that's involved in this case.
A. Well, in the case of chronic myelogenous leukemia, which is the disease that Mr. Riddle had, it has been virtually conclusively demonstrated that it originates in the pluripotential stem cell.
Q. Since we have got a disease entity, then, among this constellation of leukemias, let's stick with CML, and if you would start me through this process of a healthy person developing CML.
A. To the extent that I can, it is a lot easier to work backwards.
Q. Either way, but if you would chart it out for me, please.
A. Okay. What I have sketched here is a schematic of normal blood cell differentiation. If you would like I can use that to describe what we know about CML.
Q. Yes, sir, please.

A. The principle for virtually all leukemias, not necessarily the lymphomas, but for the leukemias, we know that the cell of origin, the target cells reside in precursor compartments that's generally called the stem cell compartment in the bone marrow. In the case of CML we have a great deal of evidence to indicate that this here is the cell of origin.
Q. When you say this here, can we go back and try to figure it out?
A. The pluripotential stem cell.
Q. All right, sir.
A. The evidence for that is really quite impressive. Now the actual cell type that is associated with the disease in the peripheral blood early on in the evolution of the disease is a relatively mature granulocyte, which is going to--this is really a line or continuum of differentiation and maturation that occurs primarily in the bone marrow, the lymphoid series which goes on outside the bone marrow.
But in the case of CML, the principal cell type that is associated early on with the disease is an accumulation or an abundance of cells of relatively mature level of differentiation,

relatively mature for leukemia cells, and they would be at the metamyelocyte level usually, or even some mature cells, the granulocyte series.
So although the target cell for the development of the disease originating cell is back here, the first-
Q. Back here being the?
A. In the pluripotential stem cell compartment, the manifestation that one sees early on in the disease is in the granulocytic series in terms of the cell type that's first described. Now one of the reasons that we know that CML arises at this level-
Q. Being the pluripotential?
A. At the pluripotential stem cell level is that at various times during the disease in some patients one can look for what's basically the heterogeneity of the origin of all the cells in the blood in the lymphoid system, and usually the erythrocyte is the most useful for this. A normal person has erythrocytes produced from many different stem cells, so it is a very heterogeneous population. If, however, they are being produced by single cell, then you will see a monoclonal distribution if you use appropriate markers to look

at it. In other words, you would see that cells are being all produced from a single cell as opposed from many.
We also have chromosomal markers, and in the case of CML the most prominent is the Philadelphia chromosome. At various times during the disease one can see the Philadelphia chromosome appearing in cells of different lineages, including cells in the lymphoid lineage, primarily the B-cell. The significance of that is there is only one cell type that can give rise to both of these, and that's at the level of the pluripotential stem cell.
So the relationship between the cell that causes or is responsible for the development of CML and the actual cells that one sees in the peripheral blood early on is different. In other words, these cells are relatively mature, but the cell of origin is a pluripotential stem cell.
Q. Is there a precursor cell to the pluripotential stem cell in an adult?
A. In an adult, no.
Q. So I guess in laymen's terms we would call the pluripotential cell the father of all the blood cells?
A. Yes. Yes.

Q. Now if you would, tell me--and if you need to make a separate chart we will--how the development of CML as you understand it is different from the development of AML, or acute myelogenous leukemia?
A. Most of the knowledge that we have on both of these model systems comes from work of Phillip Fialkow and his group in Seattle. Some others contributed to it, but that's the primary group that's done most of the work in this area. The acute--you said AML?
Q. That's right.
A. The acute myelogenous leukemia is a leukemia that the cell type that one sees is a relatively--is an immature, it is actually a blast cell, an abnormal blastoid cell, so instead of having relatively mature cells as being the manifestation in the peripheral blood, one has an accumulation of blast cells. So differentiation is blocked at an earlier stage.
The cell of origin in AML has been more difficult to pin down because we don't have the same definitive characteristics that we can look at such as the Philadelphia chromosome and some of the clonal markers that we do for CML. In general the far

majority of cases that have been studied today show an origin either at the multipotential myeloid cell, which is right here, or at a committed level. By multipotential I mean the cell is capable of giving rise to different lineages but has very limited capability for self-renewal. So it is committed to these lineages. It cannot produce B-cells, for instance.
We are talking about production of erythrocytes, granulocytes, platelets, the myeloid series, if you will. Some originate back here. And the reason that we know that is because again looking at clonal markers we occasionally can find involvement of normal--essentially normal erythrocytes precursors when we have frank leukemia myeloid cells present. And so it is obvious that this would have to be the cell of origin.
Q. This being?
A. The myeloid, the committed multipotential myeloid stem cell.
Q. Pronouns will kill us, Doctor, if we don't do this.
A. This is difficult for me to do from a chart, put the words in. Some arise at a later level in which one apparently is looking at cells that are

either committed only to the myeloid or the monocytic line, or I suspect that there are some instances, although at present I can't remember where, where there might be a rare instance of where erythrocytic leukemia actually came from a committed cell, although I think the evidence is clear for the myeloid multipotential cell being near the origin there.
Q. Is there any way that you know to tell whether in the case of AML the stimulating event that actually leads to the production of acute myelogenous leukemia actually occurred in the pluripotential stem cell or the myeloid stem cell, the multipotential myeloid stem cell?
A. In those patients that have been examined, I would say somewhere around 95 percent have been demonstrated to arise at this level. There is a small percentage, a few cases that one cannot say definitively that they arose at this level. There is some evidence they may have arisen in the early stage.
Q. I may not have communicated my question very well. The changes that you observe that you say lead you to conclude that the leukemia originated in the myeloid stern cell, could that change have

actually occurred in the pluripotential stem cell and yet only manifested itself in the myeloid stem cell and not the lymphoid stem cell?
A. To answer that question the way you phrased it would require that one be able to prove a negative, which is really not an appropriate--it's impossible within the context of scientific inquiry. What one can say is that, first of all, in the case of CML, that does arise at pluripotential stem cell, it has been--that has been relatively readily demonstrated and demonstrated, corroborated. In the case of the AMLs there is only--there is very little evidence to suggest that the pluripotential stem cell can in fact be involved in some minor number of cases. And there is considerable evidence linking this as the cell type.
Q. This being the myeloid stem cell?
A. The myeloid stem cell for the far majority of cases. Independent of that, in terms of mechanisms of causation that we know of, there is fairly demonstrative evidence, I would say, extremely strong evidence to suggest that this is the target cell and not the pluripotential stem cell.
Q. You are saying the myeloid stem cell is the target cell, not the pluripotential stem cell?

A. Yes.
Q. When you say the target cell for mechanisms, what kind of mechanisms are you including there?
A. Virtually anything that involves potential alteration of the genetic information within the cell. For potent alkylating agents you are talking about mutation events.
Q. Strong alkylating agents would be like chemicals for chemotherapy?
A. Chemotherapy, chemotherapeutic agents. However, I should state that there are no leukemia models that we have that suggest that there is a single initiating type of paradigm or single event that DNA leads to the development of leukemia. Leukemias tend to be multifactorial in their characteristics. It is clear that multiple events have to occur in order for them to evolve.
MR. HOBSON: Let's take a couple of minutes, because this is very technical. I suspect that our court reporter has some questions about words, and I would like to get those cleared up right now if we could.
(Whereupon, there was a recess.)
Q. (BY MR. HOBSON) I would like to come

back now, Dr. Irons, to the chart you have drawn us that we discussed the myeloid leukemias off of. You have drawn out the cell lineages that lead to those diseases, and we talked just briefly about mechanisms. I would like for you now, if you would, to go back and put in here the different mechanisms that you are aware of that lead to the production of CML and AML.
A. We do not know ultimately what the mechanism is that leads to the development of CML. The Philadelphia chromosome is a reciprocal translocation between chromosome 9 and 22. That translocation involves two gene or gene areas, one, the able proto-onco gene, and the break cluster region associated with 22 that are put close together where they are normally not found. And there is--it's at least tempting to conclude that that influence on the able proto-onco gene is important with respect to at least the progression and development of the disease.
What's not entirely clear is whether the Philadelphia chromosome was causal or not. There is certain evidence to suggest it might not be, even though there is compelling indirect evidence to at least lead one to assume that it plays a role. As to

what other events lead up to that, if in fact there are any, is not clear.
With respect to the acute myelogenous leukemia, certainly in the secondary leukemias, those secondary to drug therapy or in the case of benzene, there is considerable evidence to suggest that aneuploidy may play an important role, although again we don't have a definitive mechanism available for any leukemia. Aneuploidy meaning the selective loss of chromosomes and/or the loss of portions of those chromosomes.
In secondary acute leukemias, AMLs, there is a very high proportion of those have losses of either chromosome 5, chromosome 7, or 5 Q minus 7 Q minus, which would mean a portion of those chromosomes, whether or not initiating events in the form of alkylation mutation play a role in the evolution of these tumors has not been established.
I think there is at least equal if not greater evidence to suggest that these nondisjuctional events are implication errors, play as important if not a more important role in the development of leukemias than any hypotheses involving a direct mutational theory.
Q. Now I want to stick with my game plan.

Do we need to use any of the charts, and the one you have now created, to talk about the lymphocytic leukemias?
A. Not really.
Q. Tell me, if you would, your understanding of the development of the lymphocytic leukemias in man.
A. Let's take ALL because that is of significance with respect to--I have to apologize, the diseases are straight but the names aren't. I think we are talking about Mr. Sluder. The majority of ALLs arise from a committed lymphoid precursor cell, and that could mean either of these two cell types.
Q. Being which ones?
A. Either the Pre T lymphocyte precursor, if you will, or the Pre B lymphocyte precursor, or in some cases the lymphoid stem cell multipotential prelymphoid precursor, if you will. The majority of cases arise in the case of a T-cell leukemia, at least there is a presumption they arise here.
Q. Here being?
A. At the level of the Pre T lymphocyte, committed cell, the T lymphocyte lineage. In the case of Mr. Sluder he presents with the Calla

antigen, or the common lymphoid antigen, which is an antigen expressed on a variety of different cell types not limited to the lymphoid series, but it has been associated with staging those, the degree of differentiation of those tumors. That would lead me to think or suspect that it might have arisen at this level.
Q. Being the lymphoid stem cell?
A. The committed lymphoid stem cell. It is multipotential in that it likely can give rise to both, either B or T. I don't know of any gene arrangement studies on Mr. Sluder, which really eliminates the possibility of coming up with a definitive answer to that.
Q. Now is there a difference in your mind between the chronic lymphocytic leukemia and acute lymphocytic leukemia?
A. The evidence for chronic lymphocytic leukemia to the best of my knowledge is it arises primarily at this stage of a committed Pre B cell.
Q. What's the indication to you that that's the origination of the CLL?
A. Based upon studies using immunoglobulin gene arrangements in those cells.
Q. Is there a difference in your mind

between the lymphomas and the lymphocytic leukemias?
A. Yes.
Q. Explain those differences for me. And if we need to do a similar type of chart to show the development of lymphomas, I would like to do that.
A. Why don't we proceed with this, and if you want me to do another chart, we can do another chart.
Q. Fine.
A. One can get into semantic arguments about a lymphoma or leukemia, especially in animal models where the distinction is often not clear at all. In man one can have lymphomas that may have a leukemic episode and present with cells in the peripheral blood and take on characteristics of a leukemia and vice versa where you get infiltration of tissues from, say, an acute leukemia that begins to invade other tissues which can also include the secondary lymphoid. If it is all right with you, I would just as soon not--I'll try to distinguish between those semantic arguments and issues and lymphomas in general.
Unlike the myeloid compartment of the bone marrow, which is associated with the production of granulocytes, macrophages, monocytes, platelets,

and red blood cells, most of the maturation associated with the normal development of the lymphoid system goes on outside the bone marrow. In the case of B-cells you do have some development going on in the bone marrow, and you have final commitment and terminal differentiation occurring in the peripheral lymph nodes and/or spleen in response to stimuli.
In the case of the T lymphocytes the precursor migrates from the bone marrow to the thymus where it undergoes a similar, although not by any means identical, differentiation process, again terminal differentiation, a functional activity going on in the secondary peripheral lymphoid organs, not in the bone marrow.
In the case of a non-Hodgkin's lymphoma, depending upon whether you are talking about immunoblastic, a B-cell or T-cell, you are talking about different cells, the cell of origin is most probably these cells in the peripheral lymphoid organs. They arise in the lymph nodes or occasionally in the thymus, and they are external to the bone marrow.
Q. These cells being the B-cell or T-cell?
A. Yes. The B and the T-cell are

different from the other cells of the blood and immune system. Arguably the monocyte falls into this category, but these cells are capable of continued proliferation given appropriate stimuli in the periphery.
Q. Again being B-cell and T-cell?
A. These cells are committed to responding to appropriate stimuli and to proliferating and affecting either the production of antibody or cell killing in response to infection. There is a very elaborate network that normally keeps these cells and their proliferation in check, and that is what goes on in the peripheral lymphoid organs. The development of various lymphomas occurs in cells within those organs.
Q. In a person who presents with this, I guess, multiple diagnosis of lymphoma and lymphocytic leukemia where it appears to go back and forth, how do you express what's going on in the body in terms of what's going on in the cells? In other words, identifying the disease versus what's going on in the body, and if it appears like they have lymphocytic leukemia at one time and then they appear they have lymphoma the next, what is the difference that's really going on with the body as you see it?

A. Well, usually the progression of the disease from diagnosis gives you a handle on at least what the architectural site of origin is. In other words, if it arises in a peripheral lymphoid organ as opposed to the bone marrow and there is no involvement on the bone marrow, that's characteristic of a lymphoma, for certain types of lymphomas, the majority of them.
For some types you may have involvement of the bone marrow. Certainly in the case of multiple myeloma it's very likely that the disease arises in the bone marrow, even though it is a disease of committed B-cell precursor.
One also looks for antigens that may help you in deciding what particular category you are going to place it in terms of identifying T-cell and the level of differentiation.
Q. Is it possible in your view for a person who, say, comes in and presents at one time like they have a lymphoma, later looks like they have a lymphocytic leukemia because of the changes you described, really what happened was you just didn't catch the first time that it was looking like a lymphocytic leukemia? In other words, they tend to go back and forth, don't they? They appear like they

are a lymphoma, then a leukemia, then a lymphoma.
How do you know which came first?
A. If you have a biopsy of a peripheral lymph node that demonstrates the characteristics of a lymphoma and you don't have any evidence of bone marrow at that time, you are looking at a leukemia, you are looking at initial presentation of a lymphoma. Whether or not that at some time during its natural history involves the peripheral blood or invades the bone marrow is not necessarily indicative of the origin of the disease.
Q. You are saying, then, that a person who truly has a lymphocytic leukemia will always have bone marrow involvement?
A. Not necessarily always, but it certainly is going to be the predisposing factor you are going to look for. In certain cases that's not--and depending on the specific disease type, the sub-classification, that's not necessarily always true.
Q. Now I would like to go back for a moment and talk about the control mechanisms. You raised multiple myeloma, and I don't want to get to that yet, though I will, and some other diseases before we finish today. Once one of these changes in

one of these cells that leads to one of the leukemias occurs, does that automatically mean that that person will have that disease manifest itself?
A. No, I don't think that's by any means a given. In fact I would say the general sum knowledge in experimental clinical immunology, that's not the case.
Q. And there are reasons biologically that these individual changes don't always lead to disease, correct?
A. Certainly. Most of which we really don't have a clear understanding of. But it is clear there are multiple control mechanisms and the system is designed, to put it very crudely, to provide for multiple points of control over the process.
Q. It's almost as though God foresaw that these diseases could occur and built in mechanisms to deal with them so you don't get the disease every time this error occurs, if we put it in that perspective?
A. That's right.
Q. It's those defense mechanisms that I would like to go into next with you. Explain to me the ones that you have a feeling for. I think you said some are unknown, but the ones that you have a

feeling for, knowledge about, tell me about the defense mechanisms that you know, again trying to limit it to the leukemias.
A. Okay.
Q. Although it may apply to other things as well.
A. The first and obvious defense mechanism, we are dealing with a system that is producing huge numbers of cells every day. It is characterized by the most rapid replication or cell division that you are going to find in a million cells normally. In this whole expansive territory on this graft that I have called the differentiating bone marrow and immune cells, we are dealing with cells that are, and again excluding the lymphocyte series, virtually aptotic. .They are programmed on an irrevocable differentiation process that leads to the production of functional end stage cells that can't divide.
Q. I think you said earlier the exception to that is the T-cells, B-cells and maybe the monocytes?
A. Maybe. There is argument here. There is replication error going on in these cells normally.

Q. These cells being otherwise the myeloid tissues?
A. The other myeloid precursor cells, the rapidly dividing cells that are identifiable in the bone marrow.
Q. You left some of those out?
A. Yes. If you want me to itemize what we know about the morphologically identifiable types, I can do it, but otherwise-
Q. That's fine.
A. At least until we get toward the end of this differentiation spectra we are looking at rapidly dividing cells, so we are producing bone marrow cells through a series of amplifying and division steps producing blood cells. These cells are dividing so rapidly, and it's been appreciated for virtually 100 years that there is some error going on in there. If you look hard enough you can find cells that have either imperfect nuclei, have died as a function of the division process, so there is replication error that occurs in these cells.
Q. Let's try to put that just kind of in perspective, I guess. You have put here--I'm having a little bit of trouble with your writing there from this distance. What was this cell here called?

A. I called it the committed erythroid precursor.
Q. Basically there is a cell with that title that ends up being an erythrocyte in the circulating blood?
A. For the erythroid precursor there are a couple of different stages involved in the maturation of this cell, so this as a term really refers to a couple of different potential cell types. But for the purposes of this discussion, that's accurate.
Q. What I would like to do is for one of these cells that's the precursor cell at this level of division, how many ultimately circulating erythrocytes in the ball park are we talking about originally from one cell?
A. That is the subject of quite a bit of discussion. It depends a lot on influences that occur in terms of differentiation here.
Q. The person who is not ill would just ordinarily--just ball park it for me. You are talking a thousand, a million, a billion?
A. I suspect we are talking on the order of hundreds to thousands.
Q. And what's the time period that it takes to go from that one cell to the hundreds of

thousands, again ball parking it? I know it changes with different conditions.
A. Ball parking, somewhere between one and three weeks.
Q. And when you say rapidly dividing cells, you mean rapidly dividing-
A. Rapidly dividing cells.
Q. There are errors that occur?
A. There are errors. But these cells, as I said, are irrevocably programmed to produce end stage cells. They are not capable of immortality. If a replication error occurs, the worst thing that can happen to that cell is it dies, which for the system is of minimal importance.
Q. Unless they are all defective?
A. Well, in which case then you have a rapidly developing anemia, or pancytopenia if you are talking about everything.
Q. I diverted you onto that little rabbit trail, so let's get back to the main subject. You were talking about the defense mechanisms that are built in, and you have gotten us to these rapidly developing cells and why the defense mechanism is needed in even a human that's healthy or apparently healthy. Tell me now more about the defense

mechanisms, please.
A. Well, that particular process is in and of itself a defense mechanism because you have--a cell is at maximum state of susceptibility to either spontaneous or external influences that are going to produce the types of errors we are talking about earlier while it is dividing. And in fact for most known chemical leukemogens the replication is the period of time in which the cell is susceptible to that kind of damage.
Q. Say that last part again, please.
A. For most leukemogens, agents that we know cause leukemia, certainly for chemicals and drugs, the time of susceptibility is the time of replication. It's also the time of susceptibility for the cells spontaneously.
Q. I want to be clear here because my first impression when I heard you say that was that the chemical leukemogens only react with cells after they are in this committed stage. That's not what you intended, is it?
A. That's not what I mean at all. As a matter of fact, in the case of benzene the manifestations of cytotoxicity are bone marrow suppression that occur from high-level exposure occur

because cells out here that are not targets for the development of leukemia are in fact eliminated or killed off. That is what creates the anemia. That's what creates the suppression in circulating cells. But that is of no consequence with respect to the development of leukemia directly. So that is a protective mechanism, it's the mechanism that protects us daily from developing leukemia as a normal consequence of just living. It's for the large majority of us.
Q. Now there are other defense mechanisms as well besides the one you just discussed. Tell me about the others that we know about.
A. The other defense mechanisms involve the level of activation within the compartment of cells that is capable or has some capability for immortality, and that is collectively the stem cell compartments.
The pluripotential stem cell compartment, this cell is characterized by the- virtually within the context of the cells of the blood and immune systems. It has virtually limitless horizons with respect to differentiation. It can produce any of these cells. It also can produce itself. We are basically allotted during

embryogenesis a fixed number of these things, and they continue to keep us going for our whole life. It is, however, characterized by an incredibly strong resistance to division. Pluripotential stem cells do not divide very often. And there is a division history, replication history, which is to say that after so many divisions the evidence suggests that the cell progressively loses its pluripotentialness and takes on committed--characteristics of the committed stem cell populations. As we move down this line going from pluripotential stem cell to committed definable hematocritic precursor cells that produce the blood cells, we have a process whereby the cell begins to take on restricted differentiation capabilities. It no longer is capable of becoming everything. It is progressively restricted. And at the same time it has much more limited capability for any kind of self-renewal. That's lost relatively quickly. We are looking here at a spectrum, we are not looking at--we can talk about the isolation of these populations and their discussion as specific entities, but I think it is more realistic to consider it a spectrum of activity. And you can

define a cell type in terms of probability that it will do one thing or another much more readily than you can, say there is this absolute population that has X characteristics.
Q. The differentiation depends on stresses and responses to stresses as well, so the population can change depending on different circumstances, correct?
A. Yes. Yes. That's how we respond to-
Q. Infection?
A. Infection. That's how we respond to insults or anemia. And it's a highly regulated process.
These cells, the myeloid stem cell, for example, is a multipotential cell; that is, it has the capability of giving rise to the erythroid, granulocytic, monocytic, and megakaryocytic lineages. It has a greater potential for division. It has much more limited potential for self replication or self-recapitulation of self, and in fact at this stage we are talking about a cell that is basically committed. It cannot go back.
These cells, the committed precursor cells, the myeloid, monocytic, or what have you within this committed framework, are the stem cells

that are rapidly dividing. They have very limited differentiation potential. They still have the potential to--they certainly have the capability of becoming immortal under the wrong influences, but once you get past the stem cell compartment, there is no evidence that they have capability to become immortal. They are now irrevocably committed, not only in terms of lineage but in terms of replication history, to end up as a terminally differentiated cell.
Q. When you say these cells have the ability to become immortal?
A. They are appropriate targets for an influence, the replication error or any other hypothesized mechanism for the development of cancer. They are appropriate cells for the evolution of leukemia.
Q. Now your studies that you have done you have looked both at the ability of chemicals to attack these cells and cause these changes and you have looked for the toxic response of these chemicals on the body, two different things, right?
A. If I understand you correctly, yes.
Q. That's what I would like for you to differentiate for me now. Isn't it true that even

though you may have one of these leukemias caused by one agent, another chemical agent may affect whether or not the disease actually manifests itself because of toxicity and other forms that have to do with this control mechanism?
A. That is certainly a reasonable hypothesis, and it's one, in fact, that has been put forward to at least explain the difficulties in producing certain types of leukemias in animals. I should qualify that. Whether or not that in fact proves to be the case is not clear. We don't have enough information on the panoply of regulatory processes that are involved to really say that with authority. It is a logical assumption based on our current knowledge.
Q. Your work that you have done, for instance, with benzene has shown that benzene is toxic to the immune system?
A. In very high concentrations, yes.
Q. At least you have been able to demonstrate the toxicity at high concentrations, but when you lower the concentrations finding effects in the immune system becomes very difficult, does it not?
A. Finding significant effects in terms of

an effect on health, yes.
Q. It doesn't mean that lower concentrations don't produce an effect, it is just it gets very difficult to measure and say it is a distinctive effect from what might be current by chance alone?
A. I'm sorry, could you repeat the question.
Q. Yes, sir. You said--where I'm going with this is that something like benzene may well and probably is toxic at lower concentrations. It's whether or not the toxic effect is significant statistically and able of detection by the methods that you have that you have a problem with.
MR. ZATZ: There is no question pending.
Q. (BY MR. HOBSON) That's what you are saying, isn't it?
A. That's an oversimplification. It depends on what you mean by toxicity. If you are talking about a measurable end stage, alteration that holds resistance to infection, yes. If you are talking about effects that occur at a different level, say in terms of the differentiation of cell types, that's not at all clear.

Q. What I'm really asking you is when you say that's not at all clear, it means you haven't been able to develop the techniques or you don't have the capacity to make the differentiation of whether it occurs or not, you just don't know? You can't say it doesn't, and gets back again to proving the negative that you were talking about earlier?
A. There is certainly--basically we can't say that it doesn't, but there is no evidence to suggest that it does.
Q. Now one issue, of course, I need to address with you is whether or not benzene produces leukemia as a causative agent. And by asking that I want to know if you believe that benzene exposure can cause any of these genetic changes to lead to any of the leukemias. Would you give me your opinion on that, please.
A. Are you asking me whether benzene leads to the development of a specific leukemia or the genetic changes in general or what's the question?
Q. The question is, is benzene a true carcinogen when we talk about the leukemias, and if it is, in your opinion, which of the leukemias?
A. I think there is reasonable evidence to conclude that high-level benzene exposure can lead to

the development of acute myelogenous leukemia and several variants of acute myelogenous leukemia.
Q. Variants being?
A. Erythroleukemia, metamyelocytic, probably not monoblastic, although I wouldn't rule it out. Virtually the variants situated in the FAB classification with acute leukemia.
Q. What is it that you have seen in your opinion in the way of the discussion we have had about these cells that lead you to that conclusion?
A. We are talking about two different sets of evidence that lead me to that conclusion. One is my general knowledge of the role of replication in this process, the mechanism of cell differentiation in hematopoiesis, and my knowledge of the epidemiology and clinical literature associated with benzene exposure.
Q. Which defect is produced by the benzene exposure that leads to the AML?
A. It's going to occur in this population of cells.
Q. Are we talking about now the bone marrow compartment after the pluripotential cell?
A. Yes. We are talking about committed cells, either multipotential or unipotential stem

cells.
Q. Now what is the effect in those cells?
Are we talking about a change in the genetic makeup,
or what is it?
A. This is based on my knowledge of how
benzene and its metabolites affect cells most likely
to be due to replication error involving
nondisjuctional events.
MR. HOBSON: It's a good time for a
break.
(Whereupon, there was a recess.)
Q. (BY MR. HOBSON) Dr. Irons, I would
like for you to explain to me, if you would, please,
how you mean the terminology of the nondisjuctional
events, the errors that you just talked about.
A. What was the beginning of the
question?
Q. I would like for you to explain what
you just told me about benzene causing the
leukemias. I think you said at high concentrations,
and you mentioned some errors that were made and
there were nondisjuctional events.
A. In my opinion the most likely mechanism
whereby benzene produces leukemia is through the
development of replication errors during division,

replication errors being primarily aneuploidy, the loss of a chromosome, or technically to gain as well, but in this case we are talking loss of a chromosome during division and/or the loss of part of a chromosome.
Q. What is the basis of saying that? Have you done work that demonstrates this?
A. Benzene metabolites are very effective producers of aneuploidy in dividing cells.
Q. And aneuploidy means changes in chromosomes?
A. Yes.
Q. Which ones of the benzene metabolites are you talking about here now?
A. Hydroquinone, p-benzoquinone, benzenetriol, and if in fact transmuconaldehyde is a real metabolite of benzene, its chemistry is consistent with that as well.
Q. Have you looked at these metabolites individually to see if they are individually the cause of these replication errors, or do they only have the replication error effect when more than one of the benzene metabolites is present?
A. You can produce the effects in isolated cells with hydroquinone and p-benzoquinone or

benzenetriol. That's been demonstrated. Certainly the mechanics of it has been demonstrated in my laboratory. The effects on chromosomes has been demonstrated in the laboratories of Dr. Martin Smith at Berkeley and by David Eastman. Changes consistent with that are produced in whole animals following the administration of metabolites in high concentrations, but you have to use very nonphysiologic doses in order to produce toxicity.
Q. Should hydroquinone be considered a carcinogen as benzene is?
A. No.
Q. That's what I'm trying to get at. What is it about your work that says that benzene, which is metabolized to hydroquinone, and if hydroquinone is one of these things that causes a replication error, what establishes hydroquinone as being--and benzene going through metabolic changes being a carcinogen?
A. Although benzene is a very simple molecule if you look at it, has very complex metabolism, it's clear that the effects of the quinone metabolites that I described are potentiated in vivo if they are considered concomitantly with phenol, which is another metabolite of benzene, in

fact it is really the first major metabolite. If you administer phenol and hydroquinone together you can enhance the effect you see with hydroquinone by itself, but it is extremely difficult to produce bone marrow toxicity with the benzene metabolites when you are administering them de novo.
Whereas when you administer benzene you have a situation in which the metabolism and pharmacokinetics of the production of the metabolites is such that it predisposes to their concentration in the target tissue, which is the bone marrow. It is very difficult to produce that with the metabolites by themselves because they are pharmacokinetics, and inherent toxicity really preclude creating the situation that is compatible with producing bone marrow damage without incurring other types of toxicity like central nervous system toxicity.
Q. Do you have any experience, then, with the result of concomitant exposure to benzene and phenol?
A. Benzene itself and phenol?
Q. Yes.
A. No.
Q. What would you have in the way of an opinion as to the results of that kind of exposure?

A. It would be very complicated. I would not want to hazard what would happen. You are looking at certainly different levels. You are looking at primary metabolism in the bone marrow, secondary metabolism--excuse me, not bone marrow, the liver. Secondary conjugation events that are going to be effectively competed for in the liver that's going to change the disposition in the metabolism, secondary metabolism. You have got secondary and tertiary metabolism on the quinone metabolites in the bone marrow. That would be an interesting experiment, it would also be a very, very difficult one to do.

Q. What about concomitant exposure to benzene and hydroquinone?

A. I think it would be exceedingly difficult to deliver hydroquinone to the bone marrow under any circumstances through exogenous administration that would be compatible with the production of significant bone marrow damage without having very serious CNS effects. I don't think administering benzene is going to change that. It may exacerbate hydroquinone toxicity.

Q. Would concomitant exposure to benzene and phenol alter the metabolism of benzene metabolic

pathways?
A. It's hard to say. It would depend on the relative--first of all, it is going to depend on the relative affinities of benzene and hydroquinone for the cytochrome P 450 in the liver. I'm not familiar with the details of the kinetics of those two with respect to initial oxidation to really say anything authoritative on that.
Q. Just so I make sure I'm asking the question that you are answering, when I say changes in the metabolic pathway, I think you could answer that one of two ways, that the metabolic pathways themselves are changed, or that the relative direction down one of the metabolic pathways is changed?
A. I doubt you are going to see any major changes in metabolic pathways with any of these scenarios. You are talking about competing influences that may change the relative production of a given metabolite in a given place, and I think that to hazard an opinion on those influences would be very, very difficult.
Q. Now the information that you just gave me about how benzene is metabolized and the influence of the metabolites, is that based on studies in

animals or in man or both?
A. Primarily animals, but also some human tissues, some cell work, but primarily animal work.
Q. I think you have published in the past that while these metabolic pathways may be somewhat similar in animals and man, and even similar from animal species to animal species, the relative path--the amount that goes down each one of these paths relative amounts changes from species to species and animal to animal and from animal to man, right?
A. As a general statement, yes, but there are specific cases where it is relatively straightforward to determine what those differences may be. And they may be slight, they may be great, it depends on the nature of the species that you are working with.
Q. In talking about man, which animal species is the closest animal model for metabolism of benzene in man? I'm talking about now these relative percentages and the pathways.
A. That's a very difficult question to answer in a single statement. In terms of overall rate of metabolism, I would say you are probably--you are looking at a greater rate of metabolism, say, in

the mouse in generic terms than you are going to be in, say, the rat, which is probably similar to or less than you are going to find in the monkey, which is going to be similar to or approximate to that which we see in man. That's very general. You can't really apply that to a specific pathway or a specific compound.
Q. Perhaps you have anticipated where I was headed. Why don't we do these studies in monkeys?
A. To some extent there have been attempts to do studies in monkeys. I think there have been several attempts to demonstrate bone marrow toxicity, at least one that I think was conducted by the American Petroleum Institute in, I believe, the early '70s.
Q. In monkeys?
A. I'm not sure whether they used a monkey or not. I think they tried, but I haven't looked at that study in years, so I really couldn't.
Q. Tell me which study you are thinking about as best you can describe it.
A. It's known as the Zoo Study, if you will. They basically attempted to produce bone marrow toxicity through the acute or subacute, I

forget, administration of benzene to a variety of different species and attempt to look for a better model.
Q. Have you considered using monkeys?
A. The trouble with using monkeys is they are extremely expensive, there are ethical considerations you have to deal with when approaching using primates, having the appropriate facilities to do them, there aren't very many institutions that can do long-term monkey experiments. I have considered using monkeys, but I have elected to look directly at human cells and to compare metabolic and mechanistic aspects of cell differentiation replication between rodent models and man.
Q. Doing the same kind of studies in monkeys, primates, that you have done in mice or rats, though, certainly studying the monkey, all those things being equal, cost and that sort of thing, give you much better estimates of what's going to happen to man, doesn't it?
A. I think to conduct similar studies to what I have done in mice is almost impossible if not virtually impossible. It is just not logistically feasible. I could be wrong, but I don't know of a facility in this country that can do studies of that

magnitude with that number of primates.
Q. But now a number of the studies that you have done dealt with relatively small numbers of rats and mice, haven't they?
A. True. But it depends on what questions you are asking. If you want to talk about comparative metabolism, those could be done in monkeys, yes.
Q. Now you mentioned that you have looked at some human cells.
A. Yes.
Q. What have you done along that line?
A. We are developing defined stem cell assays, we are characterizing differentiation and proliferation of human cells in culture systems that are analogous to those that we have developed for mouse cells.
Q. So these are not human studies, but they are studies on human tissues?
A. They are studies on human tissues, that's correct. And they are developmental. They are in progress, they are not by any means completed studies.
Q. I want to come back for a moment now to benzene creating these replication errors that you

talked about earlier. Once I'm in your field it's more difficult for me to phrase my question so it can mean something to you, so bear with me, if you would. Besides benzene being able to cause a change in chromosome, the replication error you told me about, does benzene toxicity or the metabolites of the benzene from a toxicity standpoint also play a part in the development of leukemia?
A. I'm sorry, but I'm not following the question.
Q. I'm trying to get to, I guess, benzene causes the chromosomal changes which sets in motion these developments of malfunctioned cells?
A. Metabolites do.
Q. The metabolites of benzene. Does the toxicity of either benzene or benzene metabolites also contribute to the induction of the disease at the end point? In other words, does it affect the immune system so that the policing of these cells is also stopped?
A. I think you would have to be at such a high level of exposure that you would have problems associating with host resistance and immune dysfunction long before you would have the development of a frank leukemia. But again, this is

pretty much speculation. Effects of benzene metabolites on isolated cells of the immune system have been well characterized and have a dose response nature. We have done most of that work, a good deal of it at least.
But again, we are talking about a system that has numerous fail-safe and networking mechanisms and demonstrates an isolated effect on a group of cells, and showing it has any effect whatsoever on immune surveillance or immune response is totally different. And my impression of benzene is it is immunosuppressant only in high concentrations.
Q. Tell me something like--high concentrations is a relative term. How can you quantitate that?
A. You see frank evidence of suppression in the bone marrow, frank evidence of toxicity to the immunogens, so you are talking exposures that are consonant with the production of demonstrable pathology in target organs.
Q. Are we talking now about exposures that occurred through, say, the inhalation of benzene vapors, or are we talking about some other method of delivering benzene to an animal system?

A. It is a heck of a lot easier to do it when you inject the stuff than it is with inhalation. You can do it with inhalation, but it is far more difficult to get a reproducible effect.
Q. What levels are we talking about at the tissue level?
A. At the tissue level, in terms of dosing, we are looking at exposures in the area of 100 to 300 parts per million for a concentration for inhalation. And injection is a totally artificial game in terms of trying to describe what any relevant exposure is from the standpoint of inhalation. But we are talking nonphysiologic dosing regimen to be sure.
Q. You have said several times that we are looking at populations here, populations of cells, populations of animals. When you carry out your animal studies with benzene and you find statistically significant changes, if we talk about for a moment the production of leukemias, have you done that in animals, produced leukemias from benzene exposures?
A. I have produced some lesions in the mouse that are certainly equivalent to myelodysplastic changes. Whether or not they are

frank leukemias is an argument that pathologists may carry on for some time. But certainly it is possible to produce myelodysplastic changes in the mouse. The mouse is a very bad clinical model for extrapolation to man in terms of a disease entity, but you can certainly produce changes reminiscent to myelodysplasia in a mouse.
Q. What percentage of the population of mice would you find these changes, these myelodysplastic changes in?
A. It's going to depend on the dose. I would say to some degree or another concentrations certainly above 300 parts per million or greater you are going to see the majority of the animals showing changes. And you are going to have a significant number showing changes at 100.
Q. And is this concentration in the air or what you are injecting?
A. No, that's air, inhalation.
Q. You say greater than 300 the majority have changes, myelodysplastic changes?
A. Somewhere between 1 and 100 you basically plateau out. Between 100 and 300 you plateau out.
Q. Below 100 parts per million do you not

find any changes?
A. You can see some. You can see some hematopoietic changes associated with transient suppression in and around 50.
Q. And when you say you can see some-
A. You by no means have the same--you don't have myelodysplasia in the majority of animals at 50. You may have some at 50 that demonstrate some myelodysplastic changes.
Q. Is this true in this system as it is in other systems that below 50 you may see changes similar to the group at 50 and above, but you can't attribute those changes to the exposure because they are infrequent?
A. I've never seen any myelodysplastic changes in animals below 50. I have not conducted major exposures of animals below 50 because I have had a devil of a time producing these changes between 50 and 300. The problem with benzene has been producing basically persistent lesions in rodents at any concentration, and that was my goal with these experiments.
Q. That gets back to the selection of the model?
A. It gets back to the selection of the

model, but I think it also is likely to be a function of the exposure regimen as well.
Q. And then what regimen were you using?
A. Continuous exposure and also exposures involving periods of time where the animals have between, say, four and seven days in between exposures.
Q. When you say continuous exposure, are these 24-hour exposures?
A. No. We are talking either exposures for six to seven hours a day every day, or exposures for, say, three to four days a week with days off in between.
Q. Did you find any difference in those two exposure regimens?
A. At the higher concentrations there are some differences. What's remarkable to me is that exposure to transient--exposure for three days a week compared to, say, exposures for seven days a week, that there is not that much difference between the three-day exposures versus the seven for benzene itself. That is to say three days at high level produces almost as much damage as seven days' exposure at high level. You lose it when you go down. At 50 parts per million I don't recall any

definitive resolution to whether there is a difference, but at higher concentrations it is important.
Q. Is this work published?
A. Some of it is in abstract form, the rest of it is not.
Q. Where was that work done?
A. CIIT.
Q. When, approximately?
A. 1987, '88. We are talking now about peripheral blood values, bone marrow cellularity and histopathology. We are not talking about bioassay-type studies. We were looking for evidence of effects that were associated with any kind of damage rather than simply looking for leukemogenesis.
Q. You told me how long these exposure sessions took place. Were these over a lifetime of the animal, or were they one set period of time? How did you do that?
A. I forget the exact paradigms for all the various combinations. We did do one group that were exposed for, I believe, 12 weeks, 12 to 16 weeks, and then held them for, I think, a year. This was similar to a paradigm that was used by Dr. Eugene Cronkite of Brookhaven National Lab in which he

exposed animals for a brief period of time and held them.
Those studies demonstrate that there is a very complex dose-duration product that's associated with the production of certainly proliferative lesions in mice. Those appear to be more effective than long-term exposures to very high concentrations that have been done in a classic bioassay type of paradigm.
Q. I'm not sure I understand what you have told me there on that series of experiments. You say you took some animals that you exposed for three days and left them off for many days. You took another series, exposed them seven days?
A. Six or seven, I forget exactly.
Q. And you did this for a long period of time?
A. For up to 12 weeks.
Q. Both regimens?
A. I believe so. I have certainly done both experiments. Whether they were done in the same context or not, I can't remember. But we have done both.
Q. Then you held these animals for a year?
A. Yes.

Q. And then looked at the peripheral blood to see if there were any changes that you could find?
A. Yes, and histologic changes in bone marrow.
Q. Were their different exposure levels for these two regimens as well?
A. Between 50 and 300, yes.
Q. Did you find any persistent changes at the end of this one-year holding period?
A. There were some very impressive myelodysplastic changes in the animals certainly at the 300 parts per million level. There were some at the 100 parts per million level. I would be hard-pressed to establish that there were the same types of changes at 50. Between 100 and 300 there were plenty.
Q. What was the difference, if any, between the two exposure regimens, the short term versus the longer term?
A. That's where I can't remember whether or not we did those. We carried them out that far. As I recall, certainly exposures between 17 and 300 under a typical six- or seven-day week paradigm exposed for 12 or 16 weeks and held will produce long-term myelodysplastic changes. The problem with

interpreting that data is we are talking high exposures, we are talking plenty of evidence for infection and distinguishing between a myelo proliferative disorder and response to infection and one associated with the development of a long-term proliferative lesion that might, for instance, be typical of myelodysplastic syndrome in man is very controversial and in fact is one of the reasons why that study has not yet been published. The interpretation of the findings is very controversial.
Q. I take it you ran a control group?
A. We ran a control group.
Q. What did you find in the control group with relation to myelodysplastic syndrome?
A. There are some proliferative problems, myeloproliferative disorder and lesions in the spleen of some of those animals that could be associated with infection that was also demonstrated in those animals.
Q. Was it less marked in the control group?
A. I would say it probably is, but the diagnosis is controversial.
Q. The-
A. The history of the mouse as a model for

leukemogenesis has been fraught with difficulty in extrapolating myeloid lesions. It is a very difficult task, and you will not find consensus among pathologists.
Q. Would the exposure regimens that you used in these animals, the 300 parts per million benzene vapor or 50 parts per million benzene vapor, would those exposures lead to changes in the animal's blood or lymph system, immune system, that would make them more susceptible to disease, infectious disease?
A. In my opinion, yes, but that's an opinion. Yes. And that is a problem with respect to thoroughly defining the model. I suspect there are within that group of animals, and I forget the number we started with, between two and three leukemias, but I would be hard-pressed in a scientific form to defend those against alternative interpretations of the histopathology.
Q. Now the exposure regimens that you used that were strictly benzene vapor and air for the series of tests you just talked to me about?
A. Right.
Q. Have you done any work to distinguish between benzene vapor exposure being delivered to the target organs and skin exposure?

A. Skin in my experience is a very poor route of exposure except under conditions of perhaps high-level exposure prolonged contact with the skin. I have never considered skin as a viable route for a study. It is hard enough to produce toxicity that you can reproduce when you are administering the inhalation.
Q. What is the last series of skin studies for benzene that you looked at?
A. I have not personally done skin studies with benzene. I have elected to use inhalation.
Q. You have seen others, though, who have done skin permeation studies?
A. Skin permeation, yes. What's the question?
Q. If skin permeation is a viable route of exposure to benzene?
A. Not in any experimental system that I know of.
Q. What about for man?
A. My opinion is that under conditions of prolonged exposure to high levels of benzene, prolonged exposure being contact with liquid benzene in the skin, that it probably is a significant route of exposure. I don't think it constitutes an

important route of exposure relative to the lungs in the gaseous phase. The lungs are much more important if you are exposed to benzene in the air.
Q. What about benzene as a component of a solvent, have you seen the studies that have been done showing skin permeation of benzene contained in solvents and how the solvents affect the transport of benzene through the skin barrier?
A. I believe I have. I don't recall the specifics, but I do, I have seen those.
Q. In those studies the fact that other solvents are present actually makes the skin absorption route for humans much more significant, doesn't it?
A. My impression is that skin absorption for benzene was relatively small under most conditions. I would have to look at the data to render any more of an opinion than that.
Q. You talked about myelodysplastic syndrome in connection with these mice, I think, a while ago. Tell us what you mean by myelodysplastic syndrome in the context that you used it.
A. Myelodysplastic syndrome is basically an abnormality that describes a constellation of potential symptoms that is associated with long-term

bone marrow damage. It encompasses terms that are now archaic such as preleukemia. It can involve one or more multiple alterations in circulating peripheral blood cells in terms of number and behavior, and has a reasonably high rate of transformation into AML.
Q. Can you use your chart that you prepared for us to explain myelodysplastic syndrome?
A. Only to the extent we are talking about a recurring abnormality in the differentiation and production of the various cell types. We are talking about basically long-term abnormality in differentiation and production, so we are talking about, again, cells in the stem cell compartment.
Q. So you are talking about an effect that occurred in the stem cell compartment that is manifested out in the peripheral-
A. Yes.
Q. What kinds of changes lead to myelodysplastic syndrome? Are they the same kinds of changes that lead to leukemia, or different?
A. In general they are the same kinds of changes. We are talking about bone marrow damages, bone marrow suppression, followed by an abnormal- what is apparently an abnormal differentiation

pattern for the myeloid cells in the bone marrow primarily. It's consistent with high-level exposure. Certainly chemotherapeutic agents are known to do it. There is in fact to my knowledge no demonstrative evidence that you will get secondary leukemia in the absence of the development of frank bone marrow suppression and/or myelodysplastic changes.
Q. Say that last part again, please.
A. That secondary leukemias, leukemias arising secondary to chemotherapy or exposure to benzene, the evidence is very strong that you basically have to have significant bone marrow suppression and/or the development of pancytopenia or myelodysplastic syndrome as a prerequisite for the development of AML in those patients.
Q. That leads me to two questions. What is the basis for saying that? Is that based on animal models?
A. No, that's human data.
Q. Which human data?
A. Primarily the chemotherapeutic literature, as well as those cases for which one can define high-level exposure to benzene, but primarily the chemotherapeutic literature in which patients are

clearly defined and followed from the beginning of treatment. So you have got a handle on exposure, you know what their exposure was, and you follow the progression of their disease.
Q. How soon after the exposure to the chemotherapeutic agents does the myelodysplastic syndrome follow?
A. I think it varies considerably because you have to distinguish between transient suppression of the bone marrow which you are going to see during chemotherapy.
Q. That's the purpose of the chemotherapy?
A. It has that effect, yes. What you are really talking about is persistent changes that occur after cessation of treatment.
Q. I need for you to put that in more, I guess, laymen's terminology. What do you mean?
A. If you have suppression, if you have thrombocytopenia secondary to chemotherapeutic treatment, that's a relatively frequent event. If it persists after treatment has ended, it is likely to be indicative of a persistent problem. And it's very difficult to predict what you are going to see. You may see thrombocytopenia, you may see lymphocytopenia, a combination there of a variety of

different possibilities exist. But persistent changes after cessation of treatment would at least be indicative of potential myelodysplastic changes.
Q. A person has chemotherapy, which is going to lead to myelodysplastic syndrome. Do they always then continue with the myelodysplastic syndrome?
A. Not necessarily. It is highly variable. Some may persist with isolated changes such as thrombocytopenia for years, some may spontaneously recover, some recover with further treatment. A high percentage will go on to develop acute myelogenous leukemia, or they can also have myelodysplastic syndrome, there is nothing that prevents them from doing that.
Q. Are all of the people who die with leukemia after administration of the chemotherapeutic drugs, have they expressed the myelodysplastic syndrome?
A. All is a very strong word. I would say that it's my understanding of the literature that there is certainly evidence of bone marrow suppression and/or blood dyscrasias in almost every patient receiving alkylation therapy in large doses. And one would expect that for those going on to

develop leukemia, AML, that virtually all of them are going to have some anlage.
Q. Some-
A. Some evidence of bone marrow suppression prior to the development of the leukemia.
Q. Of course, prior to the development of leukemia encompasses the whole time period from the administration of the drug until the development of leukemia?
A. An expression of leukemia.
Q. Isn't it true, though, that some of these people who get their chemotherapy and develop the myelodysplastic syndrome, then they recover so that you don't find an expression of the myelodysplastic syndrome or even any of these changes in the peripheral blood, peripheral blood is essentially normal, they later develop leukemia, acute myelogenous leukemia even?
A. I would question whether or not you are talking about completely normal because in studies that have been done of patients receiving chemotherapy and/or radiation therapy, in at least one study done with Michael Dexter in Testa Laboratory in England has demonstrated fairly significant stern cell behavior in patients years

after cessation of therapy with almost normal peripheral blood picture, but I think thrombocytopenia is relatively persistent even in these patients. So I'm not sure I could agree with the statement as you--the question as you phrased it.
Q. Doesn't that then argue against what you just told me earlier, that you always have one of these changes in the peripheral blood prior to developing an AML that's caused by benzene?
A. No, because you have demonstrated some frank damage to the bone marrow to begin with.
Q. But if you have had benzene exposure at even 50 parts per million, you have demonstrated some toxic effect to the bone marrow, have you not?
A. Not necessarily. I think the likelihood is that between 50 and 100 parts per million you will in some people. You will in some mice.
Q. And if you look at a large enough population of people, there are going to be some people that that expression of toxicity may occur below 50 parts per million?
A. It depends where you are on the dose response curve, and I would say you are on the lower end. I am comfortable with the idea that somewhere

between 50 and 100 parts per million is likely to cause bone marrow damage. The evidence between 25 and 50 is really slim, and below that is problematic.
Q. This takes us really to the concept of individual susceptibility that out there on the tail of the population there are some people who are going to be outside the norm, just like there are some animals outside the norm.
A. That's correct. In the case of the model we are talking about, I think we see those people in the relatively low--small number of individuals that will develop AML following very high-level exposure to benzene. The majority of cases of benzene toxicity described in the literature are not leukemia. We are talking about bone marrow suppression, we are talking about anemias, pancytopenia, aplastic anemia, and a portion of those will go on to develop AML. We have seen the susceptible population when we see the incidents of AML following high-level benzene exposure.
Q. The term high level is a relative term depending on the individual susceptibility to exposure?
A. I'm talking 100 parts per million and greater, and perhaps 50 to 100 parts per million.

Q. When you say 100 parts per million or greater, is that parts per million years?
A. 100 parts per million absolute concentration of exposure for some duration of time.
Q. That's the next question, what duration?
A. I don't think we have sufficient evidence to make a clear statement on that except to say we are talking repeated exposures. Certainly the cases that have appeared in the literature have been individuals that have been exposed for periods of time in excess of six months, and the majority of them exposed for long periods of time.
Q. At more than 100 parts per million per eight-hour workday?
A. Not necessarily integrated, but certainly exposed to levels between 50 and 100 parts per million or greater. We have some studies, as I'm sure you are well aware, from Turkey and elsewhere where the exposures are horrendous.
Q. What about the worker who goes out and gets exposed to benzene on a regular basis for a period of, say, 15 minutes a day one time to 200 or 300 parts per million, where does he fit in the pattern?

A. The only experience that I can lend to clarification of that has to do with animal studies in which I have tried to take the dose regimen issue from days exposure down to hours of exposure, in looking at exposures of 45 minutes to an hour and a half, interrupting and looking at different regimens. And I have found it incredibly difficult to produce reproducible bone marrow toxicity in mice at exposures less than an hour and a half to two hours at a time. I know of no data to suggest that you can maximize saturation of metabolism or effectively produce the same biochemical scenario that will exist with prolonged exposure in that kind of a regimen. That is not to say that it might not happen, but I certainly don't have any evidence to support it.

It's unlikely that you are going to have efficient metabolism of benzene for the production of--optimal production of metabolites that can get to the marrow and undergo further metabolism unless you saturate benzene metabolism first, and that's a process that takes--that may have some species differences, but one would expect it is going to saturate later in man than it is in a mouse, so we are talking probably greater than two hours.

Q. Of course, you could achieve that by having several lower-level exposures? In other words, an exposure for ten minutes one hour, another ten-minute exposure the next hour, if you are talking about reaching the bone marrow with a certain dose?
A. No, only if you abide by an accumulative exposure paradigm, which I don't think is consistent with this particular situation at all. Metabolism requires consistent exposure because you are going to eliminate it as rapidly as well, so that if you are exposed for a few minutes one hour and then a few minutes the next hour it is as though you are starting over again.
Q. What is the biological half-life of benzene for humans as you have been able to determine?
A. I would have to look that up. I don't have that information at my fingertips. There have been some studies done by Maths Berlin in Sweden that come as close to defining that as any, and they are reasonably--they are certainly consistent with what's been seen or predicted from rat studies.
Q. But if you say, though, that an exposure one hour and an exposure the next hour is the same as starting over, the biological half-life

would have to be a matter of a few minutes as opposed to a couple hours?
A. I would have to look at that data again to comment.
Q. How can you make the statement that one hour to the next hour starting over if you don't know what that data is?
A. I'm telling you from my experience with the mouse. It is incredibly difficult to produce bone marrow toxicity with that kind of a regimen.
Q. This is measurable toxicity that you see in the peripheral blood?
A. Or the bone marrow.
Q. Or the bone marrow?
A. Yes.
Q. And you are looking how?
A. Hemologic analyses, histopathology.
Q. What specific parameters would you be looking at?
A. Peripheral blood counts, lymphocytes, granulocytes, platelets, typical CBC.
Q. And you are looking for statistically significant difference in changes?
A. Yes. Bone marrow cellularity.
MR. HOBSON: Lunch anyone?

MR. ZATZ: Sure.
(Whereupon, there was a recess.)
(Whereupon, the last question and answer were read.)
Q. (BY MR. HOBSON) Dr. Irons, I want to ask you a few questions about some other diseases, if I could. Multiple myeloma, would you tell us what multiple myeloma is in humans to you as a disease condition and how it relates to these blood cells that you have drawn in your chart, if it does?
A. Multiple myeloma is, I think, most appropriately classified as a lymphoma, although it arises in the bone marrow and is a malignancy of the B-cell lineage, B lymphocyte lineage, that leads to the production of differentiated immunoglobulin producing B-cells also known as plasma cells.
Q. Have you been able to determine what kind of a defect occurs that leads to the production of multiple myeloma in humans?
A. I think the prevailing opinion at the present time is that the target cell is a committed B and B lymphocyte lineage. We are talking about a committed B lymphocyte.
Q. Can you be more specific? Is this a chromosomal change and do you know where the change

occurs or anything like that?
A. The information that I believe has led to that general understanding has to do with the rearrangements that are seen in the immunoglobulin chains, the genes that code for the immunoglobulin chains, and the level of maturation associated with those specific changes.
Q. Are you aware of any mechanism or any agent that causes that mechanism to change, at least, and leads to the development of multiple myeloma?
A. Definitively, no.
Q. Well, do you have an opinion about any agent or causative factors that go into the development of multiple myeloma?
A. Multiple myeloma is one of the most frequent types of hematopoietic or lymphoid neoplasms that is seen in man, certainly with old age, and from that standpoint is a relatively frequent occurrence. There has been some question raised as to whether or not multiple myeloma can be caused as a function of chronic benzene exposure. At the present time I don't think that there is certainly compelling evidence to indicate that it is, although it certainly has been suggested. And at this point I have not yet reached an opinion on it.

Q. Another disease, Hodgkin's disease.
Would you tell me your understanding of Hodgkin's disease and how it fits into this blood system, if it does?
A. Hodgkin's disease is from a cellular standpoint a very complex entity. There are a number of different variations, but it appears to be a disease of deregulation, if you will, of normal lymphocyte behavior, primarily T-cells, and involves constellation of other cells as well in the way the lesion is expressed. It is a disease of the peripheral lymphoid system. It arises from, I think, most likely T-cells in the peripheral. There is still some debate as to causation, although I think most authorities agree we are looking at primarily a T-cell lesion.
Q. Is this a lesion that starts out in one of the precursor cells, or is it one that starts out in the ultimate T-cell itself?
A. Most likely a T-cell.
Q. How is it that so many T-cells can be converted if T-cells are one of these groups of cells that don't reproduce themselves, or are they?
A. They are.
Q. So in other words, you are saying the

T-cell reproduces itself, it has a genetic defect, and the defect is repeated also?
A. The lymphoid cells are distinguished from the other cells that are descended to the myeloid lineage in that they retain the ability under certain circumstances to proliferate. They have, as I said before, very elaborate control mechanisms that are apparently at work to keep them from doing so in an unregulated fashion, but they have that capability. From that standpoint they are not in the same category as the other cells of myeloid lineage.
Q. Are you aware of any efforts in the case of Hodgkin's disease to see if the lesion, if that's the right word, occurs further up the cell division line back over into some of these stem cells?
A. I would not be surprised at all if in fact there was research under way to delineate the target cells in Hodgkin's. At the present time I'm not familiar with the latest literature in that area.
Q. Are you familiar with a disease called myelofibrosis?
A. Yes, sir.
Q. What does myelofibrosis mean to you in the context of the system?

A. Myelofibrosis is a condition that's associated with the fibrous displacement of normal hematopoietic tissue in the bone marrow and frequently involves enlargement of the spleen and extramudullary hematopoiesis. Myeloid metaplasia is a better way to describe it in what's typically called myelofibrosis. It is, as I understand it, an extremely rare condition.
Q. Put that in terms of what is going on in the body structurally, if you would, for myelofibrosis. What happens with the disease with these various entities in the different parts of the blood forming system?
A. There is an abnormality that involves the production of fibrous or connected tissue sclerotic activity in the bone marrow, and this is associated with the production of hematopoietic tissue, which is normally located in the bone marrow, to the spleen and perhaps other organs as well, sometimes the liver. The spleen is the principal organ. One sees production of blood in the spleen, one can also see the same types of sclerotic changes in pathology in the spleen as well.
Q. Are you saying that myelofibrosis, then, is damage to the house where the blood is built

as opposed to damage to the cells that actually make up the blood and turn it into the different components of the blood?
A. I think our knowledge base with respect to myelofibrosis is very weak and sparse. On a very crude level that's certainly one hypothesis that could be explored. As to causation of myelofibrosis, the only--I don't know of anything that causes myelofibrosis.
Q. Are you aware that benzene has been implicated as a cause of myelofibrosis?
MR. ZATZ: Objection to the form. You can answer.
A. I am aware that there are cases, extremely few, that have been included in studies that purport previous benzene exposure. To my understanding there has been no demonstration that myelofibrosis is in fact associated with benzene exposure.
Q. (BY MR. HOBSON) Is that an area you have looked at at all, or is this what you have picked up in casual reading on other subjects?
A. I have read extensively in the benzene literature associated with both the bone marrow damage caused from acute or prolonged exposure to

high concentrations as well as leukemia and mechanisms of leukemia genesis. I don't see anything that would lead me to conclude that myelofibrosis is a consequence of benzene exposure.
Q. Is myelofibrosis a fatal disease in humans?
A. It can be, yes.
Q. Is it always?
A. Always is a harsh word. I doubt--always applies to many things.
Q. How about usually, then?
A. Usually. I'm not a clinician, but as I recall reading that literature it has a fairly dismal prognosis, although I think it can go on for extended periods of time, and in some cases it may go on for ostensibly normal life span, although again I'm not a clinician, so from that standpoint I probably shouldn't even be commenting on it.
Q. Would you know if a person who has been diagnosed with myelofibrosis would go on to develop either leukemia lymphoma or multiple myeloma?
A. I believe in some percentage of cases that I could not quote, we could look it up, I have got Jandl right here, some percentage of patients with myelofibrosis may go on to develop AML. With

respect to the other diseases you mentioned, I'm not sure.
Q. Can you enlighten us as to a mechanism for going from myelofibrosis to AML?
A. No, I can't.
Q. You mentioned earlier myelodysplastic syndrome, and I've seen you abbreviate that as MDS?
A. Yes.
Q. To save my tongue can we refer to myelodysplastic-
A. That's fine.
Q. You said, I think, in the beginning of the deposition that MDS is really replacing more archaic terms, I think you said, such as preleukemia, and you mentioned some other things. Tell me how you meant to use MDS as far as what it encompasses. What diseases that are historically recorded would you put in the MDS category?
A. Well, the classic one that is in there is refractory anemia, or sideroblastic anemia, but there are several different ones that are put under that heading. When I think of MDS myself in the context of bone marrow damage, I sometimes include the more classic transient phenomenon such as thrombocytopenia or lymphocytopenia. Myelodysplastic

syndrome or the syndromes usually have two or more of those that accompany the disease process, but I think they are all evidence of bone marrow damage either transient or blocked. Myelodysplasia is most appropriately associated with an abnormality in, I think, stem cell differentiation.
Q. Aplastic anemia, is that a disease that you would put under the umbrella of MDS?
A. It would depend on the context. Most usually, no. But aplastic anemia has variations as well in terms of the severity of the disease. And basically the suppression of various cell lines can vary considerably. Aplastic anemia can be a symptomatology associated with severe MDS.
Q. Is MDS different for humans than it is for mice and rats or other test animals?
A. Yes. I don't think we have an appropriate model for MDS that certainly is extrapolated from the cross species. We have seen changes in the mouse that are very reminiscent of changes that could be associated with MDS in man, but it is by no means an exact model of the disease in man. I don't know of any other.
Q. You don't know of any other what?
A. Model for myelodysplastic syndrome.

Q. In man?
A. I know of no animal model that completely reproduces the disease as it is seen in man.
Q. Doesn't that cause you a great concern in extrapolating your findings from rats and mice to man when we start talking about myelodysplastic syndrome and the development of these different kinds of cancers of the lymph and blood?
A. No, not legally. Basically you are working with different pieces of information. You are looking at from a mechanistic standpoint the behavior of isolated murine and human stem cells behave in similar fashion. There do not seem to be any major differences in their behavior. The clinical entities between the clinical entities of a mouse leukemia model or any other animal leukemia model appears to be both strain and species specific.
You can't translate directly to man the clinical manifestations of the diseases, they are very difficult to do. One has to rely a great deal on human literature to evaluate what goes on in man, I agree, but certainly you can use the information we have on the behavior of the various cell populations

to interpret and to refine those observations made in man.
Q. I think you mentioned earlier today that one of the things that you have relied on in forming your opinions as far as literature is the human epidemiology?
A. Yes.
Q. What specific studies can you cite me to in human epidemiology that you have relied upon?
A. For what question?
Q. Start off with the abilities of benzene to cause any leukemia in man.
A. I'm sorry, I don't understand the question. Do you want me to cite for you which papers have anything to do with leukemia in man and benzene exposure or specific leukemias? I'm not-
Q. I'm interested in the human epidemiology studies that you rely upon in forming your opinions as you expressed earlier in the day. I think you said that benzene causes acute myelogenous leukemia in your opinion and not other kinds. If you have human epidemiology that supports that, I would like to know what it is that you find significant. You said earlier before you would opine that AML is caused by benzene exposure you would want

to see some precursor situations, and I would like to see the human epidemiology you have that supports that that you rely upon.
A. The literature I rely on with respect to the relationship between benzene and AML and not other forms of leukemia are the Rinsky study, which I'm sure you are familiar with. Although I would not in the strict sense call it epidemiology, the collections and case studies of Dr. Viglianni and Dr. Aksoy. The Yin study in China, and to a certain extent the Wong study.
Q. Which Wong study?
A. I would have to in order to give you specific citation, I don't think I brought that one, so I would have to give that to you.
Q. Are you talking about the Wong study that was done for the Chemical Manufacturers Association of Benzene Exposed Chemical Workers?
A. I believe so.
Q. Now if I remember right for the Wong, Chemical Manufacturers Association Workers, none of these men had AML?
A. That study has some problems with respect to interpreting the control group and also with respect to the exposure. That's why when I

mentioned it I qualified it. There is something unique about that population. Even so, I don't think the study demonstrates a relationship with the other types of leukemias, but it does--it is anomalous to the extent that it--there are no AMLs in that study, you are correct. It is definitely a unique study in the benzene literature.
Q. You mentioned the Yen study?
A. Yes.
Q. The Yen study, the Chinese study, they found a statistical significant excess of CMLs, did they not?
A. That is what they reported, yes.
Q. Do you not believe that?
A. No, sir, I don't.
Q. Why do you think it is untrue?
A. The Chinese, the Yen study was conducted by collecting diagnoses that were made at approximately 27,000 different sites throughout China--2,700 maybe, I think I'm off. The diagnoses vary considerably from one place to another in terms of sophistication and also with respect to accuracy. In the case of AML it is not really a major issue. With respect to CML it is very important because they don't have cytogenetic

analogies as part of the diagnostic paradigms that they use in general. From that standpoint it is very likely that cases of myelodysplastic syndrome in one version or another are going to be misdiagnosed as CML because they don't have the sophistication for diagnosis of CML that we commonly require in the west.
Q. I thought I understood you to say earlier that if a person has MDS they are more likely to develop AML and not CML?
A. That's correct.
Q. How is it that you can have a person with MDS and misdiagnose it as CML?
A. Because you are looking at a chronic proliferative syndrome that may involve relatively mature granulocytes.
Q. So you believe that it is more likely to misdiagnose a leukemia as a CML than it is as an AML?
A. It is not more likely to--you are not more likely to misdiagnose an acute--could you repeat the way you said it so that I can answer it--answer the question directly?
Q. Right. CML frequently goes into a blast crisis which leads to death?

A. That's correct.
Q. If you see it in a blast crisis you are going to call it an AML. if you don't know about the previous history, that's a misdiagnosis?
A. It's possible. That's more likely than confusing an AML with the CML. If one is dealing with an immunoblast or a lymphoblast crisis you are not likely to misdiagnose it. And if you have got cytogenetic backup you probably will not misdiagnose it because you can confirm in fact that it was a CML. What I'm saying is that whether you have got chronic protracted abnormalities associated with myelodysplastic syndrome and/or chronic abnormalities resulting from bone marrow damage, that the distinction between myelodysplastic syndrome and CML can be very difficult. In fact there have been studies conducted in the United States that demonstrate that in the absence of that kind of cytogenetic sophistication, well over 90 percent of cases can be misdiagnosed.
Q. You are familiar enough with epidemiology to know that the comparison group being a general population comparison group, if errors are made in the study group, the same kind of random errors such as misdiagnosis are going to be made in

the reference population and those should cancel out; isn't that the theory of epidemiology?
A. That does not hold in this specific case because I would fully expect that in populations exposed to high levels of benzene you are going to have an increase in myelodysplasia, you are going to have bone marrow lesions and suppression and an increased incidence in myelodysplastic syndrome.
Q. Has this been followed up as far as you know in the Yen study to confirm there has been this misdiagnosis?
A. The NCI, I believe, is currently conducting a study in China now to clarify issues such as that. I don't know what stage they are.
Q. You don't know?
A. I don't know what stage they are at the present time.
Q. Are you aware that other epidemiological studies have found significantly excessive amounts of CMLs in benzene exposed workers?
A. No.
Q. Are you aware of statistically significant increases in leukemias in benzene exposed workers besides Wong studies of the CMA workers where no AMLs were found?

A. No. Excuse me, could you repeat that last question.
(Whereupon, the last question was read.)
A. My answer is the same, although I would qualify that by saying that where the epidemiology studies in the rubber industry show an excess of certainly what appears to be lymphoid neoplasms. But the issue of exposure, I think, is one that is not consonant with benzene. There may be some benzene exposures that are documented in some of those studies, but it's a much more complicated exposure paradigm than where you have got relatively clear-cut exposure to just benzene. Those studies are different. Their findings are different.
Q. I don't understand that answer. You need to tell me, if you could, a little bit more about what you mean about these exposure paradigms that are not just benzene in rubber workers.
A. Well, any situation where you have potential for exposure to benzene, certainly based upon our knowledge of benzene toxicity of the blood and bone marrow and its relationship to AML, it certainly serves as a point of departure for making assumptions as to potential relationship to causing

leukemias of different types, even though the studies demonstrate fairly clearly a relationship with AML. When you look at the rubber industry where benzene exposure has been hypothesized as playing a role, one sees a different pattern of leukemias and lymphomas, including lymphoid. My initial impression with that literature prior to reviewing it in detail was that I certainly didn't have a strong opinion, but I freely related the opinions of others on whether or not there were other tumor types associated with benzene exposure in those populations.
But after reviewing the literature in detail and taking into account the potential exposures, I'm convinced that there is evidence to suggest, fairly strong evidence, that benzene is not the causal agent in those studies. That you are looking at a different exposure situation and different constellation of diseases than you see with benzene.
Q. Do you have an opinion as to what the cause of these other disease pat-terns in rubber workers would be if it is not benzene?
A. I can relate to you in the case of certainly the Checkoway study that a greater

association was found with carbon tetrachloride and with carbon disulfide than with benzene. My interpretation of that is not that carbon disulfide and carbon tetrachloride have been demonstrated to cause these lesions, but certainly benzene is not an age actor in that exposure scenario.
Rubber industry and tire manufacture is an incredibly complicated industrial picture from the standpoint of evaluating exposures. Exposures, I think, are very complicated, and I see no evidence in that literature taking it in its entirety to suggest that benzene is the cause of that particular pattern of lesions.
Q. Would you even go so far as to say that those kinds of disease patterns that you found for rubber workers, the increase in, I think you said the lymphomas, are those occupational diseases?
A. I think there is reason to be concerned about exposure in the rubber industry. I think that the studies suggest there is an increased incidence of lymphoid neoplasms. I do not know why, and it is inconsistent with my knowledge of the benzene literature where in fact we have reasonably clear cut exposures to benzene.
Q. Are you reasonably well satisfied,

though, that you can say no lymphoid neoplasms are occupational related even though you can't say which agent in this complex environment was the cause?
A. There is reason to conclude that there are excesses in lymphoid neoplasms. With respect to individual ones I would feel much less certain about speculating on specific causes.
Q. I'm not even asking you to do that. I'm saying, look at the group. If you had a study and it reported 15, would you be in a position to opine that any one of the 15 was an occupational disease not even knowing what specific agent was the cause?
A. If you had previous studies which demonstrate specific increases in the specific tumor type, yes. I would not want to do it on the basis of a collection of different types of tumors. I don't believe that's biologically plausible.
Q. Isn't that where you are, though, on the lymphoid neoplasms of rubber workers?
A. To a certain extent, yes. We could look at individual studies, if you want. If we are going to talk about specific types I would like to, but the problem there is that in the conduct of most of those studies the object has been to get enough

power to see something. So there has been a lot of lumping, and the lumping makes it very difficult to figure out exactly what's going on. Is there an increase in the general categories? There appears to be.
Q. The lumping of the different kinds of diseases dilutes the ability to remove that confounding factor; is that what you are saying?
A. The lumping does not allow you to look at specific causation for specific tumor types.
Q. And it is a confounding factor?
A. Lumping is a confounding factor, yes.
Q. And the same token if we start looking at a specific disease category and looking at exposure levels necessary to produce a given disease, if we lump different exposure categories together, that's another confounding factor that dilutes our ability to answer that question too, isn't it?
A. I may not be speaking to your question because I'm not sure I understand it, but analysis of exposure is one of the weakest aspects of epidemiology in general. Very hard, very difficult.
Q. Getting back to the diseases for a moment. Do all CMLs demonstrate the Philadelphia chromosome that you talked about earlier?

A. No. There is a very small percentage
that do not.
Q. Where do we classify the CMLs without
causing a Philadelphia chromosome?
A. About half of them, or roughly half of
them, can be shown to have the same molecular lesion
in terms of the rearrangement of genes that occurs in
Philadelphia chromosome positive CML. There is
another proportion of those that very well are missed
diagnoses in the sense that a recent study conducted
4 at the University of Chicago demonstrated that in
some 27 collected cases in the Philadelphia
chromosome negative CML, 26 of them, I think, it was
either 25 or 26, were rediagnosed as myelodysplastic
syndrome. So without the cytogenetics or the
molecular genetics that diagnosis is not simple even
in the United States.
Q. Explain to me again. You said that the
Philadelphia chromosome is a specific chromosome, and
yet-
A. It's a translocation. It's not--it's a
translocation of bits and parts of 2, 9 to 22.
Q. And then you said that there are some
of the CMLs that have a translocation that resembles
the Philadelphia chromosome?

A. They have a molecular change that's very similar to what's seen in the Philadelphia chromosome associated with the cluster region and the proto-onco gene, but they don't have the demonstrable--we are talking about a very refined molecular change that is not demonstrable physically in the eyes of the Philadelphia chromosome, but they have the same molecular changes that are seen with individuals who have the Philadelphia chromosome.
Q. Whose work is that that you are talking about?
A. Name that comes to mind right off the bat is Kurzrock at Texas. There are several others, but she's done quite a bit.
Q. Do the CMLs that have the Philadelphia chromosome and the CMLs that have the similar translocation manifest themselves in the same way?
A. As I recall those studies, they are very similar. They are very similar characteristics. In fact the motivation of doing that type of detailed analysis is from a prognostic standpoint.
Q. From a-
A. For prognostic reasons.
Q. So the CMLs that either do not have the

Philadelphia chromosome or don't have this other translocation similar to it you say are misdiagnosed as MDS?
A. Not necessarily all of them, but there is certainly compelling evidence to suggest that that's a major problem for that relatively small proportion of cases. I'm not saying that all of them are misdiagnosed. I'm saying that there is evidence that in the absence of that kind of information it is a very difficult call.
Q. Do humans who develop MDS later develop CML?
A. Not to my knowledge, no.
Q. What about other leukemias besides CML?
A. AML? Certainly myelodysplastic syndrome secondary to bone marrow damage. Chemotherapy has a very high predisposition to transform into AML.
Q. Are you not aware of any studies that show people who have received chemotherapy go on to develop CML?
A. Not in the absence of combined radiation therapy.
Q. Requires both?
A. Yes. And there have been--that

literature base is very large.
Q. Does radiation produce CML in humans?
A. Yes, there is evidence for that.
Q. How do you explain that biologically on your chart here that you have prepared?
A. In terms of what mechanistic understanding we have of it. First of all, it is a finding, it's a fact, it's an observation that appears to be very reproducible throughout the literature. Taking that as fact, if one looks at the processes that are involved in damaging cells, chemotherapeutic agents tend to--cells are more susceptible to most chemotherapeutic agents during cycle when they are dividing.
Radiation, on the other hand, tends to--cells are actually more susceptible when they are at rest. So radiation has a much greater potential, if you will, for targeting a predominantly resting population, and that's the best explanation that I can give for it. Radiation can also affect dividing cells, but there are no chemicals that I know of that are associated with the production of CML.
Q. What other arrays of blood and lymph diseases are you aware of associated with radiation exposure, ionizing radiation?

A. Ionizing radiation causes a large variety of different tumor types within the hematopoietic system in the lymphoid system. The only disease process, the only malignant disease associated with the hematopoietic and lymphoid system that I would say categorically can't be caused by radiation is chronic lymphocytic leukemia, CLL. There is no evidence that anything causes CLL.
Q. Can you explain mechanistically why ionizing radiation can have such a broad effect, whereas other agents, chemical agents, cannot?
A. Not with any--it would be wild speculation. Radiation doesn't follow pharmacokinetic or metabolic pathways. You are not looking at activation, you are not looking at distribution that can be compartmentalized in any number of different ways. You are looking at potentially different combinations of molecular mechanisms that could be involved. With radiation these things are pretty well simplified, and that may or may not impact on why radiation has such a broad spectrum. I would say the fact that radiation with target resting cells is fairly important probably plays an important role.
Q. How would you design an experiment to

show that a chemotherapeutic agent attacks dividing cells as opposed to resting cells or vice versa?
A. Well, most of the changes that are seen associated with chromosomal abnormalities require cell division for those abnormalities to occur. T-cells are more susceptible in terms of cytotoxicity during that period, and I think you will find it is generally--it's a generally accepted concept in the medical scientific community that most chemotherapeutic agents act on cytocells. There are some exceptions, but by the same token these appear to be T-cells that are most susceptible to the effects.
Q. Isn't that looking at the result as opposed to the effect, the kind of studies you just described?
A. You are faced again with the--you can't have it both ways. You are faced again with the problems, just the innate problems of the field, you can't prove a negative. It is very difficult to prove a negative. You can only do so if you have got evidence to demonstrate that all your other technology and techniques are sufficiently sensitive and specific to see if it was there. That's where I think we are with CML.

It is really clear what T-cell type CML is. It is not proved to be anywhere near as easy to demonstrate a relationship for other target cells for other diseases.
Q. What evidence do you have that radiation affects cells at rest?
A. I would have to go back in the literature and find that for you. That comes out of the radiation biology work that was done subsequent to surrounding the studies done following the bombing in Japan during World War II. Some of Gene Cronkite's work looking at radiation sensitivities. I can find that for you, but I don't have it at my fingertips now.
Q. Don't all those studies require at least one cell division to occur before you can find a result?
A. I can tell you for a fact that if you don't have replication you are never going to see malignancy because if it doesn't divide you are not going to have it.
Q. Doesn't that in and of itself say that you don't really know that radiation effect is coming from attacking a cell at rest?
A. The susceptibility of the cell

populations to the cytotoxic effects of the agent can be demonstrated in the case of radiation resting cells are as sensitive, if not more, slightly more sensitive than dividing cells. For chemotherapeutic agents dividing cells are clearly the most sensitive population.
Q. That's what I was asking earlier is how would you design a study to demonstrate that?
A. By looking at the relative dosimetry of the effects of whatever agents you want on predominantly resting or dividing cell populations.
Q. Isn't that just answering in a circle?
A. That is how you would go about designing an experiment to look at it. I'm not quite sure what you are driving at.
Q. Earlier you listed for me some of the metabolites of benzene, and I didn't hear you say catechol.
A. Catechol produces some effects on the lymphoid system when you look at it in various tests. It doesn't appear to be as effective in interfering with cell replication as the others, which is why I didn't discuss it. We were talking about replication effects, and in that context catechol, although it has some activity, it's much

less potent than the others, than the quinones.
Q. If I understood what you told me earlier, the toxicity of the benzene metabolites in humans to you is not of consequence?
A. Independently?
Q. Yes.
A. No, I think you would be hard-pressed to produce the same effects that you get when you expose someone to high levels of benzene.
Q. What about a person who already has an immunological disease, would the toxicologic effects of the benzene metabolites have an effect on a person who already has leukemia or lymphoma?
A. It might be beneficial if it had any effect at all, and I doubt it would be easy to demonstrate it. You are much more likely to have--in order to get high enough concentrations that could possibly deliver sufficient material to a relevant target organ like the bone marrow, you would have acute and subacute toxicity in other organ systems.
Q. What role does the benzene metabolite, or I should say the benzene metabolites, what role do they play in the regulation of the development of these different cell lines of the blood and the lymph?

A. If I knew the answer to that I could probably get a free ride to Stockholm. That's one thing we are definitely interested in determining. It looks as though some hydroquinone has effects on one particular stroma population in the bone marrow known as the bone marrow macrophages that alters lympho production. How, we don't know. That appears to have effects on in turn development of certain other cell types, but I don't think there is a scenario that paradigms what I just described to you that would impact on the development, the initiation of a leukemia. I think you have to look for a direct effect on cell population. So I would say that exactly what's going on, the jury is still out.
Q. Maybe I have been using some bad terminology in trying to communicate. If you have a material that affects either the rate of cell production or the direction of cell development, would you be calling that effect something other than a toxic effect?
A. Yes.
Q. What do you call that effect?
A. Well, I can give you--there are examples. There are a number of endogenous materials that regulate what direction these things go, or at

least their rate of--their proliferation in response to different stimuli that are involved in the physiology of controlling the regulating hematopoiesis.
Q. What do we call that change? What shall we call it if not toxic effects, what-
A. That goes on in normal everyday physiology. That's how the process adjusts to any kind of insult we may have, whatever it is. Given that, why don't you rephrase the question so I understand what you are looking for.
Q. Some treatments of disease attempt to regulate this cell division and cell development direction, do they not?
A. Are you talking in terms of chemotherapeutic agents or new experimental therapy in terms of altering regulation of hematopoiesis?
Q. Either one.
A. They are totally different.
Q. Tell me how they are different and what we call the mechanism in each case so we can communicate.
A. Chemotherapeutic agents target dividing cells, certainly those that we are talking about for the most part with respect to the hematologic

abnormalities. They kill them basically. The cytotoxicity. Some are cycle specific, some are phase specific, some are not. Those are agents that are used in the treatment of cancers. Cancers tend to be fairly rapidly proliferating so the bone marrow cells, ergo, you have a problem in preserving and protecting the bone marrow while attempting to kill off tumor cells.
There are newer therapies that are experimental at this stage that attempt to either redirect or alter the progression of diseases such as myelodysplastic syndrome and some of the leukemias by using growth factors which had been purified and isolated that are known to affect the normal differentiation processes in the bone marrow. These are highly complicated systems that we don't fully understand, and they are largely experimental.
Q. Do those kinds of effects have a name that you use? The second example you gave me of giving direction to cell development.
A. I'm not exactly sure what you mean, so I would hesitate to throw out a word because I'm not quite sure what you are-
Q. What I'm getting to is does exposure to a chemical such as benzene play any part in this

direction of cell development, cell proliferation, and indeed the development of defense mechanisms that are involved with the fighting of leukemia? Not in fighting a person who is diseased with leukemia, but a person who has had the effect and how the body's defenses are brought into play?
A. I see about three questions in there. Let me answer the one that I can focus on right away. Anything that affects cells here, takes them out.
Q. Here being?
A. In the differentiating blood and immune cell compartment where we are producing this rapid division committed cells, anything that destroys a significant number of cells in any one of these pathways, be it the lymphoid, erythrocyte, granulocyte, platelet, what have you, it's going to have an impact on the regulation of the entry of cells into this compartment. That's physiologic response. That's how we can respond to any kind of damage, whether it is loss of blood cells, damage to precursor cells, that's at some point--the system can absorb major challenges with respect to production of these cells and still effectively produce normal cells. It does

so through a number of mechanisms. Ultimately if there is enough damage out here you are going to see a reorientation of the number of dividing precursor and stem cells in this early compartment. And benzene will do that just like any number of other agents will.
Q. Now that's not really the question that I was asking, although I accept that as an answer to a question that was in there. What I want to know is now assume that we have a major insult to one of these cell systems. To communicate that information back to the stem cells to tell them to produce, to tell them which way to produce, where the need is, that's a different mechanism than the destruction of the cells out here in the peripheral blood?
A. Yes. Yes.
Q. So what I'm asking you is does benzene or its metabolites play any role in interfering with or directing that message sending back to the stem cells and giving direction as to what to do next?
A. Based on the one system that we have that's been studied which involves hydroquinone and its effects on the macrophages that I indicated, I would say that's a plausible scenario. It is certainly one we are investigating. I can't give you

a definitive answer to it.
Q. In the level that you are talking about for hydroquinone being able to affect this message transfer, this is at a level that is-different from a toxic response level?
A. No, it's not. Well, depends on your definition. It's not cytotoxic to this individual cell because the cell is still alive, but it's at a functional level within the cell. Now if you have a profound effect on that process in the bone marrow in a number of cells, you are going to have a toxic phenomenon at the level of the organism, you are going to have myeloid suppression, you are going to have decrease in circulating cells.
I know of no evidence that would indicate that this goes on at a level that would not be associated with measurable alterations in one or more cell types within the immune or the hematopoietic system.
Q. I need for you to explain that last part. I didn't follow the last part.
A. I think the changes as we know them, and we are at the very beginnings of this field, are at concentrations that are most likely--the effects are most likely to be seen immediately in terms of

suppression of cell types, either in the marrow or the peripheral blood. I don't think that there is any--there is no evidence to suggest they go on at a level that would not be measurable in terms of the effects on the cells because we are still talking about support for proliferating cells, and it is manifested in a decrease in the production of cell types or an alteration in the direction they go in. So you are going to see it. It will be associated with toxicity at the whole animal level, if that may be what you were looking for.

Q. I think you answered my question. Have you gone even so far as to look at one cause of the change out here in the peripheral blood that calls for the message being sent and seeing what level of quinone involvement there is in interrupting a message or altering the message? In other words, the quinone or a benzene, for instance, with a quinone as a metabolite was not the cause of the problem, but the quinone coming in interferes?

A. You know, I'm sorry, I thought I understood you before you clarified it. We know what concentrations of hydroquinone will result in isolated cell systems in blocking division, okay? That's relatively well worked out. That is going to

result in the loss of cells here, there is no doubt about it. Cycling cells are more susceptible to the cytotoxic characteristics of these metabolites than toxic cells, no doubt about that. I don't understand what you were asking with respect to the feedback there.
Q. There are other agents that can cause the circulating blood cells that are cytotoxic to the circulating blood cells besides benzene?
A. Oh, lord, yes.
Q. What I'm asking is if you have one of these other agents; that is, the agent that knocks down the peripheral blood, have you looked at what level of quinone involvement there would have to be necessary to interfere with the transmission of the message back to the parent cells?
A. I do not understand the question. Other agents. I don't understand how you get other agents and quinone in the same sentence. I don't understand what--the paradigm I don't understand.
Q. Take concomitant exposures, there are chemotherapy agents that are able to affect the peripheral blood to knock down the different elements on the peripheral bloods, right?
A. Yes.

Q. They have nothing to do with the production of hydroquinone in the body?
A. Yes, that's true.
Q. So now if you have a concomitant exposure to benzene, which does give you hydroquinone?
A. You are looking now exposed to a therapeutic agent and the benzene?
Q. Yes.
A. Okay.
Q. Does the resulting metabolite, the hydroquinone from the metabolism of benzene, interfere with the transmission of information back to these parent cells to do more?
A. There is absolutely no information on that whatsoever.
Q. Just don't know one way or the other, has not been looked at?
A. Has not been looked at.
Q. That's what I was trying to ask, have you looked at-
A. No, we are still trying to figure out what hydroquinone does. As soon as you start dropping in multiple agents, you have a very complex system, if it wasn't complex enough to begin with.

Q. Is hydroquinone involved in this process of communications back and forth to the cells in a person that is not exposed to benzene or another chemical agent that will result in a metabolic result of hydroquinone?
A. You mean is hydroquinone normally involved in the process?
Q. Yes.
A. Not hydroquinone. There may be other quinones or alpha beta and saturated carbonyl or keto structures that play a role in regulating this process, but to my knowledge not hydroquinone.
Q. Is the reason you focused on hydroquinone because of your interest in benzene metabolism and its effects?
A. To begin with, yes, hydroquinone has taken on a life of its own for different reasons related to understanding how cells divide and are regulated. But, yes, if you are looking for a hypothesis from me, I think hydroquinone plays a--probably plays a fairly important role in the evolution of bone marrow damage associated with benzene, which you can get from reading my papers. That's nothing new.
Q. We have yet to address another area of

your work that I wanted to go into, and that's work with butadiene. Tell me how butadiene and the metabolites of butadiene, if they do, come into the development of the blood system.
(Whereupon, there was discussion outside the record between the deponent and his counsel.)
MR. ZATZ: I assume you have some wild theory of what this has to do with this case or how it is going to lead to relevant evidence in the cases of Carter and Riddle, or are we taking Dr. Irons' deposition for some other case simply because we have him here?
MR. HOBSON: I don't think we are doing that at all. Any time you depose a man who is named as a witness in many other cases, obviously you can say it has an impact on other cases, so I won't tell you it doesn't. But at the same time I think Dr. Irons' bibliography will show he has published a fair amount of work on butadiene, and I think how all of these chemical exposures--we sat here and we have talked about chemotherapeutic agents and how they develop into different diseases, butadiene is another agent that affects the blood, and how all of these different effects from different chemicals and the

mechanisms fit together I think are important to understanding how he thinks.
MR. ZATZ: Well-
MR. HOBSON: I think that's why I'm here.
MR. ZATZ: I understand that, and I didn't cut you off in quizzing him on Hodgkin's disease and multiple myelomas and that sort of thing, but it seems to me at some point we go beyond a hematology quiz, which I think is fair game, and we get into subject matter of other cases. If that's what we are doing now, I think there are probably other defense counsel in those cases who would certainly prefer to be here when you are doing it.
MR. HOBSON: You going to tell him not to answer, or what?
MR. ZATZ: No, I'm not instructing him not to answer.
MR. HOBSON: I'm going to ask him the questions.
MR. ZATZ: My objection is simply this, just so it is absolutely clear on the record, if there is some way in which all this is going to lead to evidence relevant to these cases, I'll allow him to answer, although I don't particulary see the

relevance. If what you are doing is simply taking Dr. Irons' deposition for some other cases, then I feel constrained on behalf of other counsel to instruct him not to answer.
MR. HOBSON: Well, I guess you have to make a choice.
MR. ZATZ: Which are you doing?
MR. HOBSON: I'm taking his deposition in this case, but I'm not going to tell you it won't impact others because he has been named as an expert in other cases involving butadiene too. But as I said a moment ago, you said I was going through a lecture on hematology, I don't think Dr. Irons holds himself out as a hematologist, he is a toxicologist as I understand his credentials, and we have been talking about the effects of blood--of chemicals on the blood, and that's where I'm going. I think that's where his work has been, how do chemicals affect the blood, and I want to know what his opinions are and how he got there.
MR. ZATZ: We can call it hematotoxicology if you think that's fair. At some point if this appears to be simply an endeavor to do discovery in some other case, I may change my mind.
MR. HOBSON: Just like a woman you can

have that prerogative.
Q. (BY MR. HOBSON) I have forgotten where we were, Dr. Irons, so let's start over. Would you tell me based on your knowledge how butadiene fits into the development of diseases of the blood as you understand it.
A. I don't have any evidence to conclude, nor do I believe, that butadiene acts in any way, shape, or form in a manner similar to benzene. It is a totally different agent and it acts through different mechanisms. Unless you want me to just outline the various studies I have done and what I know about butadiene, that's the best answer I can give you. Where we could spend all day talking about benzene and what it does and probably not run out of things I could tell you, with butadiene I'm going to run out of things very quickly.
Q. Then we won't take much time. Are there diseases that arise through this pluripotential stem cell? That is, either diseases of the blood or the lymph that butadiene is associated with in your mind?
A. The number of diseases that I think arise from the pluripotential stem cell in any event are few and far between. In the case of butadiene,

in certainly the one model I am intimately familiar with, the mouse, that I have a suspicion we are looking in here.
Q. In here being the lymphoid stem cell area?
A. The lymphoid committed stem cell area, primarily down the T-cell line, but again we are talking about a totally different model in which there are very likely a number of other confounding factors. Huge strain specificity and species specificity with respect to the behavior of butadiene involving any number of different things, not the least of which in the mouse is probably retrovirus, and now we're getting into very deep soil. The mechanisms are very, very complicated.
Q. My reading of some of your recent work on butadiene says it wasn't just the retrovirus, that there are other mechanisms involved too?
A. There are multiple mechanisms involved with butadiene, I suspect. Do I know that for a fact? No. Butadiene in the mouse, butadiene is a very complicated model.
Q. What is the extent of your knowledge about butadiene effects in a human related to the blood and lymph system?

A. Would you mind if I preface that?
Q. Surely, go ahead.
A. I consider epidemiology in general to be a fairly weak discipline. The methodologies and the power of the tests that are available to us are very difficult, and it is a very difficult field to work in. When I look at epi studies, therefore, I like to look for patterns, not individual studies. Individual study may give you an effect if it is reproducible, then it is more likely to be an association that you need to look at. If you have mechanistic understanding of what's going on, it helps in interpreting that.
In the case of butadiene I see a tremendous amount of inconsistency with respect to the available epidemiology studies. Some have suggested a potential relationship with lymphoid neoplasms, others have not. Others find different neoplasms, and I don't believe that the same agent under the same conditions is going to produce different tumor types in different groups. I see no reason to believe that. So at this point I think the epi studies are of very limited value in establishing and understanding the butadiene toxicity in man. Did that answer your question?

Q. It's getting there, yes. Based on the work that you have done with butadiene, if you look at the mechanisms, can you tell me which mechanism seems to be involved in the development of the lymphomas in mice? In other words, is it at the genetic level? Is it some other effect? I know you talked a little about the retrovirus.
A. I think the retrovirus influences that disease. I'm not sure whether it is causal, but it certainly plays a role in causation based on the indirect evidence we have. A whole lot of very brilliant scientists have donated their careers to the trash heap of murine leukemogenesis associated with retrovirus.
Retrovirology in the mouse is a maze.
Based on the chemistry of butadiene and some of the metabolites we know are formed, I suspect we are probably having some effects at the level of the DNA or the Genome, but we are talking wild speculation.
It alters differentiation. I have demonstrated that certainly for the hematopoietic system.
That doesn't seem to be--it doesn't produce a very pronounced anemia, and the stuff is just not very toxic from the standpoint of acute or subacute effects, but it causes profound activation

in the endogenous ecotropic. Retrovirus is just retro and virus. If you are a mouse you don't want to be exposed to butadiene.
Q. Where are you on knowing how butadiene is metabolized in humans?
A. I would say we are not very close to knowing how butadiene is metabolized in mice. Initial studies I don't think are complete, and I think the metabolism of the compounds is far more complex than people have originally thought..
Q. So you wouldn't be able to say if the same family, chemical families of metabolites exist for butadiene as for benzene?
A. That I can tell you. No. That's easy.
Q. It is different?
A. It's different. That's probably the only yes, no answer.
Q. Tell me what function in the human body the T-cell lineage plays. I know it may be many, many but give me the major roles as you understand them.
A. The T-cell is the cell classically associated with what we call cell mediated immunity, cell mediated immune response, and that was appreciated probably as early as we had a clear

understanding of T-cells and B-cells. Over the last ten to 15 years we have come to appreciate that there are multiple different subpopulations of T-cells, and they play an integral role in regulating and mediating immune response. They are very, very active in regulating their own behavior. They regulate the behavior of B-cells. They are the pivotal cell in regulating specific immune response. Now I could go into detail. I teach the subject. We could spend the next several hours if you want.
Q. Maybe not several hours, but several minutes. How in disease does the T-cell line get involved? In other words, bring it down, if you can, to more of a layman's language in explaining what a T-cell does in playing its part in fighting off disease or in warring against cancer.
MR.. ZATZ: I'm going to object to the form of that question on this because I think it is asking you two very different things.
A. It is.
Q. (BY MR. HOBSON) There are two questions there, I agree.
A. Cell mediated immune response is immune response that's targeted at diseases or organisms

that are not effectively removed simply by producing antibodies to them. It may take up residence inside cells. So cell mediated means basically that the T-cell and the cell response is targeted towards destroying cells that are infected with a foreign agent as a means of stopping the spread of the agent.
So T-cells are involved in recognition of self and recognizing whether there is something nasty inside the cell like a virus that needs to be eliminated. And it does so by recognizing some aspect of the virus on the surface of a cell that it otherwise recognizes as part of self. It also helps regulate antibody production and has several different classes of T-cells that help regulate their own response and antibody response.
So it goes up and it comes down and you have some appropriate control over the process. It's a very complicated network involved in providing us with immunity to infectious agents that are basically intracellular.
Q. You have answered my question as to the disease aspects, primarily, I guess, infectious disease.
A. Right.

Q. Does the T-cell play any part in warding off cancer in the human body?
A. There are animal models that suggest that profound immunosuppression plays a role in the incidence or development of certain types of neoplasms, various specific mouse models, so certainly some aspects of T-cells, primarily the cytotoxic T-cells and perhaps another cell type that's not really within the T-cell lineage, the NK cell, play a role in mediating against the development of tumors, certain types of tumors for sure.
The concept of immune surveillance has been around for a long time. There are very few systems, I can't think of a single model where I would say immune surveillance has been absolutely demonstrated in that context where a single cell is acted upon by a system. Certainly the immune system plays a role in animal models in susceptibility to tumor development, certain types of tumor development.
In man the evidence is, of course, a little bit different because we can't go around setting up these nice control groups and exposing people to things. There is certainly evidence that

immunosuppression involved in treating various autoimmune diseases and in transplantation cases can lead to the development of certain types of tumors, primarily those of lymphoid origin, it certainly predisposes to that.
The problem is in almost all the studies that have been conducted you are not looking at something that is selectively immunosuppressive, you are usually dealing with the administration of alkylating agents as well, which gets back to the whole issue of secondary tumor types, and it is a very complex picture. But certainly for cells of the immune system or lymphoid tumors there is some evidence that immune surveillance plays a role, if only inferentially from the studies of immunosuppressed patients.
Q. Do you know if there is butadiene exposure in a tire plant?
A. I am not an industrial hygienist. I rely on industrial hygiene data to render an opinion. If you want to know whether I personally know if there has been a butadiene exposure in a tire plant, my answer is no.
Q. Do you know of a compound called tertiary butacatechol?

A. Tert butacatechol, I have heard of it.
Q. Do you know of any of its uses?
A. No.
Q. Do you know if tert butacatechol, as you have referred to it, how it structurally compares to the catechol that is the metabolite of benzene?
A. No. Any substitution to that ring is going to alter the chemistry of the catechol dramatically.
Q. Have you seen any information about metabolism of tertiary butacatechol?
A. No.
Q. I would like to get, if I could, your understanding of some different terminology so that I can hopefully understand how you might have used it or might use it in the future. A mutagen. How would you use the term mutagen?
A. The way it is most popularly used is the way I refer to it, and that is something that actively causes directly a mutation. Mutagenic event at the level of the DNA.
Q. And to go further, what do you mean by a mutation at the. level of DNA?
A. A specific change associated with the alkylation or deletion of a base para sequence that

leads to the misreading of the genetic code.
Q. Clastogenic?
A. Something that changes chromosomes.
Q. Is there a difference between a mutagenic agent and a clastogenic agent?
A. Yes. By the definitions I just gave you, yes.
Q. Explain clearly the difference in the use of those two terms for me.
A. Shall we cut to it? Benzene metabolite such as hydroquinone are clastogenic, they are not mutagenic in the context of studies aimed at looking at mutagenic mutagens, they are negative.
Q. What about the other metabolites of benzene?
A. Same. Para-benzoquinone is going to be very similar to hydroquinone. To my knowledge none of them have been shown to be mutagenic. Catechol is more cytotoxic than hydroquinone but less effective at clastogenic events at least related to nondisjunctional events. Phenol just kills cells. It is not effective in either category.
Q. Promote or promoter in terms of in the context of carcinogenicity?
A. If we talk in the context of

carcinogenicity it means one thing. If we talk in context of diseases of the blood and bone marrow it is entirely different, usually being that they have never been established to be relevant to the hematopoietic system.
Q. I have heard you say that before, that's why I asked in terms of carcinogenicity.
A. Usually they refer to studies done in the skin. There are some liver models in which one is looking at a paradigm in which an agent administered after an initiating event can enhance or exacerbate the expression of tumor phenotype in one model or another.
Q. When you say there is no model that has been found for the hematopoietic system, has it been looked for?
A. Certainly the same agents that have been shown to be effective in at least the paradigms that have been used in the skin models and liver models have been looked at in terms of the hematopoiesis. There is none. Certainly the compounds have been looked at in the same context, but I don't know of anyone who has specifically done initiation promotion studies because we don't have a system that is amenable to that.

The problem with promotion paradigms is they aren't definitive in terms of what you are looking for--they are a paradigm. They are an elaborate hypothesis, if you will, and if it doesn't fit the algorithm, then the biology is just as relevant as any other biology, but it doesn't fit your model so you don't know what you have got. Therefore, I would say they have been looked at in the context that the same compounds have been administered to the animals that develop tumors of the hematopoietic system, but there is no clear paradigm that can be associated with initiation and promotion.
Q. You have used many times today the term paradigm.
A. Model, pattern.
Q. I just want to make sure I don't miss that one too. The term immune system depressant, what would that mean to you?
A. Something that suppresses immune response. In general I mean something that demonstrably alters host resistance to the animal. You can say, for example, that hydroquinone as an immune system suppresses certain aspects of immune function in isolated systems. That doesn't mean

automatically that hydroquinone administered to the animal at tolerable concentrations in some way is going to automatically lead to immunosuppression, altered physiologic resistance to infection, or what have you as a function of that. So I distinguish between something that suppresses immune function and something that alters an isolated cell function.
Q. How broad would you be using the term immune system in that context?
A. The immune system is anything and everything that includes lymphocytes, macrophages, dendritic cells, a variety of effecter cells, and accessory cells, the whole immune system.
Q. Something like hydroquinone, does it affect the whole of the immune system, or a very narrow part?
A. You can show isolated effects in a variety of different cell types that are interesting that probably are important with respect to understanding what hydroquinone can and can't do. They are excellent models for studying the behavior of that compound. The consequence of high-level exposure to hydroquinone in terms of its effects on the immune system is a separate issue, and I do not know of any evidence to suggest that you can

administer hydroquinone even to animals and produce effects on immune response that don't also result in reduction in cell types, damage to the bone marrow, and a variety of other findings as well.
Q. If we talk about benzene exposure, how long after exposure ceases will you expect to find immunologic effects?
A. Immunologic effects?
Q. Yes.
A. What do you mean by immunologic effects?
Q. I'll start there. Give me a list again of the immunologic system, and then we can talk about the different effects, maybe that's a better way of doing it.
A. You mean cell types? We were talking about B-cells, T-lymphocytes, macrophages, monocytes.
Q. All of them? I think you see where I want to go.
A. If I did, this would be a little easier.
Q. I would like to know the effects that you have seen benzene produce in either animals or man, and which of those effects, if any, persist after exposure ceases, and if you know at what level

of exposure?
A. In experimental animals, most of the studies we have done looking at specific effects on immune response have been done with metabolites because it is very difficult to produce reproducible effects with benzene itself. With the metabolites I have looked at short-term scenarios, and we see recovery, we see recovery, it has been difficult to produce changes that go out for any great length of time, it's the devil to work with if you want to produce something that you can look at for any great length of time.
Q. May I interrupt you for just a second. With your hands in front of your mouth it is hard for me to understand you, and probably her too.
A. Sorry. I don't know of any studies that have looked at the prolonged effects of benzene exposure on immune response in the absence of looking at other things such as peripheral blood counts, damage to the bone marrow, what have you, that have looked at long-term. There may in fact be some and that I have read them, I just can't remember--they certainly didn't leave an indelible stain on my memory.
My own impression of benzene, as I

said, it is not a very potent immune suppressant, you have to get up there in the concentrations that are knocking cells off all over before you are going to see an effect. I do recall early in almost the turn of the century Selling and Weiskotten and a few others looked at the effects of inhalation of benzene on rabbits.
I believe there is at least one what I would call highly uninformative allusion to it decreasing host resistance to either TB or to pneumococcus or something like that in rabbits. Again I think we are talking acute exposures, or subacute exposures. We are not talking about something that's happening or the exposures were ceased and you looked at immune function.
Q. Those were exposures in the rabbits?
A. As I recall they were dosing the stuff on gauze or cotton and just holding them under, which is not very quantitative but certainly effective.
Q. What about other effects on the blood and lymph systems from benzene, can you tell me what effects that you have found that are long lasting after exposure ceases?
A. No. All of ours have been transient with the exception of continued exposure to

hydroquinone in an interrupted regimen in which we got prolonged suppression. But that was with continued exposure. I was trying to produce an equivalent of an aplastic anemia, and came as close as I think I'm ever going to with hydroquinone and phenol together. We have never done it with benzene.
Q. Are there any effects that you know of where you have followed a benzene exposure regimen for a period of time? You told me earlier about some studies that you did where you tried to quantitate or part of your opinion about the quantitative relationship of exposure to benzene and certain effects. Have you done similar-type studies where you have exposed animals over a period of time, not exposed them, and looked at any effect some months after?
A. On immune response?
Q. No, for anything, not just immune response.
A. We have looked at--basically that was the paradigm that we were using. Expose them for 12 weeks, stopped exposure and held them for a year or longer, and we get mild proliferative changes in the spleen.
Q. And your recollection was there was no

effect below 50 parts per million; is that what you said?
A. We didn't look below 50 because we have had a devil of a time historically producing reproducible toxicity at higher concentrations, so we were looking at 50. At 50 it was not very impressive. We are certainly not talking about the same magnitude changes. Given a dose response whereas you know you are having effects at higher concentrations, you could look at some of those spleens and say there are some changes here. But in the absence of a dose response you would be hard-pressed to defend that there was a great deal of effect.
Q. I had one paper this morning that we brought over at the lunch hour that goes into something--I have both here now. It's not your paper. I'll give you the published one here. Is that a paper that you have seen before?
A. Yes.
Q. And just for the record here, unless somebody wants to attach it, would you read the title so we can identify later where it was published?
A. Induction of Cytogenetic Damage in Rodents After Short-Term Inhalation of Benzene.

Q. It was published?
A. Environmental Mutagenesis, pages 29 to 40, 1986.
Q. Explain for me how what is presented in this paper fits into the context of the earlier work you described for me?
A. You are going to have to clarify that.
Q. This paper, I think, presents findings from exposures at less than 50 parts per million?
A. Yes.
Q. And it presents findings that are, I think, a year later; is that right, after exposure ceases?
A. I don't see where they were exposed a year later or they were examined a year later. It is 18 hours after exposure.
Q. It may be my memory has failed me. Why don't you say what the procedure was for dosing the animals and looking and tell me what it was, that way we won't have to guess.
A. Rats were--first of all, mice were exposed to concentrations ranging from zero to 1,000 parts per million, zero, 10, 100 and 1,000 every six hours. Rats were exposed to target concentrations of zero, 1, .3, 13, 10 and 30 for six hours, and then

they were sampled or tested at 18 hours after exposure.
Q. 18 hours after exposure ceased?
A. Yes.
Q. What levels does this study report that there was significant finding, and what findings were significant at those levels?
A. It reports significant findings at 10 parts per million for sister chromatidic exchanges and micronuclei in the rats--wait a minute, excuse me--for the mice and at one part per million in the rat experiment.
Q. Is that again for sister chromatidic exchange in rats?
A. Yes, that's what it reports.
Q. Is this any of your work?
A. No.
Q. This was done at CIIT, but by someone else besides you or your group?
A. That's correct.
Q. Can you fit in for me the results of this study into your previous statements about not finding convincing any data that shows effects below 50 parts per million?
A. What we are talking about, when I talk

about 50 parts per million I'm talking about demonstrable effects to hematopoiesis that can be characterized as being significant with respect to bone marrow damage and/or hematopoiesis. Sister chromatidic exchange is a very sensitive technique for looking at potential effects of a compound on either DNA replication or on occasion it correlates with damage, although it is an error-free process and therefore is not necessarily indicative of damage. It's often misrepresented as that, but it is not really an indicator of cytogenetic damage.
Q. Tell us in laymen's terms as best you can what sister chromatid exchange means in the context of doing these evaluations, and then if you can relate that to what it means to a cell.
A. The last one is easy. It's not really clear that it means anything necessarily with respect to damage, it's an error-free process, or ostensibly an error-free process. It involves the exchange of sister chromatids on the chromosome, and it occurs naturally, spontaneously. So what you are looking at here are differences in rates of what is naturally occurring or spontaneous process.
Q. This process that's being changed rate wise is a change in the chromosomes of the cell?

A. It is a change in--yes.
Q. And tell us again in laymen's terms, if you can, what you mean by chromosome?
A. You have two--you basically are exchanging parts of the chromosome that are the same. You have two and you are basically exchanging parts of them.
Q. I don't know that that communicates much to me. Can you do it again in more detail to tell me what sister chromatidic exchange means a little more elaborately but simple terms?
A. Chromosomes consist of chromatids which are basically matching complimentary sets of the information. When the cell divides, on occasion you get information or material from one chromosome, one chromatid exchanged with a piece of another, and if you have appropriate staining techniques that allow you to look at multiple generations of dividing cells you can measure that.
Q. Now in the context of this study that we are examining, which cells was the change in sister chromatid exchange noted?
A. It was measured in vitro; that is, in culture, in lymphocytes taken from animals that were previously either controls or exposed to benzene.

Q. And which cells were being cultured?
A. Lymphocytes.
Q. And where in the body of these animals would the lymphocytes be found?
A. Peripheral blood or spleen, I forget. Cardiac blood.
Q. Out of the heart?
A. Uh-huh.
Q. Would this be blood that's being circulated, then?
A. Yes.
Q. The heart is a nice reservoir in the animal to-pull the blood from?
A. In the mouse it is one of the few you have got.
Q. And you say that these cells were then cultured. By that I guess you mean grown or allowed to reproduce?
A. They are stimulated with a highly toxic material called lipopolysaccharide or concanavalin, either one which stimulates the cells to divide.
Q. And this effect of sister chromatid exchange that you are looking for would be one produced by the benzene exposure?
A. That's ostensibly the case.

Q. And the conclusion that it was the benzene comes from comparing the exposed animals and what you find in them to the unexposed animals and what you found in the unexposed group; is that right?
A. That's the process.
Q. Is this a real finding in your view?
A. I think it is highly questionable.
Q. Is there anything in the published report that says that this is a highly questionable finding?
A. I don't know whether it says that or not. They do qualify the nature of the dose concentration response curves, especially at the lower end.
Q. And where do you see that indication?
A. On page 38.
Q. Would you read the part that you think says that?
A. It should be noted that the shape of the concentration response curves for both rats and mice from both end points are nonlinear with the greatest slopes at the lower concentrations.
Q. Is there any other indication that you can see in the paper that would say that this is a highly questionable result?

A. One of the reasons why I think you have got the frequency distribution of the raw data which is presented which is not usually done is that you don't have a normal distribution of the effects that--of the results that go into arriving at their conclusion with respect to the dose response. The positive findings are restricted to a very few number of cells, and a great many cells show no findings outside the norm. That is certainly from my perspective a major question with respect to the validity of the results, especially at lower concentrations. It is not a normally distributed population. That suggests potential sources of error or bias may be involved in influencing that type of result.
Q. Are these sister chromatid exchange type studies that are reported here so they fall in the category of chromosomal aberrations?
A. I think it depends on your semantics. It's a non--when we talk about chromosomal aberrations--first of all, chromosomal means whole chromosome, not chromatid. Second of all, we are talking about--generally when we are talking about cytogenetic analysis we are talking about permanent error, we are talking about error in the DNA message

that is a result of a break, a deletion, a gap, a translocation.
In the case of sister chromatid exchange, you have none of those. It is ostensibly error free. So its significance with respect to cytogenetic damage or an aberration is questionable from the physiological standpoint. Where you have a clear-cut demonstration of an increase in sister chromatid exchange it suggests some response to whatever the treatment paradigm was, or whatever the stress was, whether it is compound related or handling related or what have you, it does not automatically connote an error in the DNA message.
Q. So the furthest you would be willing to go is to say that exposures at one part per million produced a measurable change?
A. As defined by this study.
Q. You, of course, are familiar with studies of the peripheral blood of humans exposed to benzene and the findings of changes in chromosomal information at exposures below 10 parts per million, are you not?
A. If you show them to me I will be happy to comment on them.
Q. With that description you are not able

to-
A. No.
Q. What would be the mechanism for these test animals that are reported here to have breathed benzene that would end up showing an effect that's manifested by the sister chromatid exchange test?
A. I don't know of a mechanism that would account for sister chromatid exchange. I know of no mechanism for that. I don't know why it happens spontaneously. I don't know why it increases following stress or exposure to various agents. Some agents that can produce it correlate with their immunogens or clastogens, others aren't.
Q. What kind of work are you doing now in the way of research?
A. Doing studies to characterize stem cell differentiation in the hematopoiesis in defined culture systems.
Q. Which systems?
A. We are developing them. By defined I mean we are not using--we are using defined growth factors in very precise conditions for examination of the influence of those growth factors on hematopoiesis.
Q. Who is we?

A. My laboratory.
Q. At the University of Colorado; is that where it is?
A. Yes.
Q. Which department are you in there?
A. I'm in the Molecular Toxicology and Environmental Health Sciences program. I have appointments in the school pharmacy and in the department of pathology in the medical school.
Q. Has this group at the University of Colorado done similar-type research in the past?
A. It's basically my group, and I have done similar research in the past. And certainly those that are collaborating with me on various aspects of metabolism and/or biochemistry have done similar studies.
Q. That wasn't what I was trying to ask.. Prior to you coming to the University of Colorado and joining this department, had any similar-type work been under way?
A. Yes.
Q. Who was doing it then?
A. Dr. William Robinson in hematology and medical oncology.
Q. What kind of work was he doing that was

similar to what you are now doing?
A. Looking at stem cell regulation primarily associated with leukemias and dysplasias.
Q. And which group is he?
A. Oncology.
Q. What is the funding source for your present work?
A. I have several different funding sources. The American Petroleum Institute I have a grant from. I have a grant from the Chemical Manufacturers Association. I have funds provided to me by the University of Colorado. And I have grants pending at numerous agencies.
Q. Right now there are three sources of funds?
A. Yes.
Q. And how long have you been at CU?
A. One and a half years.
Q. Have those been the only three sources of funds you have had in the past one and a half years for your work?
A. Yes.
Q. Did you do an RFP to the API and CMA for your funds?
A. RFP?

Q. Did you make a proposal for the work that you anticipated doing?
A. Yes. Yes.
Q. You used to be at CIIT?
A. Yes.
Q. May I ask why you left?
A. I had an opportunity to develop a toxicology and environmental health program in a state that sorely needs one and the independence to do so, and I wanted to take a crack at developing an educational program in toxicology before my career was completed, and this was an opportunity to do that.
Q. Were you restricted at what you could do at CIIT?
A. In the sense that CIIT is a research institute and my ties with academia were at the level of adjunct participation at surrounding colleges, yes, I certainly couldn't direct a training program or be involved in education, higher education. This has given me the opportunity to have the best of both worlds.
Q. CIIT kept you from being involved in higher education?
A. No, I lectured a lot, but I certainly

couldn't take on the primary responsibilities of directing an educational program.
Q. In other words, you couldn't have two full-time jobs?
A. That's right.
Q. May I ask if your income went up or went down?
A. It went up.
Q. When you left CIIT?
A. Yes, sir.
Q. There has been discussion about your being paid to be here today. Where does the money that you receive go? Does it go to the University of Colorado or to you?
A. It goes to me. I have a contractual arrangement with the university that allows for 15 percent of my time to be spent consulting, and in fact I spend about 10 percent of my time doing that. It is both a requirement as far as the administration is concerned and pay. It is something they allow me to do. If I didn't do it I would probably hear about it as well.
Q. Is there direction given to the kind of consulting you must spend your 15 percent for?
A. No.

Q. The funds that you receive consulting, does a portion of those go to the university?
A. No.
Q. When you were at CIIT and you acted as an expert witness in cases, did the moneys you received then go to CIIT or to you?
A. First of all, to my knowledge, and I'm not an attorney, I never acted as an expert witness while I was at CIIT. I gave one deposition at CIIT while I was there. I received no compensation for it in any way, shape, or form, and neither did the institute.
Q. What was that deposition about?
A. It was in Skeen two.
Q. You testified in that?
A. I ultimately testified in Skeen two, yes.
Q. Were you away from CIIT when you testified?
A. Yes.
Q. You were paid directly for your services then?
A. That's correct.
Q. Was one of your activities at CIIT to slow down the development of OSHA regulations

regarding chemicals?
A. That's nonsense. That's utter nonsense.
Q. Was that a purpose of work at the institute?
A. No.
Q. Well, I need to show you something. Do you know of a Mr. F.B. Thomas, Dr. Thomas at Shell?
A. Yes, I do. He is no longer at Shell, I believe, but, yes, I do know him.
Q. Were you at CIIT in 1987?
A. Yes.
Q. Did you attend a joint meeting of the IISRP Industrial Hygiene Committee, and the IISRP Butadiene Steering Committee in Edinburgh, Scotland, in 1987?
A. I gave a talk at that meeting. I didn't attend other than to present a talk.
Q. Let me show you a letter which purports to be from Dr. Thomas, and attached to it is a summary of that meeting. Let me direct you to the last page, although feel free to read the whole thing if you like. Frankly I'm not convinced that all of it is accurate, but I want to ask you about the last item.

A. What's the question?
Q. Would you read the last item out loud so we can put that in the record.
A. Irons believes the data from CIIT to date have without doubt slowed OSHA regulatory procedures down and have also set some limits on product tort liability.
Q. Did you say that?
A. No.
Q. So that's just incorrect?
A. I suspect that what he was doing is extrapolating from my comments on the mouse model in arriving at those conclusions. I have been in contact with OSHA people during my studies with butadiene and have discussed the mouse model continually. My scientific opinion is the mouse model has a number of very complicated and species specific findings that may or may not be of relevance to man. From that standpoint I don't think the mouse--original mouse data should be assumed to be necessarily relevant to man.
I can see where someone in industry might draw this conclusion from those comments, but it is certainly not an object or a motivation for my work. My object for studying butadiene is to

determine what it does.
Q. Would you say that the institute work accomplished this, if not your work, then?
A. No. No. If our work slowed down regulatory procedures, it's in the context of evaluating the data they had, they elected to review it further. That would be the only way in which it could possibly have any effect.
Q. I think the top paragraph there on the same page, doesn't that deal with contacts with OSHA and the mouse model?
A. Yes.
Q. What was the answer to that question? You might read it into the record so later we'll have it all together.
A. Irons noted that he has been asked by OSHA scientists about whether a chemical viral interaction can be modeled by a multi-hit mechanism, suggesting there is some concern at OSHA about defending their mouse lymphoma risk assessment. It should be recognized, however, that dismissing the thymic lymphoma still leaves all of the other tumors seen in the NTP bioassay.
Q. Do you recall that discussion with OSHA?

A. Yes. I have discussed with them whether or not it would be appropriate to characterize chemical viral interactions in various models.
Q. And I would like to know your response to that, whether you can or not.
A. We are talking very complicated here. And at the present time I think we are either talking about concomitant models, interactive models, the virus may act as a cocarcinogen, it may act independently, there is just no way to reliably model it in that context given our knowledge base at that time.
Q. Do you have an opinion as to whether or not butadiene should be regulated as a carcinogen?
MR. ZATZ: Objection to the form.
A. Butadiene is a potent mouse carcinogen. It certainly deserves regulation as one would deal with any other potent mouse carcinogen or compound found to cause cancer in experimental animals, no doubt about that.
Q. (BY MR. HOBSON) Are you aware that the American Conference of Governmental Industrial Hygienists has recommended lowering the TLB for benzene?

A. For benzene or Betadiene?
Q. Benzene.
A. I have heard that. I have not seen it directly, but I have heard that.
Q. It's in the latest Archives of Industrial Hygiene, the notice of intended changes--the notice may not be there, but the documentation is there, and they recommend, as I recall, going to a tenth of a part per million times average TLB. Would you have a position one way or the other on that?
A. As a policy?
Q. Yes, sir.
A. No.
Q. You have no opinion?
A. I don't think it relates to causation. It's a policy issue. It doesn't impact one way or the other on causation.
Q. What would be your view, then, about regulatory activities and causation?
A. I think they are two separate exercises. They have totally different criteria and totally different--they are functionally totally separate. I don't think that a regulatory standard says anything about causation.

Q. Why wouldn't it say anything about causation?
A. Because there are paradigms that are used de facto in establishing regulatory standards based upon risk assessments for which there is no evidence to support or refute the assumptions made in the risk assessments. The one thing you can say about a regulatory standard is the standard itself is probably--you can be virtually certain within reasonable scientific certainty that the exposure level that's set is far below that that's going to be related to causation just by nature of the assumptions that go into the risk assessments.
Q. Have you read Mancuso's second edition of his epidemiology book Occupational Epidemiology?
A. No, I haven't.
Q. Would you have any opinion as to whether regulated exposure levels should have a certain margin of safety built into them?
MR. ZATZ: I'm going to object on two grounds. Number one, I think you are asking him opinions about policy matters as opposed to toxicology matters, and we have not proffered him as an expert on what American regulatory policy ought to be; and secondly, based on the use of the word should

in that question. You can answer.
A. Given the lack of scientific basis for many of the assumptions that go into setting standards, I think that margins of safety are certainly called for.
Q. (BY MR. HOBSON) I think earlier today you told me that in your work that you have done you have seen replication errors, chromosomal damage in laboratory animals at 50 parts per million?
A. I have seen damage to bone marrow and to hematocritic organs at those concentrations. Certainly mitotic abnormalities at 100 parts per million and probably at 50. I can't remember precisely. I suspect I have probably seen them at 50.
Q. Given the difficulties of this animal model and extrapolation to man, do you feel comfortable, then, that the same exposure levels for similar-type effects are appropriate for man?
A. I think that exposure--first of all, the types of changes we are talking about, which involve bone marrow damage, are related indirectly to the metabolism of benzene, which is a requirement. And although I can't cite you the specific kinetic studies that I have been involved with or have been

done, because I can name the studies actually but I can't remember the data right now, it's been years since I looked at it, my conclusion would be the mouse is a much more sensitive model with respect to bioactivation and short-term or subacute toxicity than the rat or man is likely to be. So I would say the mouse is likely to be a more susceptible model for subacute damage bone marrow toxicity than either the rat or man.
Q. What about for chronic?
A. I don't think that you get chronic changes in the absence of subacute changes.
Q. That's where I wanted to go with you next. Earlier today you said that the acute myelogenous leukemia in man must have certain precursors before you believe it would be related to benzene exposure?
A. In secondary leukemias, yes.
Q. Explain to me what you mean by that in secondary leukemias.
A. I think, first of all, the evidence, the epidemiologic evidence provides very strong rationale as a basis for that conclusion.
Q. Which conclusion?
A. That in fact one has to have

significant alterations in the stem cell compartment related to bone marrow toxicity before you incur the risk of developing AML secondary to exposure. I think it requires a physiologic response on the organism that alters stem cell regulation, and I presented today in the context of other questions several lines of evidence to support that.
Q. That raises two questions I would like to ask you. Number one is, can benzene produce leukemia in man as a primary result? You said in your answer to my question a moment ago leukemia was a secondary leukemia.
A. It can't be primary if you are exposed to benzene. By definition it is a secondary. Secondary means not spontaneous. It is secondary to some exposure, be it a drug, be it benzene, be it radiation, what have you.
Q. I didn't understand how you were using secondary there.
A. That's what I mean by secondary.
Q. Secondly, what are the markers for effects on the stem cell regulation that you would put in the category of a precursor to the development of AML?
A. Well, if I had an absolute marker that

I could point to in stem cell population and say this meets to AML, this would be a very prognostic tool. We don't have anything like that at the current time. One of the things that I would want to see first, and in fact as part and parcel of what I'm doing now, is alterations in the way stem cells are regulated that would in fact be consonant with the physiologic response to the damage caused by an agent such as benzene.
Q. Let me ask the question this way: You have got an AML, you have got a worker exposed to benzene, list for me the factors that you would want to see before you would say that the AML was caused by the benzene exposure.
A. I would want to see accurate exposure data that demonstrates exposures that are significant in terms of potential bone marrow toxicity. If you have exposures at 100 parts per million or greater repeatedly, I'm going to be satisfied with the exposure scenario. Between 50 and 100 I'm going to be concerned about it. If it's below 50 you get into real foggy territory with respect to evidence, and somewhere lower--somewhere between 25 and lower there is just no evidence to support it.
I would want to have some indication of

latency. If the disease develops I'm going to be much more happy or confident about the latency issue if we are dealing in the area around ten years from initial exposure, it's going to be in a typical Gawssian fashion becomes less and less clear as you get closer to initial exposure, and at about three years I think it is impossible to draw a conclusion as to whether or not the exposure could be related. That's also way off the end of the Gawssian curve. I believe Rinsky in his study used 5, and this is certainly following up of five years.
The type of leukemia, if it is an AML, to me that's important obviously. if in fact the cytogenetic abnormalities that are associated with what classically have been referred to the C-type chromosomes that I have referred to initially, 5 Q minus 7 Q minus 5 or 7, missing that is consistent with secondary leukemias, it is inconsistent with spontaneous leukemias, that would be a factor that would weigh heavily in terms of me reaching a conclusion.
Q. Would you only find those abnormalities in AMLs?
A. 5 Q minus and 7, to my knowledge they are heavily weighted towards myelodysplastic

syndromes and AMLs. It is because of the growth factors on those chromosomes are associated with regulation, specifically the myeloid lineages. Sub type can be useful empirically for reasons which nobody knows.
If it is an erythroid leukemic variant that heavily predisposes towards benzene if in fact you have a reliable exposure history. I don't mean a simple erythroid hypoplasia in the presence of a myeloblastic AML, I'm talking about a clear-cut erythro leukemic.
But I would not exclude virtually any type of AML as being possible, with one exception, and I wouldn't rule it out entirely, but there is certainly no evidence of monoblastic type.
Q. Anything else? The reason I ask that is for some reason in my mind I got the feeling earlier you were telling me that you would not attribute benzene as a cause of AML unless the person had aplastic anemia.
A. You are talking about the presentation of the patient at the time of diagnosis. If you had had the ability to clinically monitor a patient from the beginning of exposure to the onset of leukemia, it is my opinion you would very likely and almost

always, if not always, see some evidence of bone marrow suppression. We are not talking exposure to trace amounts, we are talking about real life response to bone marrow suppression incurred as a function of exposure to benzene concentrations that will do that. That's consistent with the literature on chemotherapeutic agents, and I think it is consistent with certainly the case histories that were presented in the early benzene literature.
Q. Would the person with AML know they were sick from what you would consider a significant enough bone marrow suppression to be a link to benzene exposure in the past?
A. I would say there is a fairly high probability that there would be some evidence of bone marrow suppression, but it is not absolute--I don't think it is absolutely a requirement because the effects on stem cell, if in fact you had a clinically nonsignificant effect that resulted in an altered physiologic regulation of stem cell activity, you could then continue to have prolonged--you could have a lesion at the level of stem cell differentiation that would persist, and it is conceivable that an individual might not seek treatment for the initial episode. I think it is conceivable.

I don't think that if you did bone marrow taps and looked at stem cell regulation using even existing assay procedures that are relatively crude that you would have any trouble seeing them because certainly the chemotherapeutic literature suggests that to be the case. But that's not normal routine diagnostic procedure.
I will not tell you that you absolutely have to have a clinically significant presentation. I'm not sure. That may be the case, but I'm not prepared to say that as an absolute. I do think if you look for it with all the means at our disposal you would see.
Q. And the patient might or might not know that they have some outward symptoms?
A. For some proportion of patients I suspect that's the case. Certainly the prevailing opinion in the medical community is the majority of these patients are surely going to present.
Q. When you talk about the bone marrow suppression that you would need to see for an AML, are you talking about suppression of all the different cells or a particular line?
A. It is highly variable, highly variable. It is even variable in animals, but it is

more variable in man.
Q. It could be the pancytopenia, thrombocytopenia?
A. Yes.
Q. Would it have to be long lasting?
A. This is rank speculation. I certainly would want to see long-standing changes at the level of the stem cell, I expect to see them. I suspect that the clinical presentation could be relatively short.
Q. What about if you have a person who has had a history of exposure to benzene at a level several hundred parts per million for some time period and a person who presents then with AML, same person presents with AML some years later?
A. Several hundred parts per million?
Q. Yes. An exposure that you would accept of being capable of causing the disease.
A. Okay.
Q. And the person presents with AML some years later after the exposure has ceased, say ten years again to fit into your exposure profile, and at some point in between presented at a physician's office for, say, thrombocytopenia which was not long lasting, or pancytopenia which was not long lasting;

in other words, one of these markers occurred and then disappeared, does that tell you anything about whether or not benzene played a part in the causing of the AML?
A. If you show me an individual who has had documented exposure to several hundred parts per million for any significant period of time that meets criteria that I'm comfortable with and develops AML, as far as I'm concerned there is no reason to assume that it was not due to that exposure.
I would say you would have some qualifying concerns if it was totally--if it was a cytogenetic presentation that was typical or spontaneous, but nevertheless you have got predisposing evidence to suggest that there could be a relationship there. I would fully expect to see some presentation. You can't be exposed to several hundred parts per million benzene and not develop some hematologic abnormalities. I don't believe it is possible at those levels.
Q. I forgot to ask you about another disease earlier.
A. Can we do this very quickly?
Q. Hairy cell leukemia. How does hairy cell leukemia fit into these different diseases?

A. It is a totally different disease entity existing ostensibly at the level of in a quasi-world with the monocyte and the macrophages and the so-called reticulum cell. It doesn't fit into this paradigm.
Q. Are you familiar with any epidemiological literature that links Hairy cell leukemia and exposure to benzene?
A. No. I'm familiar with literature that links it to HVL2, the human T-cell varium.
Q. You have to go?
A. I have to go.
Q. Before we go I would like to get the court reporter to mark as Exhibit 1 your chart that you drew, that's this legal sheet. This is the chart that you drew which we are marking as Exhibit 1; is that right, Dr. Irons?
A. Yes.
Q. I would like to mark as Exhibit 2 the paper that's from the CIIT group that we discussed, Induction of Cytogenetic Damage in Rodents After Short-Term Inhalation of Benzene. That's the paper we discussed earlier, right?
A. Yes.
Q. Then as Exhibit 3 I would like to mark

the Shell document that I questioned you about. And Exhibit 3 is the Shell paper I questioned you about, correct?
A. Yes.
Q. Anything else that's handwritten, or just the articles?
A. One handwritten note.
Q. Could I get those two notes and mark those. Anything else that's handwritten that you have?
A. That's it.
Q. And then these are just articles?
A. Yes.
Q. Let me just ask if you would leave this with the court reporter and let her copy this and then return the original to you, would that be all right?
MR. ZATZ: Is that all right?
Q. (BY MR. HOBSON) As long as you agree to maintain the originals?
A. Okay.
Q. We'll mark these as the next attachment as a group exhibit. And you brought Jandl's book with you?
A. Yes.

Q. Blood Toxicology Textbook of Hematology. And which edition is this?
A. The only one.
Q. Let's see, '87, copyright date, correct?
A. Yes.
Q. Bring anything else about the case with you?
A. No.
(Whereupon, documents were marked Irons Deposition Exhibits 1 through 4 for identification by the reporter.)
(Whereupon, at 4:05 p.m., the deposition was concluded.)

I, RICHARD D. IRONS, Ph.D., do hereby state under oath that I have read the above and foregoing deposition, and that the above transcript and accompanying correction sheets, if any, constitute a true and correct record of my testimony.

RICHARD D. IRONS, Ph.-D.

STATE OF COLORADO
ss.
CITY & COUNTY OF DENVER

Subscribed and sworn to before me by the said RICHARD D. IRONS, Ph.-D,, this- day of 1990.

My commission expires_

CERTIFICATE

STATE OF COLORADO
ss.
CITY & COUNTY OF DENVER

I, BARBARA WISHART, a Certified Shorthand Reporter, a Registered Professional Reporter, and a Notary Public within and for the City and County of Denver, State of Colorado, do hereby certify that previous to the commencement of the examination of the said RICHARD D. IRONS, M.D., a witness called for examination by the plaintiffs herein in the said suit in the said District Court, was duly sworn by me to testify the truth in relation to the matters in controversy now pending and undetermined between the said parties so far as he should be interrogated concerning the same;

That the said deposition was taken in shorthand by me at 1290 Broadway, Suite 700, City and County of Denver, State of Colorado, on the 15th day of August, 1990, at 10:21 a.m., and was reduced to typewritten form under my supervision;

That the foregoing is a true transcript of the questions asked, the testimony given, and the proceedings had;

That I am neither attorney nor counsel, nor in any way connected with any attorney or counsel for any of the parties to said action, or otherwise interested in its event.

IN WITNESS WHEREOF, I have hereunto set my hand and affixed my notarial seal this 28th day of August, 1990. My commission expires November 26, 1990.