14TH JUDICIAL COURT, PARISH OF CALCASIEU,
STATE OF LOUISIANA
Case No. 94-2695

DEPOSITION OF RICHARD D. IRONS, Ph.D.

RAYMOND McFARLAND, et ux,
Plaintiffs,
VS.
HARTFORD ACCIDENT and INDEMNITY COMPANY, et al.,
Defendants.

PURSUANT TO NOTICE and the Louisiana Rules of Civil Procedure, the above-entitled deposition was taken on behalf of Plaintiffs at 4150 East Mississippi, Denver, Colorado, on **Friday, December 15, 1995**, at 10:30 a.m., before Dyann Patterson-Labo, Registered Professional Reporter and Notary Public within Colorado.

APPEARANCES:
For the Plaintiffs: HERSCHEL HOBSON, ESQ.
Baggett, McCall & Burgess 3006 Country Club Road 70605
Lake Charles, Louisiana 70707-7820
For the Defendants: GREGORY W. BELFOUR, ESQ.
Jones, Tete, Nolen, Hanchey, Swift & Spears, L.L.P.
Post Office Box 910 Lake Charles, Louisiana 70602-0910
Also present: Jim Tillson

I N D E X

EXHIBITS
(No exhibits were marked.)

P R 0 C E E D I N G S
RICHARD D. IRONS,
having been first duly sworn, was examined and testified as follows:
EXAMINATION
BY MR. HOBSON:
Q. Dr. Irons, you're still at the University of Colorado, are you?
A. Yes.
Q. And I take it your capacity there hasn't changed since your arrival, or has it?
A. I don't remember exactly the last time we met. The only change that has happened since I have been there is I have taken on responsibility as director of a cancer-causation program in the Cancer Center.
Q. Tell me about that project.
A. I am program director and responsible for our -- the coordinating and promoting of research in cancer causation and carcinogenesis within the Cancer Center. It's an NCI-funded Cancer Center.
Q. The Cancer Center is funded by NCI?
A. It's an NCI-funded Cancer Center. It's the University of Colorado Health Sciences Center's Cancer Center.
Q. And as the director of this project, is it

your job to do research for the NCI, or is this a sort of an administrative post to make sure money keeps coming in?
A. No. It's to coordinate research, to encourage collaborative activities amongst faculty, to look for potential opportunities for faculty members to obtain grants, and to encourage researching mechanisms of carcinogenesis.
Q. But your job with the NCI cancer-causation project is not to do research, correct?
A. I wouldn't say that's a fair characterization, but it does not support directly my research activities.
Q. One of the things I really want to cover today with you, and I would like to kind of start there, I guess, is your research activities. What current research activities do you have funded?
A. I have a grant from the National Institute of Health. I have a grant from the American Petroleum Institute. I have a grant that I am coming off of in 1996 from the National Aeronautics and Space Administration.
Q. You say you're "coming off of"?
A. It's ending -- it's ending this year. I don't know whether the effective date is December or April. I have -- I have had a -- this last year a

grant from the National Cancer Institute. I finished a grant from the Chemical Manufacturers Association. I have had a number of grants from pharmaceutical companies, the most recent one I believe was Hoffmann-La Roche. I have a number of grants pending.
Q. Any others that are currently underway?
A. I think that -- I think that covers it.
Q. When you use the term "grant" in context with this -- these research activities, is it correct to say that that terminology means that these different entities contract with either you or your administration to do certain kinds of work for which they pay?
A. No. That would -- you could say that for contracts and grants. All of those, with the exception of the NCI -- actually, calling the NCI project a grant is probably a misnomer. That's a contract. Everything else has been a grant.
Q. What is the distinction that you make between a grant and a contract?
A. A contract is a specific agreement -- an agreement to do a specific, characterized piece of work to measure A, B, or C, to specifically do an individual test or project. A grant is to study a specific area. It may be very general, it may be relatively specific, but the experimental design, the things I measure, the

approach I take is at my discretion.
Q. And to get a grant, you provide a proposal of what you intend to do with some, as you say, more broad parameters describing the kinds of efforts that you intend to make with the moneys that are going to be provided to you?
A. To varying degrees, and varying institutions have different criteria, and depending upon the nature of the proposed work, it may be more specific or less specific as the case may be.
Q. Now, the grants are made with agreements that surround them, are they not?
A. Sometimes yes, sometimes no.
Q. I mean, when you take grant money from the NIH or the API or NASA, it's just given to you with no strings attached?
A. It depends. You have lumped those three, and they actually all three are different. In the case of NIH, there is a process which is called extended budgetary authority, which I am subject to, which basically gives me the freedom to manage that grant and the funds made available to it in any manner that I see fit within the context of the work product that I am expected to perform.
So whatever budgetary proposals I put forward

in the original grant application I can change if in fact that's a -- if I find it necessary in order to perform the work as I get into it. In the case of NASA, it's fairly stringent with respect to the specific use of funds and what you propose to do.
In the case of API, it's extremely -- it's like NIH in the sense that there are no specific provisions with respect to how I reallocate or use funds once the grant is underway.
Q. But I think you said even with the NIH grant, which I heard you say is called the loosest arrangement of all, is there still a work product that is involved in the project?
A. The work product is what I am expected to do based upon what I proposed to do, and that's evaluated in the context of interim reports and final either renewal of the application or in prospective applications for other funding.
Q. Let's start with the NIH grant. When did that grant funding begin, approximately?
A. I am in the third year of a five-year cycle.
Q. So this is -
A. So it would be September -- this is '96 - 1994.
Q. And you expect that to continue through when?

A. 1998.
Q. What is the nature of the work product for the NIH grant?
A. It's a study of mechanisms of leukemogenesis associated with exposure to chemotherapeutic agents and benzene.
Q. The API grant, when did that start and when do you expect it to end in the current arrangement?
A. I believe it started in 1990, and it's been renewable by year, and I have just been informed it will be renewed for '96.
Q. What is the nature of the API grant work product?
A. Improving the biological basis for evaluation of risk assessment of leukemogenesis and mechanisms of leukemogenesis.
Q. The NASA grant, when did it begin, approximately?
A. I believe it was 1992.
Q. And you say that's in the process of ending now?
A. Yes.
Q. What was the nature of that grant work?
A. Risk characterization associated with extended space-flight environment.

Q. The NCI contract, can you tell me approximately when it began?
A. 199 -- I think it was middle to end of '94.
Q. And that project ends in '96?
A. It's ending now. It's basically done. There is still some paper-writing to do.
Q. And what was the endpoint for that NCI contract?
A. Characterization of alterations in cytokines associated with benzene poisoning in Chinese workers.
Q. The CMA grant that you finished, when did that begin, approximately?
A. I think it was 1993, but it could have been '92.
Q. Approximately how long did that project last?
A. Three to four years.
Q. What was the nature of that work?
A. Characterization of mechanisms of butadiene leukemogenesis in the mouse.
Q. Now, you say that you either have had or have grants from some pharmaceutical companies?
A. Yes.
Q. Would those be grants or contracts?
A. Grants.
Q. What was the nature of those?

A. Either characterization of mechanisms of murine leukemogenesis or lymphomagenesis associated with exposure to specific drugs, or potential toxic effects of specific or prototype drugs on human bone marrow function.
Q. Are these primarily chemotherapeutic agents for cancer treatment?
A. No.
Q. What kinds of drugs were they?
A. Some are proprietary at this stage, and I am bound by confidentiality not to discuss their nature. Some are agents that are used for a variety of purposes that have either some -- there is some question with respect to whether they're toxic to bone marrow or not. Some have been chemotherapeutic agents.
Q. The NIH grant that is currently underway, can you give me the approximate annual funding level for that?
MR. BELFOUR: Excuse me, Herschel. I don't know whether it's appropriate for those kinds of information -- that kind of information to be revealed. I'll leave that up to Dr. Irons, because I don't know if there are any confidentiality provisions in there.
MR. HOBSON: Well, I would hope that the United States Government grant would not have any

secrets in it about how much the United States is paying Dr. Irons, but if there is something like that, speak up.
A. Well, there are issues with respect to overhead and indirect cost that make parity between grants very difficult to compare. What I am trying to do is determine what it is I am getting versus what the institution is paying.
Q. (BY MR. HOBSON) Well, I truly was curious to know what the overhead factor for your research is and how it varies from grant to grant.
A. Some of that information I am not at liberty to discuss. The overhead -- certainly the negotiated overhead rate for the University of Colorado Health Sciences Center with NIH is, I believe, 51 percent. I believe, including consortium arrangements, that grant is approximately at the level of 200,000 -- I see about 200,000 a year in direct cost.
Q. You say "in direct" cost as two words?
A. In -- those are direct costs, that's the budget that I have to work with.
Q. Now, this NIH grant, is it more fund work than just work that you are doing?
A. No.
Q. Are they activities of the university?

A. No.
Q. The API grant, can you tell me approximately what the funding level for that has been and is?
A. It's probably on the order of 170- to 180,000.
Q. Per year?
A. That I see.
Q. Per year?
A. Yes.
Q. And is there a different overhead rate for the API grant?
A. Yes.
Q. The NASA grant, can you give me approximately how much funding there is for that grant, or was?
A. That was approximately $100,000 a year, but it had some very strange indirect cost issues, so I can't -- I can't give you an accurate assessment of what my annual budget was for that. It varied from year to year.
Q. The NCI contract that ended, or is ending, approximately what funding level did it reach?
A. That I -- I don't remember the specifics on that. It was basically a supplies-based contract, so the costs were relatively -- or the funding level was relatively small.
Q. When you say that something is supplies-based

for funding purposes, are you saying that there is no money in that NCI grant for salaries?
A. There are no salaries -- I don't think there is any salary money in the NCI grant.
Q. Would that be the only one of these grants for which there is no salary funding?
A. Yes.
Q. Now, for you to take a contract that is salary based -- I'm sorry, that is supply-based only, do you have to get some special dispensation from your authorities there at the university?
A. No. I pay overhead on it.
Q. I'm sorry.
A. I pay overhead on it.
Q. What does that mean?
A. The university is paid an overhead rate that is within the guidelines for the university for indirect cost recovery, so whether the funds are spent for personnel or not is of no concern to the university.
Q. The CMA grant that you finished, can you give me approximate funding level for that grant, please.
A. I think my annual budget was between 50- and $70,000.
Q. Various pharmaceutical grants, I take it that they vary depending on the work that you were asked to

do?
A. Yes. On average, I would say they're about $100,000 a year.
Q. That's for all of them or is that each?
A. That's on average, so at any given time.
Q. Now, when it comes to how you're paid at the university, does the amount of research that you are participating in and get funded for affect how much you're paid?
A. No.
Q. It just depends -- affects whether or not you get retained, I guess, or are you not expected as part of your duties to entice research to the university so that your department has funds to do research?
MR. BELFOUR: Objection, vague and compound. I heard at least two questions, Herschel. I'm not sure which one you want to ask him.
Q. (BY MR. HOBSON) Well, let me ask it again. Is part of your job at the University of Colorado to attract research moneys?
A. I have three major generic functions or missions as a faculty member at the University of Colorado: teaching, research, and service. And I am expected to provide a balanced response to that mission in all three categories.

Q. And someone in your position who is unable to attract research is less likely to be retained at the university; is that fair?
A. Well, I have tenure, so that the actual amount of research dollars that I bring in does not have any bearing on my security or my salary.
The fact of the matter is that in an academic environment, if you are not actively publishing in the peer-reviewed literature, you basically don't exist, you don't have -- that is a major role in demonstrating that a faculty member is current, productive, and competent in his or her area.
Q. Old adage of "publish or perish"?
A. Yes. And you can't publish without research funds.
Q. What was the percentage of your time, say, in the last year, that you spent actually teaching at the university?
A. Probably on the order of 30 to 40 percent.
Q. And the percentage of your time you spent doing research?
A. Probably on the order of 40 -- probably on the order of 40 percent.
Q. And then your service time would be what?
A. 15 percent. Probably spent less this year,

but that would be -
Q. That, in my notes here, doesn't add up to 100 percent. Is there something else you do?
A. Yes, administration.
Q. What time do you spend doing administration?
A. Whatever the remainder is. That would be committees and administrative responsibility.
Q. What would qualify in your duties as service work?
A. Government advisory work, consulting either with government or industry.
Q. Is there some limit that the university puts on you for percentage of time for service work?
A. I have a contractual limitation of 15 percent.
Q. Maximum or must-do?
A. Maximum.
Q. Is that based on time spent or money?
A. Time.
Q. For doing work like the giving of the deposition here today, is that university-related, then?
A. As a service, it is considered as fulfilling a service component of my performance of activities as a faculty member. It's independent of my contract as a university employee.
Q. The moneys that you will be paid, then, for

today's deposition go to whom?
A. To me.
Q. Not the university?
A. No.
Q. And your preparation work and other consultation concerning this lawsuit goes then directly to you and not through the university?
A. That's correct.
Q. Who sets the rates for your time? You or the university?
A. I do.
Q. Do you bill that as an individual or do you have a business entity set up to deal with that?
A. As an individual.
Q. Do you pay any parts of the moneys that you get for this service work, doing depositions, and working on lawsuits, to the university?
A. I compensate the university for services used, such as library facilities, that type of activity.
Q. No percentage of what you bring in goes to the university as fixed overhead similar to what the university would charge in a grant or a contract?
A. No, sir.
Q. Let's talk a little bit about your research activities in general. I know that -- at least I think

I know part of your work has been in looking at myeloid stem cells and how those myeloid stem cells and their offspring react to benzene metabolites and drugs. Have I said that correctly?
A. That's certainly part of what my work is about.
Q. What I would like to do is understand where you are in that research, as far as whether or not you have been able to isolate the myeloid stem cells any better than when I took your deposition before, which was, I don't know, four or five years ago, just try to understand how far you have come in that research of knowing about myeloid stem cells and how the -- how they function at the university-research level that you have done. Does that make sense to you?
MR. BELFOUR: Excuse me, Herschel. Four or five years ago in connection with which case, so perhaps we could refer back to that?
MR. HOBSON: I don't even remember what it was.
A. I don't remember.
MR. BELFOUR: If we don't have a reference point, it's hard to say how it has changed since the reference point.
MR. HOBSON: He can tell me how it is now.

I'll understand the differences, I think. If not, I'll ask him how it has changed.
A. It has changed considerably in the last four or five years. It's changed dramatically in the last couple of years.
Q. (BY MR. HOBSON) Where are you in isolating myeloid stem cells and determining how the myeloid stem cells develop into mature cells?
A. We can isolate -- we now have developed technologies that allow us to isolate a defined set populations of stem progenitor cells to virtually 99 percent purity based upon the criteria that we use. We can distinguish within the stem cell compartment subpopulations of early primitive stem cells. We can distinguish committed myeloid progenitors and lymphoid progenitors from uncommitted cells, and we are in the process of looking at specifically the early events that are associated with exposure to drugs and metabolites on those cells.
Some of this work is -- much of this work has recently been published; some of it is not published; but certainly the purification schemes and cell-specific metabolism and toxicity associated with myeloid differentiation is published.
Q. And when you say the early stem cell

progenitors, are you talking about, then, the level of the kinds of cells that precede the myeloid stem cells?
A. Yes. Myeloid progenitor cell is a relatively late cell in that process.
Q. How early, in getting back to the earliest of the cell development, have you been able to go in isolating these stem cells?
A. Well, we're in the process now of validating some of our recent developments and results, but I would say that arguably we're at a stage where we can distinguish what is referred to as the long-term repopulating cell from cells that have either intermediate or short-term repopulating ability. So we're beginning to actually subdivide what has historically been referred to as the pluripotential stem cell. It's not really one cell. It's a hierarchy of cells. It's a heterogenous population of cells that have varying propensities and potentials within that compartment.
Q. As I read the literature that I see published, some by you and some by others, I see different terminology used which to me describes the same thing, and so I have trouble sometimes following from one study to the next, or even one conversation to the next with the same person; that I am using the same terminology

for the same item, and I don't want to make that mistake. So I would like to take a minute, if we could, and just agree on some terminology so I can try to follow what you are telling me.
When you say "pluripotent stem cells," is that a proper term?
A. The problem you're discussing is not one that is unique to you. It's a product of a field in the literature that has progressed explosively in the last 20 years, so terminology shifts regularly based upon increased knowledge.
What you will see in the literature referred to as a "stem cell," "pluripotent stem cell," "totipotent stem cell," varies from person to person, depending upon how current they are with the literature and what particular point in time or history you're reading whatever they're saying.
"Pluripotent stem cell" is a useful term.
Some people say "totipotent" is more current for referring to the ultimate cell. I still use "pluripotent" in that context, but as I have just said, pluripotent stem cell really refers to a compartment, depending upon what criteria you assign to that cell and what the characteristics are.
I tend to also use "stem cell" to refer to

that compartment, except that in certain environments, such as talking to a high school class or a lay population, I may use "stem cell" to mean a variety of different cells; I would not necessarily use it for in a technical setting.
Q. "Compartments," that's another term that I see from time to time that I am not sure that I either understand or can follow from one conversation to the next or one article to the next. How do you use the term "compartment"?
A. It varies, but basically "a compartment" defines a set or category of characteristics. In the case of stem cell or pluripotent stem cell compartment, what I mean is that all the cells within that compartment have nominally certain characteristics that historically have been used to describe what was thought to be a cell. They may differ with respect to certain potentialities, but they all have certain characteristics within that compartment.
You can use "compartment" to define differentiation state or status. For instance, the committed progenitor cell compartment could mean a myeloid progenitor cell; it could mean lymphoid progenitor cells. They're different cells, but they all are at comparable stages in differentiation.

One can say lymphoid compartment or myeloid compartment, and there you're talking about a lineage-specific compartment. And so basically you use compartments to divide up the landscape in such a way as to make it easier to discuss the characteristics of cells that are within these compartments. There is no universal -- for some there is some universal criteria, but there is no absolute criteria for how you want to define a given compartment. It depends upon how you are using it.
Q. If you will bear with me and try to work through this so I can try to come to some understanding of how you use the terms, then hopefully we will have some level of communication. It won't be probably your fault, it will be more mine that there won't be any communication, but if you will bear with me, I would appreciate it.
If we talk about the pluripotent stem cell compartment -- first of all, is that a proper terminology?
A. I would say so, yes.
Q. Then that would be a set or a collection of cells, as you use that term?
A. Yes.
Q. Would all of those cells in the pluripotent

stem cell compartment be noncommitted?
A. Yes.
Q. I take it there comes a time, then, when the pluripotent stem cells, as they develop, they do commit?
A. Yes, and I use -- commitment is consonant with movement into progenitor cell compartment.
Q. How many progenitor cell compartments would it be appropriate to talk about, then?
A. In the simplest -- in the simplest -- simple is not always the most accurate; but in the simplest context, lymphoid and myeloid; but within the myeloid compartment, you have multiple levels of differentiation and commitment. The myeloid compartment contains cells that are variously referred to as multilineage cells. The myeloid progenitor cell is a multilineage cell. At one point in history many people thought it was the stem cell. It's capable of giving rise to erythrocytes, ultimately erythrocytes, granulocytes, macrophages, platelets.
The lymphocyte progenitor cell compartment is understood at the mature end, if you will, probably better than it is at the immature end, in some degree - to some degree.
There are some subtle differences between the apparent differentiation of lymphoid cells among

species, so the structure of the lymphocyte progenitor cell compartments differs from one species to another. It certainly appears to be.
Q. Now, you said that in simple terms there are these two committed progenitor cell compartments: the lymphoid and the myeloid?
A. Yes.
Q. Now, is there a possible third progenitor cell compartment or have we pretty much isolated the two cell compartments?
A. There is a potential -- well, it depends on how far back you want to go on ontogeny. There is some evidence that there is a third compartment early on with respect to differentiation of stroma and fibroblast during embryogenesis, that is a more primitive cell than the hematopoietic pluripotent stem cell. There is, depending on the species, evidence to suggest that the initial committed progenitor cells for lymphoid differentiation between B and T cells is different, and that those are not necessarily analogous compartments. So if we're talking about committed progenitor cell compartments, you can either think of it as being two or three, depending upon how you view lymphoid differentiation.
Q. If we're talking about for humans?

A. Yes.
Q. Have you described for me this pluripotent stem cell compartment for the noncommitted cells and then the lymphoid and myeloid progenitor cell compartments for humans, or is it different?
A. In humans it appears that -- well, the myeloid -- commitment to myeloid differentiation in humans is as I have described.
In the case of lymphoid differentiation, there are some -- there is indirect evidence, although it's fairly compelling evidence, to suggest that B cells undergo a defined commitment step to a progenitor cell -- a B lymphoid progenitor cell. The T cells may not; and, in fact, T cells may directly -- or may be derived directly from a pluripotent stem cell or that that differentiation process takes place in the thymus and not the bone marrow.
Q. Are you saying there are two pathways then to this T cell?
A. It's either/or. We have evidence in mouse of a commitment step at the level of the bone marrow. In humans, so far no one has been able to demonstrate evidence of a commitment step at the level of the bone marrow.
Q. You talked from time to time about murine

systems?
A. Murine.
Q. There you mean rodents?
A. Mouse.
Q. I must say that as I have gone back and looked at other depositions you have given, sometimes I have trouble understanding in your answer if you are talking about the human or if you are talking about the mouse, and I don't know sometimes if the questioner realizes which one you're answering. So that's what I would like to try to work through that with you too if I could. For humans have you identified any of the components of the pluripotent stem cell compartment?
A. In terms of functional characteristics and differentiation, yes. That's been a major focus of ours, has been the reproducible isolation of subpopulations of human stem cells for characterization of function and metabolism and toxicity.
Q. But they're pluripotent stem cells, not myeloid or lymphoid?
A. That's correct, that's correct.
Q. And can you now in your laboratory isolate the pluripotent stem cells to the same, like this 99 percent purity that you were talking about earlier?
A. Yes.

Q. And how far have you been able to describe the development of the pluripotent stem cells and isolate them?
A. We're in the process of taking the earliest cell population we can define by differentiation characteristics and to validate its function as what is referred to as a long-term repopulating cell. In the current parlance of experimental molecular hematology, there is less focus on pluripotent stem cell and much more focus on function; and the ultimate function that is of concern with respect to functionality today is what is referred to as long-, intermediate- or short-term repopulating ability, because these are the characteristics that are important in bone marrow transplantation.
Q. People that have diseases like leukemia, where you can essentially eliminate their blood system, and then the hope is to put in this cell and generate a whole new bloodlike condition that does not have leukemogenic properties?
A. That's correct.
Q. Are you at that stage?
A. Yes, yes. As I said, we're in the process of validating our purification schemes at the level of long-term repopulating cells now.

Q. What do you mean by the difference -- that's a bad question.
What is the difference between long-term repopulating and short-term repopulating, as you use it?
A. It's probably best discussed in the history of the field of transplantation. Early on it was recognized that there was a subpopulation of bone marrow cells that obviously contained the stem cell population. Bone marrow transplantation originally involved simply the -- and still, in many cases today, does involve the transplantation of whole bone marrow, which obviously contains some stem cells, and it's an effective approach. But in terms of trying to enhance and improve the ability to successfully transplant bone marrow, there has been a major effort to characterize the function of those cells and to purify them.
Both in terms of animal studies, and certainly indirectly in human studies, it's been possible to demonstrate that cells within the so-called stem cell compartment have different characteristics with respect to their ability to repopulate a host.
If you lethally irradiate a mouse and/or use radiation and chemotherapy to essentially kill off his native bone marrow, it was discovered relatively early on in the -- well, the late '80s, that there was -

there were cells that had to be there if that animal was going to survive for six months or a year, but if those cells were the only ones that were given to the mouse, he wouldn't survive for two weeks or a month or three months.
And it's been found that there, in fact, are populations of cells that are different with respect to their ability to provide survival following lethal irradiation for different periods of time. So the most primitive cell is one that is capable -- is called a long-term repopulating cell, and that's basically, by definition, a cell that will enable the animal to eventually completely recapitulate normal bone marrow function and survive indefinitely.
There are shorter-term repopulating cells that are necessary for immediate survival, but they're not capable of indefinite or perpetual maintenance of bone marrow function. So you really need a heterogeneous population of cells to provide that function if you are going to effectively reconstitute bone marrow.
Those are the characteristics that we're using now functionally in terms of providing validation for the subpopulations that we're looking at in the laboratory.
Now, what I defined for you is what has been

seen in mouse. We have now assays that are surrogates for the same type of function in humans, so that we can look at the ability -- to a certain extent, we can look at the ability of stem cells -- human stem cells to provide what is called long-term repopulating ability in culture that appears to be analogous to what we see in terms of the isolation of cells that are capable of repopulating, for instance, a mouse.
Q. When you say "culture," you're talking about outside the human body?
A. Long-term culture, yes.
Q. Now, the long-term repopulating cells, are they still in one of the committed progenitor compartments?
A. Yes, they're way back. They're at the very, very beginning of the pluripotent stem cell compartment. Committed progenitor cells are not long-term repopulated cells. They're virtually -- they play some role in the intermediate repopulation, but they're not even what I think you could refer to as intermediate repopulating cells. They have a fixed short-term life-span on the order of a few weeks at the most.
Q. Say that again about the life-span.
A. On the order of a few weeks.
Q. The long-term --

A. Short-term. Short-term are certainly no longer than a few weeks.
Q. I'm sorry, that's what I missed.
Now, the short-term repopulating cells, do they fall in one of these compartments?
A. They -- well, again, here there is some fuzziness with respect to the difference between committed and functionality. Committed progenitor cells have some short-term repopulating ability. If you give committed myeloid progenitor cells to an individual or a mouse, they're going to immediately make cells of the granulocytes. So they participate very early on in that process, but they fall within -- certainly in the mouse, within the 14-day window, and in humans probably comparable to that.
Q. Can you tell me if there is a difference in how thoroughly you have been able to, I guess, characterize or identify the progenitor cells in these committed compartments, compare the lymphocyte or the lymphoid progenitor cells to the myeloid? In other words, can you do one better than the other, or have you got them both pretty well lined out, in your view?
A. Well, we have asked different questions, depending upon the results that we have gotten. We have certainly characterized the myeloid progenitor cell

quite well in terms of functionality, ability to clonate -- the ability to form clones of cells in response to growth factors of cytokines. We certainly know their response to exposure to a number of agents, certainly the metabolites of benzene. We have followed and are currently tracking the response of cells in the pluripotent stem cell compartment as they track into the myeloid compartment and the effects of these agents on those.
In the case of myeloid differentiation, we've been able to characterize cell-specific metabolism and the enzymes that are present as a function of differentiation in those cells which confer upon the myeloid progenitor susceptibility to an agent such as benzene.
We've been able to characterize and contrast some of the enzymes that are present in myeloid cells from lymphoid cells, and we've been able to characterize and contrast the functional clonogenic responses of lymphoid cells versus the myeloid progenitors in terms of exposure to many of these agents.
We still have further studies to do in terms of looking at cell-specific metabolism for a variety of other types of processes in lymphoid cells.
Q. You mentioned that for the myeloid line, that

you have done quite a bit of work with the -- or at least some work with the metabolites of benzene?
A. Yes.
Q. Have you also done the same work with the lymphoid or lymphocyte compartment?
A. Yes and no. We have focused primarily on the myeloid progenitor cell differentiation process because that's where we have seen effects, but it's not fair to say that we haven't looked at early events in lymphoid differentiation, because we have, to the extent that these agents have not produced demonstrable alterations in those cells.
We have looked at cell-specific metabolism in the myeloid progenitors, because there are very specific enzymatic changes that occur consonant with commitment to myeloid differentiation that are important for the secondary or tertiary metabolism of benzene. We don't see those enzymes present in lymphoid progenitors or lymphoid cells. We have also seen -- we see a different pattern with respect to the enzymology of those cells.
We have also seen direct changes in differentiation that are functionally associated with myeloid differentiation that don't occur on the other side with respect to lymphoid differentiation.
Subsequent to those studies, we have devoted

most of our time to looking at myeloid differentiation because that's where the action is and that's where we see effects.
As I said, there are still other studies that we have in mind to do to look further at lymphoid differentiation, although we're looking at, at this point, a lack of effect rather than positive responses in these systems.
Q. Have you also looked at metabolites of benzene and their interactions with cells from the pluripotent stem cell compartment?
A. Yes, and we're in the process of doing that now.
Q. No results yet?
A. I have some results. They're not published at the present time, but yes, we do have some results.
Q. Do you see any effects?
A. Yes, we see some fairly profound effects associated with shift in differentiation toward myeloid cells. The first effect we see is a change in the pattern of differentiation that occurs in these cells, and it's consistent with a push into the granulocytic committed compartment.
Q. When we talk about the effects of the metabolites of benzene on either the myeloid, the

lymphoid, or the progenitor cell compartments, what effects are you looking for? If you could just kind of tell me what those are.
A. Well, a pattern has been observed and characterized that is very demonstrable in the evolution of secondary acute myelogenous leukemia. So there are a pattern of changes that have been described, both functional and genetic, that occur in the progression and development of AML, secondary to exposure to chemotherapy or to benzene, that define stages or events that appear to be very important within the process. One of those is a shift in response to growth factors, so the cells respond differently to specific growth factors. In particular, one appears to be an early event and characteristic of agents that are associated with the production of AML.
There are also changes that occur with respect to differentiation or the maturation process that are consistent with functional changes in cytokine response that we're looking at, and these are events that precede -- that apparently precede the types of changes that are associated with fixed or permanent changes, such as cytogenetic alterations, where again, in secondary AML, there is a fixed pattern that has been demonstrated that appears to be very strongly expressed in secondary

leukemias.
Q. You mentioned that there are growth factors.
How do you use that term in this context?
A. Technically, we're talking about cytokines.
These are low-molecular-weight molecules that function as, if you will, intercellular hormones, or these are molecules that allow developing cells to communicate with each other and that regulate the differentiation process.
Q. That regulate the differentiation process?
A. They regulate differentiation and growth.
There are cytokines that act very -- there are cytokines that appear to be responsible for survival of stem cells.
There are cytokines that are called early cytokines in that they're responsible for maintaining early response -- the response of early cells in the commitment process.
There are cytokines that are of intermediate level of differentiation that support the growth of maturation of cells that are committed -- that have just been committed.
And then there are late-acting cytokines that are primarily associated with late -- with end-stage maturation of committed cells, single-lineage cells.

And then there are a variety of cytokines that cannot do any of those but can influence the behavior of those I just talked about.
So it's a fairly complicated paradigm, and if you're going to look at the functionality of these things, you have to look at them -- basically, it's an elaborate dissection and reconstitution process, where you look at how they behave individually and how they behave together.
Q. My mind was conjuring up all kinds of questions to ask you as you went along, and I probably will tend to jump around a bit.
What you're telling me that you've been able to find, is this now for rodents or is this for humans?
A. There are some subtle differences between rodents and humans, and this certainly is not all our work. It's been known for some time that survival - there are subtle differences in cytokines that appear to be effective at promoting survival of stem cells. In the mouse you're looking at a cytokine called interleukin-3, and one called stem cell factor, which appears to play a fairly important role as far as the mouse is concerned.
In humans it's interleukin-3 and GM-CSF, or granulocyte/macrophage colony-stimulating factor. Stem

cell factor doesn't appear to be a major player in survival of early stem cells in human. As one gets further downstream, the selectivity and the responses of cytokines appear to be fairly comparable between the species.
Q. One of the thoughts that came to my mind that I am sure you can answer for me is that in, I guess, a healthy human being or healthy mouse, this system of the blood and its regeneration has a constant ongoing effect, right?
A. Yes.
Q. And so you have got pluripotent stem cells that are differentiating and developing into those that go down the lymphoid line and those that go down to the myeloid line, and all this is going on simultaneously within the body, right?
A. Yes.
Q. Did you say that the metabolites of benzene tend to shift the production of the pluripotent stem cells from or more toward the myeloid compartment then?
A. More toward myeloid and away from erythroid, so that there is what is an apparent stimulation of granulopoiesis, or the production of granulocytes. This has been reported in animals before. It's been interpreted variously by different authors, but there is

this -- there is evidence out there of this quizzical increase in granulopoietic activity in hematopoietic tissue from mice exposed to high levels of benzene, which has not been entirely understood. And what we're seeing is, there is a shift away from erythroid and a shift toward granulocytic granulation.
Q. You say that for humans, as well?
A. Well, yes.
Q. When you say that for humans, as well, is this in a test system or in human beings?
A. It's in a test system.
Q. Now, it occurs to me that if you have got a living entity that has a need for myeloid and lymphoid cells being produced on a day-to-day basis to survive, and you create a shift to the myeloid compartment and, as you say, more to the granulocytes, does that not stress then the lymphoid side or the lymphoid compartment?
A. Not necessarily. A priori, there would be no reason to reach that conclusion. It depends on the nature of -- it depends on the nature of the insult and the exposure. It also depends upon the duration of exposure.
And what I think is ultimately where you may,

in effect, have that situation develop -- and this is - this is at this point speculative; we certainly don't have sufficient data to make a definitive statement - but there is some evidence that at the earliest stages of stem cell development there is a promotion of differentiation. That may very well play a role in the eventual failure of the bone marrow -- that is to say, in the case of the evolution of a refractory anemia or aplastic anemia, if you will. It may play a role there, because you may, in fact, be shifting the propensity of the earliest cells to stay undifferentiated and capable of recapitulating. Whether or not that has -- we certainly at this point don't have any evidence that there is downstream any specific stress on the lymphoid system.

As a matter of fact, we know from animal studies, as well as human exposures to benzene, that, for example, erythrocytes are a relatively late effect in the pantheon of toxicities that are associated with bone marrow toxicity from benzene; certainly not an early effect. And yet we have evidence that there is a primary shift away from erythropoiesis that occurs functionally at the level of those cells, but it is not enough to produce an anemia in any short period of time; and in fact, the animals and humans can survive

following fairly egregious exposures to benzene for long periods of time without demonstrating any clinically significant toxicity.
Q. Tell me, for your research that you have done involving the metabolites of benzene, how you carried out these various studies of evaluating the effects of the metabolites on the different pluripotential stem cells and the myeloid and the lymphoid. In other words, do you isolate these particular cells out and conduct an experiment? Do you test more than one at one time? I mean, how does that work?
A. It depends upon the nature of the specific question we're asking or the study that we conducted. Early on in murine studies the first thing we did was look at clonogenic activity in response to defining cytokines. So we were looking at their functional ability to form colonies of cells that differentiated and formed the specific types of lineages we're talking about.
We started out with whole bone marrow, without any purification scheme, and we used those functional clonogenic activities as the definition of what the cell populations were which we were looking at. So we were indirectly looking at cells by looking at their function and by looking at the effects of various metabolites or

combinations of metabolites on individuals to find cytokine-stimulated clonogenic activity so we could say something about the functions that were being affected. After seeing very selective effects on myeloid differentiation in that system, we went to -- we began to employ purification techniques to hone in on the cell populations and to allow us to be more focused on the physical cells, as well as their individual function. At that point, we began moving into humans and invested a great deal of effort developing the purification schemes we were talking about, because in humans it's much more important in looking at cell-specific function to be able to isolate these cells for a number of reasons.

First and foremost, when we do a study in a mouse, we have the ability to completely evacuate the bone marrow, so we don't have to look at a sample of mouse cells. We can look at the universe of -- totality of bone marrow cells from a mouse. We can take a femur and look at every cell that is in it.

In humans, we are taking aspirates of femoral bone marrow. They are representative of the bone marrow. And in order to provide for standardization from one sample to another and one person to another, we had to develop purification procedures that allowed us

to be looking at the general population of stem progenitor cells and to know how many we had there from one sample to another. We also need to subfractionate those into defined populations if we're going to look at metabolism and look at individual function.
So we began our human studies with the background we had from mice, looking at individual - the effects of individual metabolites and compounds on cytokine response, then on semipurified cells, and then into humans, looking at semipurified and now highly purified cells, cytokine response, their ability to form colonies, differentiation, antigen expression, and expression molecules on their surface, as well as the functional characteristics.
I hope that was responsive.
Q. Well, I think it was. I just wanted to make sure you finished.
Now, obviously from the description of the grants and contracts that you told me about, one of the interests that you have had is the endpoint of leukemia.
A. Yes.
Q. How do you go from these observations of functionality, and any other parameters that you have told me about, to knowing when you have got a cell that

is going to be leukemogenic, or have you gotten that far?
A. We know from -- I mentioned briefly before there is a very strong pattern associated with the development and progression of dysplasias and leukemias that are secondary to exposure to chemotherapeutic agents or benzene. That, superimposed on our general knowledge of cancer biology today, and of the types of events that occur in the progression and development of cancer, gives us -- allows us to set forth hypotheses as to what we would expect to see at a given stage in the leukemogenic process, in the development of leukemia. We are in the process now of looking at the individual steps in that which occur early on. For instance, one would predict from in vivo animal data, from human data associated with exposure to chemotherapeutic agents, the early events associated with altered cytokine response as an early effect. One can see changes associated with functionality of cells that predispose towards the types of lesions that are seen in the progression of AML, and we have then used those changes to generate hypotheses as to how the cells should behave in culture.
And what we found so far is that there is very good concordance between the very earliest events that

are seen in culture and events that are predicted by in vivo experiments in mice, as well as in vivo/in vitro studies of patients that have developed dysplasias secondary to chemotherapy.
Q. Let's see if I can distill that in my mind a little bit.
If we take a human being who has leukemia, and let's say acute myelogenous leukemia, you can find in that person's circulating blood a leukemogenic cell; is that right?
A. I think the way you have used the terminology may be misleading.
Q. Help me with terminology so that I would use or could use terminology you would use.
A. A patient who has developed leukemia is going to have circulating leukemia cells. There are going to be, for that given place in time in the natural history of that patient's disease, abnormal cells that are what we refer to as leukemia cells. They are -- they originate. They may not, however, represent precisely the cells that the leukemia originated from. So in other words, the bone marrow cells that are the -- the original cell -- and I should say cell, because virtually all human leukemias are monoclonal. The original cell, certainly for the vast majority of

secondary AMLs, can be found to originate at the level of the committed myeloid progenitor, so there is a committed myeloid progenitor cell at the apex of this amplification scheme that is associated with the clonal development of an AML.
In the case of CML, or chronic myelogenous leukemia, it's a cell within the pluripotent stem cell compartment. Now, some individuals will argue with that and say that even in CML, it's not a pluripotent stem cell; however, in terms of clonal origin, it is a cell with bifunctional characteristics that can give rise to either lymphoid or myeloid cells. So whether or not it is in fact, a long-term repopulating cell or an intermediate-term repopulating cell is not of any significance with respect to defining its bifunctionality.
In the case of myeloid cells, there is certainly, for the vast majority of cases, evidence that we're dealing with a multi -- a cell of multilineage potential that is restricted to the myeloid pathway. Those cells are the cells that are the neoplastic transformed cells. They give rise to abnormal cells. They can also replicate. They may have longer life spans than the normal cells and what we normally think of as leukemia cells, but the lesion originates at the

level of the progenitor cells that I am talking about.
So when you talk about a leukemogenic cell in the peripheral blood, you're dealing with a leukemia cell; but it is not, by definition, the originating - the site of clone origination of the tumor.
Q. I have heard a lot of things that I need to sort out with you, if I could.
If we could stay, for instance, for a moment here with AML.
A. Okay.
Q. Because we both know there are lots of kinds of leukemia, it might help me if we could stick with one for a minute.
Are you saying that -- let me back up.
To have an acute myelogenous leukemia, there has to be a cell which is defective that is capable of reproducing?
A. Yes.
Q. And I think you said that since leukemia - almost all human leukemias are monoclonal, that means that there had to have been, at least in the beginning, one cell that was defective, i.e., had a lesion, that led to the leukemia in the individual. Would that be accurate?
A. It has to have a number of lesions.

Q. And all of those lesions have to be in one cell?
A. They have to occur not necessarily all in precise order, but there are a number of independent events that must occur in the clonal progression of that lesion that all intersect with one cell. One cell has to basically have all those events occur before you arrive at the stage where it is either a dysplastic cell, which is on the same line, or a leukemia cell. And those changes are associated with functional changes in the survival of that cell, its response to growth factors, and its differentiating potential.
Q. So help me again. If we're talking about AML, then we have -- at some point in time there is a cell that is defective with this proper series of lesions, and this cell will replicate itself to produce a bunch of defective cells that become AML in a human being?
A. Yes.
Q. Now, that defective cell that is at the beginning point of AML, its precursor lineage that got at all these lesions at just the right point in time of its development to become AML, where do those lesions occur in the generation of that cell from its earlier lineage?
A. Certainly in terms of the clonal evolution of

the tumor, which means you are assuming that the cell - the point at which the cell becomes malignant and the clonal origin of the tumor, okay. That cell, for the majority of cases that have been studied, certainly for secondary leukemias, is at the level of a multilineage myeloid progenitor cell.
Q. Say the last sentence again.
A. Certainly for the clonal evolution of the tumor, that cell is at the level of a multilineage myeloid progenitor cell.
Q. Multilineage myeloid progenitor cell?
A. It can be as early as a cell that can go erythroid, myeloid, monocytic, thrombocytic. It may be a cell that is committed to granulocytic/macrophage-type cells, but is basically within that span or spectrum for the vast majority of cases.
Q. And so that cell may be even in the pluripotential stem cell compartment?
A. A small number of -- in a small number of cases there is some indirect evidence to suggest that is possible. In the majority of cases that have been studied, certainly for secondary leukemias, there is no evidence that that cell has pluripotent characteristics. It appears -- I would say roughly 85 percent of the weight of evidence associated with characterization of

those suggest they are committed to myeloid -- they are myeloid progenitors. One cannot rule out in a subpopulation of AMLs, per se, that there could be involvement of a pluripotent cell, but there is not substantial evidence to support that notion. There is much more evidence to support its committed progenitor. Now, all these lesions we were talking about, or events, have already occurred, because we're talking at that point the clonal evolution of a neoplastic line or clone. So we're talking about events that have preceded that.
Q. Is what you're saying now is that some of these lesions that appear in the committed myeloid progenitor cell that becomes the source of the leukemia, some of those lesions may have actually -- or a portion of the lesions may have actually occurred while this cell was still a progenitor stem cell or in the progenitor stem cell compartment?
A. That was certainly one of the questions that we set out to ask early on. That's why we were interested, and still are, in early events, because quite frankly, there are a number of independent steps that have to occur in the evolution of a leukemia, but the earliest ones appear to be the most important with respect to the potential to evolve into leukemia in the

first place. It's kind of a numbers game.
If independent steps have to occur, the probability that those steps are going to occur changes with respect to what has already happened, and it can influence what is going to happen downstream. So the earliest events are .most important.
I would say that the sum total of the work that we have done over the last three years does not support the concept you have just described in terms of a random -- or implied with respect to random events that may occur at the level of pluripotent stem cells. The earliest event we see is a -- which is not a cytotoxic event at all. This is a regulatory response, and the presence of normal cytokines is a shift in differentiation or promotion in differentiation toward myeloid.
So if I was going to put it in a -- if I was going to try to put it into some layman's context, it would be more like Marshall McLuhan's, "The Medium is the Message." If the first response that a primitive stem cell has is to go myeloid, the first thing we see is a shift in differentiation. So at that point in time it may not be a normal myeloid progenitor. I can't tell you that, but it certainly thinks it's a myeloid progenitor, and it is acting like a myeloid progenitor.

Q. Are you at the point, then, that you can say that it is biologically implausible to have any kind of secondary leukemia other than an AML?
A. I would say that -- based on our current understanding of the processes that are involved in the evolution of leukemia, that the evidence would support that, that it is biologic -- we see no plausibility for other major types of leukemia. That does not mean at this point that we can say, absolutely never, it can't be, but certainly all the evidence points to the -- to that being the case.
I think that's also consonant with the epidemiologic literature in that it explains -- merely explains what we see, by far and away, the case in terms of secondary hematopoietic neoplasms following either exposure to chemotherapeutic agents or benzene.
If you take all -- if you don't critique the validity of any given study, but you simply add up all the cases that have either been hypothesized as case reports or as quantitative retrospective epidemiology studies or case-control studies for secondary leukemias, you end up with virtually -- I did this exercise once recently -- I think you end up with about 96 percent AMLs, or myelodysplastic syndromes; and everything else that has ever been hypothesized falls into that 4

percent range.
So you have got an overwhelming body of evidence suggesting a predisposition towards AML as the principal, if not the only, secondary leukemia type associated with chemotherapy or occupational exposure.
MR. BELFOUR: Excuse me, Herschel. Could we assume that your question which you last asked was directed toward benzene and chemotherapeutic agents? As I recall, you asked if it was biologically implausible to have a secondary leukemia other than AML, and I didn't hear a qualification on that, but Dr. Irons answered, I think, with regard to benzene and chemotherapeutic agents. I don't want there to be a misunderstanding about what you were asking.
MR. HOBSON: I think you stated the question I asked. I don't know how that affects the answer you gave, but I think you restated the question I asked.
A. I may have missed -- I may have mistaken the generic nature of your question. I was speaking specifically to exposure to alkylating chemotherapeutic agents and to occupational exposure to solvents, of which benzene is the only known leukemogen. If we invoke radiation, we're talking about a totally different story, and radiation -- and there are explanations that I think provide some understanding as

to why -- and that radiation is capable of and, in fact, tends to find resting cells more susceptible than dividing cells, whereas the types of lesions that are seen in the progression of AML associated with exposure to chemicals or drugs are division-dependent processes. Without division, they don't happen.
Q. (BY MR. HOBSON) In regard to radiation, are you saying, then, that a cell that is at rest is more susceptible to radiation, if cancer is the endpoint, than one that is dividing?
A. It actually can be, yes. Certainly, in terms of the stem cell population, that's the case. That's one of the reasons why, for instance, you see a very respectable incidence of CML following radiation. CML occurs in a cell that, for the most part, is a resting cell, and radiation is effectively doing that.
Q. Is it a function of the resting cell being more susceptible or is it a function of just the statistics of how many of the stem cells are actually replicating as opposed to being at rest?
A. No, it appears to be more a function of resting cells being more susceptible to radiation-induced damage.
Q. What research do you have that points to that? How can you say that?

A. This goes way back. This is work that began in the '50s and has been the subject of a number of studies over the years that have looked at the relationship between radiation-induced damage and chemotherapeutic or chemically induced damage. The chemotherapeutic agents target cycling cells. That's the whole paradigm; that's the whole goal in the selection and design of chemotherapeutic agents: It's to spare stem cells and to target cycling cells. Radiation does not show that kind of selectivity. Radiation obviously will produce damage to replicating cells, but there is evidence to indicate that in certain replicating-cell populations, they're more resistant to radiation-induced damage than the resting ones.
Q. Doesn't that depend on where the cell is in this progenitor line?
A. To a certain extent, yes, but if you want to target a stem cell, you don't use a chemotherapeutic agent. You use, by far and away, a combination of radiation/ chemotherapy.
If you want to leth -- I mean, the classic model in the mouse is lethal irradiation. That's the model for either bone marrow -- for looking at reconstitution or bone marrow transplantation. Now, effectively, you can use chemotherapeutic

agents to obtain bone marrow oblation, but often radiation is used in conditioning; and when chemotherapy is used, you're talking real high doses of agents, very high doses of agents.
Q. Getting back to the question I was trying to ask, though, if we talk about the cells that reside in the pluripotent stem cell compartment, can you show me any research that says that those cells that reside in that compartment are either more or less susceptible when they're at rest or when they're replicating, when radiation is the agent?
A. Yes. I would have to go back and search my files, because I didn't come prepared to discuss that literature; but, yes, I can find data to support that.
Q. I take it you're going to read and sign your deposition?
A. Yes.
Q. Would you insert on whatever page this is in your errata sheet a note as to what paper you would cite me to, or papers, to that proposition?
A. Can we define a question so that I can write it down?
Q. Yes, sir. I am trying to find out -- if we talk about the cells that reside at the pluripotent stem cell compartment, as you define that terminology, I am

looking for a paper that you can cite me to that says that cells that are at rest have a different susceptibility to cells that are at replicating if radiation is the agent. Is that a logical question for you to answer?
A. Yes.
MR. HOBSON: Let's take a break.
(Break was taken.)
Q. (BY MR. HOBSON) Have you been able to locate one of these defective cells that has the lesions in place, that is capable of replicating, that would later give rise to an individual being diagnosed with AML?
MR. BELFOUR: Herschel, could you ask that one again? It may be that it's not vague, I just didn't understand the question.
Q. (BY MR. HOBSON) Sure. I am trying to find out if Dr. Irons has been able to locate -- or maybe I said isolate -- one of these cells that is defective that has lesions in place, that is capable of replication, that later would give rise to a person being diagnosed with AML.
A. If you are asking me, have we been able to create AML in the test tube, the answer is no. That is certainly an ultimate goal. We're in the process now of defining steps in the process and attempting to produce

those events and characterize those events.
If we are capable ultimately of bringing it all together and integrating it into a single model and producing AML in a test tube, that would be fabulous.
We certainly haven't done it yet.
Q. Where are you in that process?
A. We are in the process -- we have characterized early events in altered differentiation and response and we're linking now with studies looking at early cytogenetic abnormalities associated with exposure to benzene metabolites.
So I would say we're -- we are looking at the transition from early transitory changes that are temporal -- temporarily transitive to the first steps that are associated with the first known clonogenic step in the development of AML.
Q. What do you mean by "the first known clonogenic step"?
A. Certainly, the earliest event -- the earliest permanent event that has been associated with the evolution of AML secondary to occupation or chemotherapeutic exposure is cytogenetic aberrations involving chromosomes 5 and/or 7. Those are certainly the predominant pattern that is seen.
Chromosomes 8 and 21 have been implicated as

well, but they're also seen in primary leukemias as frequently as they are in secondary.
But the earliest events that are seen are associated with aneuploidy and the potential involvement of chromosomes 5 and/or 7 in the -- certainly the dominant model for secondary AML.
We're in the process now of looking at those early events. We certainly have seen aneuploidy. We're in the process now of trying to translate into a system where we can look at the evolution of those types of changes in liquid culture, looking at myeloid stem - myeloid progenitor cells and pluripotent stem cells, to see where these events occur.
Q. Tell me how you use the term "aneuploidy."
A. "Aneuploidy" basically means the addition or loss of a chromosome. One also sees interstitial deletions. These are the types of lesions that are seen in the evolution of secondary AML, and they are precisely the types of abnormalities that are consonant with the molecular effects of the quinone metabolites of benzene, and they appear to be comparable to the effects that these metabolites have on cells in culture, and they're comparable to what has been seen in benzene-poisoned workers in China.
Q. When you say the quinone metabolites of

benzene gives you -- I have forgotten the term that you used. You didn't say it gave you the same as, but you used a different modifier, and I have forgotten what you said now. Let me start over. I have lost the question myself.
Do you find that you can have these chromosomal changes in chromosomes 5 and 7 when you expose cells to the quinone metabolites of benzene?
A. Previous studies by other investigators have suggested involvement of 7. The Chinese-worker studies have looked at chromosomes 7, 8 and 9. We have just recently produced effects associated with 7. We're beginning to look at 5. 7 appears to be certainly a susceptible endpoint associated with exposure to either benzene in vivo in humans or hydroquinone in vitro in human cells.
Q. Each time I think you said -- in your answer there you used a term like "suggestive" or "suggested," and then you said "associated with." And what I am trying to find out is, Do you actually find this chromosomal change for the 7 chromosome; or you say, it's just suggested?
A. No, we see a loss of 7. We are looking to see whether it's suggested with the whole chromosome or whether it's an interstitial-type deletion. We need to

look at 5 in detail, as well. We also want to compare it to other types of chromosomes to see if there is a selective effect with respect to the chromosomes that are associated with AML, or whether it is a nonspecific effect on virtually any chromosome that just happens to be consistent with cell survival and risk of future events if it happens to involve chromosomes 5 or 7 or 8 or 21.
Q. At which level in cell development have you done work with the quinone metabolites of benzene that leads you to the finding that the 7 chromosome is damaged?
A. A number of cell lines have been looked at by other investigators. We have looked at lymphoid cell lines. We are looking in bone marrow now. All of these studies suggest the predisposition to involvement of chromosome 7 in that. In other words, 7 is certainly a target in replicating cells associated with exposure to these agents.
Q. You're looking at the lymphoid compartment?
A. We're looking at a lymphoid line basically as a surrogate for bone marrow early on to develop the technique, and we have virtually completed those studies, and we're moving into actual bone marrow cells now.

Q. So at this point are you able to take human cells -- expose human cells to the quinone metabolite of benzene and find chromosome damage to the No. 7 chromosome?
A. We have not done that. We are in the process of doing that as I speak.
Q. And the 7 chromosome is merely one of the chromosomes that has to be affected to actually produce an AML?
A. The earliest aberrations that have been seen involve 7 or 5, interstitial deletion of 5 or loss of 5, one copy of 5 and/or loss of 7. 7 appears -- 7 appears to be found more often, but I think it's been looked for more often as well, so that may be an artifact. That's the earliest event that is seen.
Other chromosomes have also been implicated, but they are not necessarily a -- they do not allow you to distinguish between primary and secondary leukemias. It doesn't mean they're not involved in certain cases of secondary, but they're also involved just as often in primaries. 5 and 7 early involvement appears to be a hallmark of secondary leukemia.
Q. Is the 5 and 7 -- are the 5 and 7 chromosomes only involved in AML or are they involved in other leukemias?

A. For the most part, we're looking at AML, certainly in the case of 5, although 5 is involved as a step in other types of cancers, such as the development of colon carcinoma, although we're looking at different genes that appear to be involved in 5 associated with colon carcinoma, and they are in the case of AML.
Q. But for the other leukemias, 5 and 7 would not be involved?
A. They are not made -- they certainly do not - there are not nonrandom chromosomal aberrations in the lymphoid leukemias that involve consistently 5 or 7. That's not to say that you may not find another leukemia type that may have involvement of 5 or 7, but there is certainly no evidence that it is the earliest change or that it is a consistent nonrandom finding in those tumors.
I mean, the chance -- the importance of a given chromosomal abnormality really relates to the genes that are affected or involved associated with that abnormality. And those genes -- the significance of an alteration in gene function is dependent upon the stage of differentiation or gene expression in that cell at a given point in time. Let me see if I can give you an example, because I know that is fairly esoteric.

In CML, the primary gene abnormality -- genetic abnormality that occurs in CML involves the production of the Philadelphia chromosome, and that reciprocal translocation is an abnormal fusion product between two genes: the cluster gene or region and the abl oncogenes, the bcr/abl. That, in turn, indirectly leads to abnormal regulation of the ras oncogene product. The ras gene isn't mutated, but the BCR/ABL fusion product deregulates the function of ras in a pluripotent cell.

If that cell was to undergo a mutation in ras, it would probably be of no biological significance whatsoever, because you already have deregulation of ras; and if you mutated the gene, it would have no bearing on the life history of that cell.

A ras oncogene mutation in another tumor in another stage and differentiation might be very, very important. So where a gene is affected in the natural history of the cell can make a big difference.

In the case of genes on 5, we know that the area 5 that's always affected in secondary AMLs corresponds to a relatively small number of genes that are very important in early steps in myeloid differentiation. If that were to occur in a skin cell, it may have no consequence whatsoever.

Does that make sense?
Q. Yes, for the moment.
The question that comes to mind next is you say "secondary" versus "primary" leukemias. How do ~.~»1 know which is primary and which is secondary?
A. In the occupational literature that's a very -- that can be a very practical, relevant question, and in fact, the occupational literature is a little fuzzy with respect to the types of findings that are seen because of the problems of assigning exposure and causation, what have you.
Having said that, you still see a very pronounced pattern with respect to the characteristics of AMLs in that literature between exposed and controlled populations.
But all that aside, where we have a much better handle on AML than many diseases is the relationship between AML secondary to chemotherapy and/or radiation therapy. There you know precisely what the exposures are, you know what the agents are, you have a captive population, you know what the primary diseases are, you know what the secondary leukemias are. And that's a very, very strong, rich, and deep literature base that superimposes a great deal of structure on our interpretation of the occupational

literature. They're very comparable.
So we can learn a lot from looking at that literature where we do have a very clear picture of the differences that are seen in primary versus secondary AMLs.
Q. Talking about the occupational exposure, I can see your rationale for the chemotherapeutic and the radiation therapies, because as you say, there you have probably extremely well-regulated exposures; at least one would hope that most of the time they are. And so you have your exposure in hand, I guess, or at least it's somewhat understood better. But if you go -- you say that in occupational that's not so.
Jumping back for a minute to the chemotherapeutic- and the radiation-secondary leukemias, is the definition of a secondary leukemia for chemotherapeutic and radiation the fact that you have got the leukemia in the exposed population, or is there something about the individual who has received the exposure that you can say, I can look at this individual and say that this is secondary leukemia versus what just turned out to be a primary leukemia that happened? Does that make sense?
A. The question makes sense, and the answer is both.

Early on in that literature -- obviously, you're dealing with epidemiologic studies and associations, and early on, the primary definition of a secondary leukemia was one that occurred in a patient who received chemotherapy for some other neoplasm, blit it became very clear very quickly that there was a prescribed pattern, both with respect to the progression and development of that disease, as well as the characteristics of leukemias that were seen that differ dramatically from what you see in the spectrum of leukemias that aren't associated with secondary -- as a secondary.

You now -- I think it is arguable that there are specific criteria that can be used to help distinguish between a primary and a secondary that are very strong. There are some changes that aren't particularly useful, but there are changes or characteristics of those that are very useful.

For example, we've been talking about 5 - chromosome 5 and/or 7. If you add all those up, one finds -- well, first of all, just take general cytogenetic nonrandom -- demonstrable nonrandom cytogenetic abnormalities, clonal aberrations, cytogenetic aberrations occurring in a leukemia.

If you use banded chromosome analysis, and you

look at primary leukemias, there is no evidence of any previous exposure to a potential or putative cause of aberrations, slightly more than half present with a demonstrable clonal chromosomal aberration, a 50 to 60 percent. If you look at patients developing AML secondary to chemotherapy, it's arguably 95 percent. Given the experimental area, you could be looking at virtually 100 percent. It's a huge preponderance.
So the presence of a nonrandom clonal cytogenetic aberration provides you with some means of discrimination a priori. Basically, you can't, if you have it, distinguish a priori between a primary or secondary. But if you have no demonstrable chromosomal aberration, it's more probable than not that that is not a secondary leukemia, because there is a much greater predisposition toward clonal chromosomal aberrations in the secondaries.
If you take it a step further now and look at the specific involvement of chromosomal aberrations in the various types of leukemias, 8 is found in both, 21 is found in both to about the same degree, on the order of, I think -- maybe 5 is low -- probably somewhere between 5 and 10 percent.
If you look at 5 and/or 7 together, you're looking at, depending upon the study -- certainly in the

chemotherapeutic literature -- 80 to 90 percent of secondary AMLs involve 5 and/or 7, and only about 15 percent, or something like that, of primary, that have a cytogenetic aberration, would involve 5 or 7. So there is a clear preponderance of that type of chromosomal aberration in secondary leukemia.
Q. If I asked my question in a little bit different way, would the answer be the same? And the question asked a different way would be -- I guess the preliminary question is, Can you have a primary leukemia in a person who has received chemotherapeutic agents?
A. I don't know whether that's a semantic question or a philosophical one.
Q. That's why I went down this road, and I wasn't sure we were communicating, because that's what I am trying to find out really, the answer to that question. I mean, if you are going to define a secondary leukemia as any leukemia that appears in someone who has had chemotherapeutic agents administered, then it seems to me to be difficult, then, to separate out primary leukemias based on what you find in those people.
A. Let's take the two to whatever percent of individuals who develop AML secondary or AML following intensive chemotherapy who have no clonal chromosomal aberrations.

Q. Say that again.
A. If you were to take the relatively small number of individuals who present with AML, one of the subtypes, that does not have a demonstrable nonrandom clonal chromosomal abnormality, we're talking very, very small number of people -- very, very small fraction.
If you took it one step further and went in and looked at specific regions of the chromosomes on those individuals that are involved in secondaries and they were intact -- in other words, they're using molecular probes now, not just using visual cytogenetics -- and found that, in fact, they were intact, I think philosophically you would have an interesting question. And that is, Do those represent primary totally independent events that have occurred independent of the toxic exposure those patients have received?
1 think that's certainly not -- it's a philosophical -- at this stage of the game, that's a philosophical question, but I think you could certainly ask it in that context.
I think the pattern that is seen in the secondaries is so striking, with respect to the predisposition, relative to what is seen in the general population, that for most of those the answer to your question is no, they're not primaries, they're

secondaries; they have a different natural history in many respects than what you see in the primaries. But it is possible, I imagine, that some -- the small number of individuals that don't present with a pattern that you can find consistent with secondaries could conceivably be primaries.
Q. But none has been reported that you know of?
A. Well, I mean, we do have negative -- we don't have an absolute concordance of 100 percent cytogenetic aberrations in AML secondary to chemotherapy. Do those represent primaries? I don't think anyone can tell you that.
Q. That being the case for the chemotherapeutic agents, would we put radiation in that same category? Is it philosophical there as well?
A. Well, you see a lot of the same changes in radiation too. With radiation you're dealing with a different dynamic, because you don't always know precisely what an individual is exposed to, so you're in the same -- you're basically in the same situation you are with occupational exposures. There is some fuzziness with respect to the exposure data, even with respect to radiation.
Q. If we're talking about people who have had radiation therapy -

A. I mean, there you do have a captive.
Q. I'm sorry. If we talk about people who have had radiation therapy where their exposure is pretty well defined from the therapy?
A. Right. What you have to keep in mind there is that as we become much more sophisticated in terms of distinguishing and designing effective therapies and figure out where the risk factors are involved, it has become clear that radiation is certainly within the therapeutic range of exposures, not anywhere near as potent as chemotherapeutic agents in inducing AML. It can happen, but it's not -- it is not as effective as chemotherapeutic agents; and, in fact, there is a major difference between combined radiation/chemotherapy and radiation and chemotherapy; and chemotherapy looks to be the big culprit there.
Q. None of the work you have done at the university or earlier at CIIT involved radiation; would that be accurate?
A. No. We have looked at radiation -- we have done quite a lot of radiation work in the context of the NASA grant, looked at interactions between radiation and leukemogenic agents in the mouse model.
We have not directly done any dosimetric studies looking at radiation with human cells, primarily

because the model that we were characterizing for NASA, we basically provided sufficient evidence that the mouse was not a good model for predicting effects in human cells.
Q. So in the NASA work, you actually exposed cells to radiation in your research?
A. That's correct.
Q. But only to mouse cells?
A. We have done some human work. We find the mouse cells are much more susceptible. We don't see the same effects.
Q. Now, if we go to occupational-exposed groups for benzene exposure, where do you find your pool of exposed individuals that you can say are secondary leukemias that you can study?
A. Now, with respect to the human occupational literature, I'll present you with a caveat, and then I will go through it with you.
The caveat is, We have talked about some of the problems with respect to assigning or characterizing exposure in an occupational setting, which I'm sure you are intimately familiar with. You can characterize the secondary -- the occupational literature with respect to benzene solvents, and you can characterize the literature with respect to characterization of

cytogenetic aberrations in leukemias in occupational-exposed populations.
In general -- generalizations are dangerous - but in general, the most sophisticated useful cytogenetic data is in case-controlled studies where the exposure characterization is abysmal, where it's self-selected, it's an anecdotal, no one measured a thing. Where we have the greatest strength of data with respect to characterization of human exposure, cytogenetic analogies are amateurish.
Q. If they exist?
A. Yes. So we're dealing with, in general, different strengths and weaknesses with respect to the study populations.
So there is some inference that is required with respect to looking at the pattern of results that are found in one versus another. Are they consistent? Are they inconsistent? Do they support each other? Do they completely corroborate each other? And often you don't have, across the literature, complete corroboration, but sometimes you do.
So, for instance, where we have solvent exposure where benzene is a component and is the only well-characterized and accepted leukemogenic agent, we have consistent cytogenetic findings in AMLs; and the

pattern is the same.
In other studies we have -- where we know the exposure is benzene, we have nondescript cytogenetic aberrations that have been described in certain tumor types that are consistent with what is seen in the secondary -- the literature associated with exposure to chemotherapeutic agents. It doesn't provide us with specific banded-chromosomal analytical data, but the types of changes -- the types of chromosomes that are involved are comparable; they're consistent with the same pattern that is seen. So there are variations in the -- in that data that, if you put it all together, is entirely consistent.
Having said that, I have recently written a review where I attempted to summarize that literature, and I believe I brought it.
Now, I hand this to you, and I don't have it memorized, but basically this is just -- this is not an all-inclusive table. The text has more information in it.
This table shows the pattern of cytogenetic characterization in secondary AML. The first several studies involve a combination of either chemotherapeutic agents and/or some occupational data as well. The last few studies involve primarily occupationally exposed

populations where solvent and/or benzene are implicated. And there are other studies as well. It's not meant to be all-inclusive, but the pattern is consistent in either case.
Q. I see the title to what you have handed me; it says, "To be published in Environmental Health Perspectives." Has it been accepted now?
A. Yes.
Q. Any idea when it will come out?
A. No, I don't have any idea. That's worse than -- that's even more ambiguous than most journals, because it's a government journal.
Q. Since I haven't had a chance to read it, let me see if I can get any questions together, having not read the paper. It may or may not make any sense. Going back to something you said, you looked at the literature, I think you said, for solvent exposure where benzene was in the solvents and where there was no other -- and I couldn't write it down as you said it -- where there is no other well-recognized or well-something.
A. There have been a number of studies that have looked at occupational exposure to solvents, and that is in quotations. Obviously, studies vary with respect to the reliability of what that means and what is defined

by that.
What I have said is that, and it's my opinion, it's reasonable to include that data in an evaluation such as this if benzene is the only defined human leukemogen that we're talking about. And when we're talking about aromatic solvents, that that's where we are. We're not talking toluene, we're not talking xylenes, we're not talking substituted benzenes.
So if we're talking solvents and benzene in the mix, then I think it's fair to assume that if we are dealing with secondary leukemias, they are benzene-derived leukemias for purposes of population analysis and for looking at the characteristics of the tumor types.
Now, since they do fit the pattern, I think clairvoyance or hindsight is 20/20. I think I'm justified in doing that because the pattern is the same. It's what is seen with the chemotherapeutic agents. So I think that it's a fair analogy and one can deal with those in the same context.
Q. Except for the plants that are manufacturing the chemotherapeutic agents, is there any other occupational exposure to a defined human leukemogen?
A. Radiation. Certainly based upon the existing literature, no.

Q. In looking, then, here at the table that you handed me, which was on page 22 of your submitted paper, can you help me go down these and say -- you pointed out some differences between whether these were chemotherapeutic as well as occupational. I think you said the bottom four were occupational only. It's a long table.
A. The first one is the -- what do they call it? The first one is one of the first studies done looking at the working group that looked at secondary leukemias that involved both chemotherapy and some occupational, but primarily chemotherapy.
Q. Can you tell me what the occupational group was there?
A. Not without going back and -- I am not sure that I discussed it in detail. Le Beau and Rowley are chemotherapeutic. Pederson-Bjergaard, the particular one I have referenced there, he has done both and combinations of both -- which one did I reference for this? This is taking a bit, because there are several in here.
Q. That's fine.
A. Tile first one is the Fourth International Workshops on Chromosomes. That involved both occupational and chemotherapeutic. I cannot

specifically tell you which populations they looked as we sit here this minute.
Q. What is the date for that publication?
A. I think it's 1984, yes. Well, as we sit here, I am not sure precisely which Bjergaard study is cited in the table, but there are at least half a dozen cited in the chapter, and he has looked at both.
Q. Bjergaard, is he an American researcher?
A. Danish.
Q. Any idea where his benzene-exposed leukemias, his secondary benzene leukemias came from?
A. He's looked at solvent-exposed. I'm not sure which population he has looked at, as I sit here. I would have to go back and review that literature to tell you specifically where they came from.
Mitelman has looked at occupational exposure.
Mitelman, Golomb and Fagioli.
Q. Golomb; is that the Golomb that is at the University of Chicago?
A. Yes. Those are case-control studies, so they define their populations in different ways, and you have to look at each individual case-control study to see what those are and how they characterize or assign their exposure.
Some of them involve occupational exposure to

what is referred to as pesticides -- which is probably the least reliable piece of information you will ever see. It boils down to a questionnaire, Have you ever used a pesticide? -- and organic solvents, which can range from leukemias in individuals who again answer a questionnaire in a self-selected fashion.
We may be talking casual use of a solvent or we're talking about occupational-exposed individuals where there is some reasonable characterization in the to exposure stream. It varies from study to study. As I told you before, it ranges from very reliable to very unreliable.
Q. Maybe I can help short-circuit some of this, and I don't mean to sound perhaps offensive; but as I do benzene cases as a lawyer, I seldom ever find anybody on the other side of my cases who will ever say that this is a benzene-induced leukemia, no matter what kind of leukemia it is, including AMLs, and no matter what I think the exposure history might have been. And so it strikes me as odd that I could go or you could go to the medical and scientific literature and find this pool of individuals that have been studied that are conceded to 2_) be benzene-induced leukemias, from which you can then 2.4 look at their blood or bone marrow and do cytogenetics to say this is a secondary leukemia.

Can I short-circuit through that and tell me whether or not you have such a group or can find such a group reported in the literature.
MR. BELFOUR: Herschel, let me just make a general objection. I don't mind your giving a preface in order to expedite things, but I don't want the record to reflect I have not objected to those comments. Since the objections to the form of the question must be mate at the time of the question, may I make it general, and you may proceed.
MR. HOBSON: Yes.
A. As I said before, the reliability with respect to the exposure history tends to be at odds with the sophistication of the cytogenetic analysis. The most recent studies that have been done of occupational - that have involved occupationally exposed populations, that have sophisticated cytogenetics, have been done in an era in which the exposure to benzene in general is far less than it was in the 1940s, the 1950s, the 1960s. If we look at occupationally exposed populations in that era -- most notably the studies done in Europe where we have an attempt at cytogenetics, superimposed upon a very clear, heavy, consistent, egregious exposure to benzene -- there is a pattern that is seen there. There are cytogenetic aberrations. They

are consistent generically with the types of aberrations that are seen either in case-control studies or in studies of people receiving chemotherapy today where the cytogenetics is much more reliable and much more sophisticated and precise.
We do not have, outside of China, to my knowledge, a population that one can point to today, hopefully, that was consistently exposed to high levels of benzene and for which we have leukemias with clear-cut, state-of-the-art cytogenetic characterization.
The Chinese study may, in fact, represent the exception to that, and certainly the results that are coming out of that study now show aneuploidy in benzene-poisoned workers involving chromosomes 7, 8, and maybe 5; we don't know yet. Certainly the characterization of cytogenetic aberrations in the AMLs is consistent with what we see in the studies that are -- the chemotherapeutic studies.
As we go forward with that study in China, we may very well be able to answer that question directly and precisely. Right now, the evidence certainly points to it.
Q. (BY MR. HOBSON) What is the existing evidence for the Chinese workers in this regard?

A. Martha Linet has reported predominant involvement of cytogenetic aberrations, from my understanding and my conversations with her; certainly involvement of chromosome 7 features strongly in those. I do not know what the remainder of the cytogenetic is in those patients, but I do know -- in the population that is being followed now, that have been poisoned by benzene, we do have predominant evidence of aneuploidy involving chromosome 7; and studies are ongoing now looking at 5, as well as other chromosomes.
Q. Dr. Linet is where?
A. National Cancer Institute, NCI.
Q. At which location?
A. Bethesda.
Q. She is doing the hands-on research herself?
A. Some of it, yes. She is coordinating that aspect of the study. I think the study director is Nathaniel Rothman, if he is the overall director of the project.
Q. Has any of the work that you referred to through Dr. Linet been published?
A. Some of it has. One of the articles -- here is a reference. I have included here a manuscript because I'm a coauthor on it. There are a number of others that are coming out and that are either submitted

or in press at the present time.
Certainly, the chromosomal involvement involving chromosome 7 is either in press or close to it at the present time.
Q. The work that you're coauthor on, is that the result of the NCI contract that you had?
A. Yes.
Q. You said that is published now?
A. It is in press.
Q. I didn't think I remembered seeing it, but I don't read it all.
A. No, it's in press.
Q. Now, that contract work that you did for NCI, did you say that that was work that was done on workers who had been benzene-poisoned?
A. Yes.
Q. How do you use the term "benzene-poisoned" in this context?
A. A combination of industrial hygiene and personal monitoring data that documents the current exposures, as well as anecdotal and retrospective industrial hygiene data, as well as clinical evidence of bone marrow suppression associated with benzene exposure.
Q. So if I can say that in perhaps a little bit

different terminology, you have got industrial hygiene exposure data in recent times that documents extremely high exposure levels, right?
A. Yes.
Q. And we're talking hundreds, if not thousands, of parts per million?
A. We're talking highly heterogeneous exposure: level, but some individuals have documented exposure well into the hundreds, I would say 400 parts per million.
Q. By "anecdotal," you mean people telling stories of its being even worse in the past?
A. I wouldn't even go so far as having to say "worse." There is a certain conspectus with respect. '_o the descriptions, with respect to what has been seen in recent times. There is a movement to reduce exposure levels, which is wonderful. It obviously -- it obviously complicates the evaluation process, but there is now a very valuable natural experiment, if you will, or unnatural experiment involving a cohort of individuals that have documented toxicity acute - subacute toxicity to benzene who are being followed, and there are individuals independent of those who have continuous exposure to benzene that certainly in the - I would have to look back to see precisely what their

exposure levels are, but we're talking double digits with respect to ppm.
Q. And then another parameter for this group that you looked at from the NCI contract was that they actually had clinical findings?
A. Within -- yes, within that subpopulation there are individuals who actually have had some clinical manifestation of benzene exposure: cytopenias, anemias, what have you.
Q. Now, did you actually analyze tissue from some of these individuals for NCI?
A. I analyzed serum for specific cytokine levels. That was my function.
Q. And the serum that you analyzed, did it come from individuals who had a clinical manifestation?
A. Excuse me, plasma. I should correct that. It's my understanding ostensibly yes, although I was blinded to the nature of the samples, so I can't tell you which samples were what.
Q. Did any of these individuals whose plasma you evaluated as part of the NCI contract have leukemia?
A. No, not to my knowledge.
Q. If we go back then to my question of, Is there any pool of individuals who have leukemia that anyone is willing to say was secondarily the result of benzene

exposure, does such a group exist that has been looked at, to your knowledge?
A. Yes, the Chinese study. There are individuals -- there are a number of individuals, to my knowledge, who are still alive who have been diagnosed with leukemia and/or myelodysplastic syndrome where the exposure is basically a pure play on benzene.
Q. And their cytogenetics have been looked at?
A. Some of them have and, again, to my knowledge, I have not seen specific data, but my conversations with Martha Linet and the summary data that have been published or is in press suggests a consistency between those findings and the literature that we have been talking about here today.
Q. But there is no way for me to go to the literature and see what this information is.
A. Travis, 1994, it's No. 23 in the set of documents I brought here this morning. I can just read you one summary sentence from the abstract, which reads -- this is "Hematopoietic Malignancies and Related Disorders Among Benzene-Exposed Workers in China," and I quote, "The hematopoietic characteristics of the benzene-exposed acute nonlymphocytic cases resembled those following chemotherapy or radiotherapy. ANLL in workers exposed to benzene may represent a distinct

clinicopathologic entity, with characteristics similar to treatment-related ANLL, including a preceding preleukemic phase in some patients." So some of that information is characterized and presented in this publication.
There is another one that should be in press. I believe the first author is either Lee or Martha Linet. I have not yet seen a copy of that manuscript.
Q. And had these Chinese workers been studied epidemiolosically?
A. Yes, and these studies are ongoing, but a lot of this is coming out now as the data is analyzed.
Q. Is there a current epidemiological report that you're aware of in the published literature that I can go to?
A. Well, this is one. Lee is another. There may be a couple by Lee. There is also, as I said, Martha Linet's publication which is in press in the same issue of Environmental Health Perspectives, and that should be available. I expected to have a copy by now, but I don't have one yet. If I had it, I would have provided it.
Q. Now, if I heard you right, Travis, in the paper that you were citing from for the ANLLs, what was the modifier he used, similar to, or consistent with?

What did he use?
A. It resembled those found in chemotherapy or radiation therapy, and he said, "may represent a distinct clinicopathologic entity, with characteristics similar to treatment-related ANLL."
Q. "Similar to" and "resembled." How "similar to" do you find them to be, the benzene-exposed workers with leukemia, or I should say, with ANLL, and those people who get leukemias from secondary to chemotherapeutic agents?
A. Certainly with respect to the frequency of any cytogenetic aberrations and the subtypes of leukemias that are associated with the different groups, they are essentially consistent. I certainly can't point to any feature that distinguishes them from AML secondary to chemo- or radiation therapy.
Q. Say that last part again.
A. I cannot point to any single feature or characteristic that distinguishes them from AML secondary to chemotherapy.
Q. So they're the same?
A. Essentially they are the same. What I cannot do, because I have not seen it, is give you the specific cytogenetic aberrations that occur in every case. I haven't seen that data. It has been described to me as

consistent with AMLs secondary to chemotherapy.
Q. Is Travis reporting on the Chinese workers, the same ones that Lee and Linet are talking about?
A. Yes, these are all follow-up studies as part of a continuing retrospective and prospective analysis of benzene-exposed workers in China.
Q. But as of yet, you have not seen the actual data analysis?
A. I haven't seen specific patient-by-patient cytogenetic aberrations. I have seen data with respect to -- wait a minute. What can I tell you? The subtypes in morphologic characteristics are reported by a patient.
Q. So in other words, you're saying that whether it's acute myelogenous leukemia or a variant of acute myelogenous leukemia?
A. Nil, M2, so forth and so on. Whether it's evidence of preleukemic changes or myelodysplasia. They simply -
Q. "They," being Travis, et al.? Are you still on the Travis paper?
A. Yes. They speak of them in general in that they have many of the same features that are seen associated with secondary to chemotherapy or radiation. They don't describe the individual aberrations in this

paper.
Q. And the features that they mention are not elucidated, they're not set out?
A. No. They talk about 5 and 7. They talk about the various populations that have looked -- the various populations that have been looked at in terms of exposure to organic solvents and involvement of those chromosomes, and they characterize those aberrations, but they simply state that the -- their data with the Chinese workers is consistent with those.
Q. How many individuals are we talking about here that were looked at?
A. Well, I am not sure -- I don't know how many actual cytogenetic analyses they have. They are talking about 32 cases of leukemia.
Q. But you don't know, of those 32, how many !»d cytogenetic analysis for this statement?
A. No, I can't tell you that.
Q. Has anyone looked at the other cancers of the blood or bone marrow besides ANLLs?
A. Yes. In what context? With respect to association with benzene exposure?
Q. No. In these Chinese workers; I'm sorry.
A. They characterize virtually all hematopoietic neoplasms in this population. One of the difficulties

that they now are faced with is looking for confounders and other potential exposure scenarios because they're dealing with 74,828 benzene-exposed individuals, and I forget the number of actual sites, but it numbers in the hundreds to thousands, so they have a huge number of occupational-exposure scenarios. They have captured virtually all the tumor types based upon the diagnostic criteria that they are able to use for those populations.
Q. So these are 74,000 workers from throughout China doing different occupations that putatively involve benzene?
A. Yes. This is probably the largest study I have ever seen. There is no "probably" about that; there is no qualifier necessary, both with respect to industrial hygiene and with respect to employment characterization and disease characterization. Having said that, even this study has some problems with respect to interpretation.
Q. For the workers that Travis reports cytogenetic analysis, is there any description of the occupation of those people?
A. Not on an individual basis, I don't believe.
Q. How about as a group? I mean, do they predomindce from one particular industry?

A. No. They have evaluated painters, they have evaluated petroleum-refinery workers, they have evaluated individuals using benzene as a glue. They have evaluated a variety of different occupational settings in which benzene is either produced or used. They have, as I said, retrospective, prospective, and current industrial hygiene data. So it's a very complicated study with a number of different types of analyses that are going on simultaneously.
This is one report. There are many that are coming out in various bits and pieces as this data is sifted through and evaluated.
Q. Is there any suggestion, that exposure level being so high in these workers, that t'-~at has the potential to give you a different outcome than if you have exposure levels to benzene that are lower?
A. I haven't seen any data that would suggest that. Now, I have not seen everything, by any means. I have seen their characterization of exposures, and one of the problems they have is that their time-weighted average cumulative exposure scenarios that are used for dosimetry purposes include a huge heterogeneity with respect to transients that they have documented. In other words, a time-weighted average exposure of 10 ppm -- for instance, 10 ppm-years in China doesn't mean

anything like it would here in the sense that in this day and age today, of course, we wouldn't be looking at a 10 ppm time-weighted average exposure, or shouldn't be. It would be much closer to the mark than it is in China, where you can have within that context exposures to sub-ppm or low-ppm levels, as well as transients into the hundreds of ppm. So it's a very heterogeneous exposure base, and they are trying to dissect that out.
Q. In the work that you have done in your laboratory, is there any way to gather information when you look at the benzene-metabolite exposures that you have had to the different cell populations? Is there any way to correlate the amounts of metabolites that you have used to the exposure level?
A. Ideally, I would hope we would be able to do that. We're not in the position where we can do that today. There are a number of issues that have to be dealt with.
First of all, in order to look at the precise effects of various metabolites on the cells, some of our early works are, for instance, looking at clonogenic responses, expose the cells for short periods of time in the absence of media protein, so that we were looking directly -- we were delivering, A, dose of the metabolite to the cells we could quantitate, and, B,

assure that that's what they were seeing.
Those same cells in bone marrow are going to be bathed in protein, bathed in media -- call it media, if you will -- that is going to compete with abstract and otherwise provide a totally different dosimetric with respect to what a given level of metabolite means to that cell.
At some point, and in liquid culture with long-term exposure now, we're beginning to get there, we're beginning to address it. We have to come up with a means of relating those types of exposure levels, concentrations to exposure levels that might be targeted in vivo.
Having done that, the next step is to take the pharmacodynamic and pharmacokinetic data that is available today both from animals and humans and try to extrapolate. No one, to my knowledge, has been able to do that adequately. It's obviously something that needs to be done if we are ever going to be able to translate this directly with respect to what it means dosimetrically. We can say some things generically now, but we certainly can't say in precise terms, quantitatively how those relate to in vivo exposures.
Q. Your work that you have done in your laboratory with the benzene metabolites, how would you

characterize the amount of work that you have done with individual metabolites as opposed to the combinations of metabolites?
A. Our work is centered primarily on individual metabolites. We have done some work on combinations of metabolites. In fact, we have pioneered looking at interactions between metabolites and the potentiation of the effects of hydroquinone biphenol. There have been other students independently that have looked at catechol and hydroquinone, and hydroquinone and the putative -- the ring-opened products -- trans, trans-muconaldehyde, in particular.
You focus where we have because we have been trying, first of all, to go from the simple to the complex, and we have seen interesting effects at the simple level. Our focus on the quinone metabolites has been primarily because they have produced effects. We have looked at some of the others, and we haven't been able to produce anything except cell death, which is not a very interesting input, but that doesn't mean they don't have effects.
Q. Did I understand you to say that when you used the quinone metabolites along with the phenol, that you have gotten greater effects, or has it been lesser effects, or can you tell?

A. We have described those types of effects both in vivo in mice as well as in vitro. It extends right down to the cell level. Phenol potentiates the effects of hydroquinone. It appears to be related to promoting peroxidase-mediated oxidation of hydroquinone to its terminal end-stage product, para-benzoatd_none. There may be some pharmacokinetic effects, as ~.~11, that various people have proposed, but we certainly can see it at the enzymatic levels, so it may, in fact, explain why you see it at in vivo level.
Phenol, itself, doesn't produce bone marrow toxicity in experimental animals; and I would say there is no evidence that phenol in occupational settings is toxic or metatoxic.
Hydroquinone, in experimental animals, can produce some effects. It's a very nonphysiological situation when you administer hydroquinone externally, but you put the two together and there is a phenomenal synergy.
Q. Externally?
A. Yes.
Q. Hydroquinone and phenol?
A. Injected together will produce hone marrow toxicity that is much greater than hydroauinone by itself.

Q. That's what you meant by "potentiated"?
A. Yes. At an enzymatic level, phenol stimulates the terminal hydroxylation by peroxidation, and we can, by adding phenol, know the presence of hydroquinone, although most of our work has not focused on the combinations as of yet. We have been looking at the isolated effects.
Q. Is there a way to drive the metabolism of benzene away from quinone in a living organism?
A. There are no known selective inhibitors of peroxidase.
Q. Can you administer something else along with the benzene, though, that will drive the metabolism of benzene away from quinone or the quinone metabolites?
A. I don't know of it, because as soon as you put a substituent on the ring, you're going in a different direction in the first place.
Q. I wasn't talking about on the ring. I'm talking about in combination with.
A. Right. I simply was saying that in terms of -- if you throw in toluene or xylene, you can protect against benzene toxicity, but that's competition for peroxidaition. After that, we're not talking about the same pathway at all. I don't know how one would selectively block that.

You can look -- what we have done is look in specific cell populations and under specific conditions where you have a different balance of detoxifying and toxifying enzymes, peroxidase versus two electron quinone reductase on DT diaphorase, which is a protective enzyme in that system. And coincidentally, the myeloid progenitor cell has very respectable mycloid peroxidase activity and absolutely no DT diaphorase activity. So susceptibility correlates -- in terms of cell populations, correlates with the enzymatic machinery that drives activation and not detoxification. So I guess what I am saying is that there are cell-specific models that provide a natural experiment to indirectly address what you are saying, but we don't have any major bullets that allow us to go in and specifically block the selective oxidation of those quinones.

Q. Going back to a little bit earlier line of questioning, and I hope to wrap it up. Your work that you have done, and the works of others that you are familiar with, still don't allow the exposure of human cells or -- stay with human cells for a minute -- to benzene or benzene metabolites and generate a leukemic cell; is that accurate?

MR. BELFOUR: I object to the question as

vague, Herschel. I'm sorry, I didn't understand what you asked.
A. What you have defined with that question is basically the holy grail of leukemia research. We are not at the stage where we can cradle-the-grave, nuts-to-bolts, step-by-step, induce a human leukemia in a test tube as a function of exposure to anything. We are much closer than I ever imagined we would be as recently as 1990. We have come a long way, and I may not have to answer that question in the negative for too many more years.
Q. (BY MR. HOBSON) Where is the gap?
A. I think the major gap is secondary events that are probably not consequential with respect -- important with respect to order and/or -- that are not probably associated with exposure to benzene or anything else. In other words, it's becoming clear there are multiple steps in the process -- some are independent, some are not -- comparable to other types of tumors that are fairly well described now, such as colon carcinoma, and that some of the subsequent events probably are not associated with exposure. They are the product of proliferation in abnormal cells that leads to secondary events.
Q. What do you mean by "the product"?

A. As I said earlier, some changes may predispose to other ones, and if you have three, four, or five independent phenomena that have to occur in some order, some totally independent order, for the actual leukemia phenotype to be expressed, those later events may h~--•-~ nothing to do with exposure to anything. They may be a function of the probability of a random event occurring in a cell that has already gotten to that stage in the process, for instance, if a late event in AML or a type of AML involves mutation of the retinoblastoma gene.
Q. You mean a late event in the formation?
A. In an actual history of the disease.
Q. Before the disease actually occurs?
A. Yes.
Q. Okay. I'm sorry.
A. It may not be related -- it may be totally separate and distinct from the processes that led to the early changes.
Q. We're talking perhaps host-susceptibility here?
A. It could be. I mean, that would be speculative. It's more likely a random event.
Q. I mean, I'm having trouble thinking of possibilities. What would some of these possibilities be for these random events?

A. We know, for instance, simply that there are a number of human tumors where the only known influence is cell proliferation. You increase the number of times a cell proliferates, you increase the probability of a replication-dependent error; or simply increasing proliferation can increase the risk of probability of cancer developing in humans.
If we have a cell that has got two or three aberrations or abnormalities in it that now leads to increased survival and replication in a cell that is capable of transformation, that next event may simply be a function of it or the probability may be related only to the fact that the cells proliferated. That would be a random 7, but with a very high -- with a different rate of probability than it would be in any other cell which doesn't proliferate as fast or in the context that we're talking about.
So that would be an example of an event where you have proliferating population that shouldn't be, and that leads to an ultimate mutational change, if you will, or cytogenetic event that is associated with the phenotypic expression of a tumor.
Q. Is it possible that what you're saying - maybe I am just off in left field; tell me if I am - that if you have an individual who has been exposed to

benzene and you have had some of these precursor steps occur, that you have got this cell sitting in a compartment; that but for having to replicate a lot, you would never see leukemia in that individual, and then this individual's bone marrow is affected by a greater benzene exposure which would require that cell then to replicate much more quickly? Is that one of the things you're talking about as a possibility?
A. No.
Q. I am off in left field then.
A. I would say that one has about the least plausible probability of any scenario, and the reason is that benzene, like other chemotherapeutic agents -- end early in the century was actually used for one, God help the patients that got it -- I don't think it would be very effective, and the side effects would be pretty lousy -- but, nevertheless, it tends to target proliferating cells. So if you are Going to imagine a scenario where you have those types of events, I think that high-level benzene exposure might actually be protective in a situation where you have already got - or at least is somewhat ameliorated.
So high-level benzene exposure may actually - in individuals who are already down that .road, high-level benzene exposure, there is no reason, a

priori, to assume that is going to potentiate. It more likely is going to suppress a proliferated cell population.
Q. Does the latency period seem to be different for benzene-induced leukemias than it is for chemotherapeutic-induced leukemias?
A. It's a little longer.
Q. Which way?
A. Benzene.
Q. By "little," are we talking order-of-magnitude?
A. No, no. For AML secondary to benzene, certainly in most populations that have been studied, I think the consensus is that it's on the order of about nine to ten years, 9 1/2 years for a geometric mean for latency.
Q. I'm sorry, I didn't get the pronoun. You said "it." What are we talking about there?
A. The latency between first exposure and onset of leukemia in occupationally exposed workers.
Q. To benzene?
A. Yes.
Q. What would it be for chemotherapeutic?
A. It can vary three to six years, is usually the mean that is tossed out. Now, with some of the newer

agents that don't act like classic alkylating agents, it's a much shorter latency. It's on the order of months, but that I don't think is appropriate for what we are talking about.
So benzene -- certainly, occupationally exposed AML, we're talking a mean latency on the order of nine to ten years.
Q. Since we're talking statistics and using mean, what would then be the standard deviation for that? What does the curve look like?
A. I think you can argue that you're way below the 5 percent confidence level by -- probably between three and five years. So, I mean, that would be the bell at one end, and it's probably close to the bell at the other, although I think you're parsing hairs there. My interpretation of that latency is both a product of the nature of the specific disease and the type of exposure, but it is not clearly one or the other.
In the case of patients receiving chemotherapy, I think it's important to remember these people are not receiving trivial doses of drugs, and the bone marrow is the target, and they are poisoned. we're talking toxicity here; we're talking bone marrow damage. Probably not even in Aksoy's Turkish population do you

have that level of toxicity associated with benzene.
Arid so the difference between three to six
and, say, nine may be related to the intensity of the
damage that was done in the first place.
Q. Take me, for instance. In my graduate school
program I had occasion to work with benzene for a few
years, and based on what I know now, my exposures were
probably in the hundreds of parts per million several
days a week for a couple of those years, at least,
because of the work I had to do. That occurred in about
1969 through 1972.
Can you tell me that I will never have to
worry about secondary leukemia from benzene exposure?
As far as I know, I was never acutely ill at the time
from benzene exposure.
A. Well, we're talking 199 -
Q. '5.
A. -- '5.
Q. 27, 28 years ago.
A. I am in the same -- I am almost precisely in
the same boat you are. We could each have an oar.
From 1965 through 1968 I was a chemistry major
with an absolute flaming love of organic chemistry that
many people took to be a sign of mental instability, but
I used benzene as a solvent, phase extractions, and

everything under God's green earth.
Q. Not with hood, not with gloves?
A. Not with gloves. Often not in a hood. But based upon the latency we're talking about, I certainly am not worried about that. There are other things in life, I think, that are much more potentially important to me, such as cardiovascular disease and a few other things as I get older; but based on being out 20 years, I don't think it's reasonable to expect that you are going to incur any major probability of developing AML secondary to those exposures.
Q. Can you rule it out?
A. I mean, absolutely, positively, never, nohow or based on a more-probable-than-not standard. As a scientist, you can't rule out anything absolutely, never, nohow; but I don't think it's a realistic prospect, and it certainly wouldn't meet a standard in terms of probability. You're way out on the curve. It's nonexistent.
Q. I hope your prediction becomes reality, not that I want cardiovascular disease.
A. Well, I was just speaking in terms of the fact that as we age, we have other things that become a lot more imminent, I think.
Q. The lesions that are created by these chemical

agents, be they chemotherapeutic or benzene-induced, some of those lesions are created in the pluripotent stem cell compartment?
A. We have no direct evidence of that, and I don't think that that's a very high probability based upon what we have seen. The transient changes we see first involve shifts in differentiation, which, by definition, involve commitment. That appears to be the first thing we can see. It is also consistent with the first types of changes that I have seen both with human cells and in animal models associated with cytokine - alterations in cytokine response that are associated with dysplasias.
The key, I think, is going to be where those chromosomal aberrations show up. Everything points to myeloid progenitor cell. That is going to be -- at least today, that is the first nonrandom permanent change associated with the evolution of AML.
Q. When you do your experiments with the pluripotent stem cell compartment cells, you're exposing those cells to benzene metabolites, correct?
A. Yes.
Q. And then after the exposure occurs, there is some period time that passes, I take it?
A. Hours to days to weeks, depending upon

precisely how the experiment is designed.
Q. When you say that you see a shaft to the myeloid compartment, what kind of time period are we talking about to see that shift?
A. The exposures can be as short as 30 minutes.
Q. When do you see the shift, how far in the future?
A. Within a matter of hours. We haven't reduced it -- we have not had as a goal to figure out whether it's minutes or hours. We've been more interested in the fact that it's less than days.
Certainly it's easy to see -- see the clonogenic assays. It was difficult to predict what was going on, because the exposures were only for 30 minutes. So -- you know, the exposure didn't take long, but the readout is days later. So you're looking at a series of events that have to occur before you see ultimately what has happened. You have a different number of cells -- well, you have to have a different number of cells recruited to respond in the first place. What we can say is that functionally, they're responding like myeloid progenitors. They weren't functioning as earlier cells; they were functioning as myeloid progenitors.
Now, in liquid culture, we're beginning to

look directly at marker changes, change in differentiation, and to correlate those things on the time -- on a timing basis. And it appears that that whole shift functionally occurs within hours, for sure, and is over in a matter of days. So you're looking at a window of time in which this particular type of transience occurs.
For hydroxycyclophosphamide, which is a metabolite of cyclophosphamide, we've been able to do it in vivo in mice, and it's comparable to what we have seen in vitro and we have used it as a surrogate. We're talking a matter of few hours. The whole process is certainly over in a matter of a couple -- two to three days, something like that.
Q. As I envision this in my mind, now, which I'm sure is far too simplistic for the work you are doing, you start off with the pluripotent stem cell compartment cells and you expose them to benzene metabolites for some period, generally a matter of 30 minutes, or is -
A. Yes, that's in one scenario. We can also look at that with continuous exposure, but that's using our newer culture techniques.
Q. But you have the exposure period, and then you wait a period of time to see those cells proliferate?
A. That's one type of assay. That's the first

type that we were using.
Now, we're looking at changes in their
phenotype with time -- that is, changes in their
expression of various genes that correlate with their
functional characteristics.
Q. Now, when you start off with the pluripotent
stem cell compartment cells, I take it you run controls
as well as having your dosed cells?
A. That's the only way we can do this, yes.
Q. So in your controls, when you look at how
these pluripotent stem cells differentiate, some
differentiate into the myeloid compartment and some
differentiate into the lymphoid?
A. Yes.
Q. And then when you look at the exposed
pluripotent stem cells, you're seeing a different
division, but still some lymphoid, some myeloid?
A. We're looking at population shifts in highly
purified cell populations. Some of those are ultimately
capable of or destined to be lymphoid. The vast
majority appear to be destined to ultimately either be
erythroid or myeloid in terms of the way they respond in
culture early on.
Q. Are you saying you do your analysis, then,
before this differentiation is completed?

A. Yes, for those types of -- yes, for the liquid culture work, yes. We can monitor -- we can monitor readily erythroid and myeloid, and in the liquid culture we're using they are -- they predispose -- the ones we're using right now are -- they predispose toward looking at the myeloid pathway.
We have to use different conditions if we want to look specifically at early differentiated events that would be associated with lymphoid. Clonogenic assays provide us with some handle on that, because those, by definition, coincide with populations that have lymphoid capability or myeloid; but for the different -- what we're doing with markers right now, the work we have done to date has emphasized looking at myeloid and not lymphoid. That's another step in the process. We are not there yet.
Q. In your system that you have set up, can you start off with pluripotent stem cell compartment cells and culture those cells long enough to see them differentiate and become either myeloid or lymphoid cells and keep them alive?
A. Okay. If you are asking, have we done that in both directions in our laboratory now in the systems we're using, the answer is no. Is that technology available? Yes, it is. If you're asking, Can you, in

the context of what the state of the art is, the answer is yes. With respect to, Have we done that, no.
9. I guess in a sense, the experiment that I would be interested in knowing if you could do is, could you take pluripotent stem cell compartment cells, expose those, for whatever the appropriate period of time is, to benzene metabolites or a metabolite; let those cells culture-differentiate; and then do a cytogenetic analysis of both the myeloid compartment differentiated cells and the lymphoid compartment differentiated cells?
A. To varying degrees, those are capabilities that exist. We are, to varying degrees, attempting to do that; not precisely as you have defined it; but generically, yes.
There are issues with respect to the technical culture and readout of lymphoid versus myeloid. That involves technologies that exist but that are difficult. You can't do them in the same system. You have to figure a way of controlling for what are essentially stochastic events in the system, because the culture conditions are different, although there is at least one technique that has been -- that has been proposed to allow both simultaneous lymphoid and myeloid development, but I have yet to see that.
Our approach is not to look for random --

cytogenetic random aberrations, but to look for nonrandom. Those that survive, by definition, are going to be the ones that are most important. They are also by definition ones that are going to be amplified and we're going to be able to see. So there is a certain - for long-term culture techniques, that's our strategy. The short-term techniques allow us to look at the potential, and that's what I described for you in terms of exposed to cells in culture to hydroquinone, look at what types of aneuploid changes or chromosome changes are associated with those cultures. We can see the potential for involvement of 7 very readily, some evidence for 8. There are other chromosomes we obviously want to look at. Whether those are the ones that ultimately are clonally expanded and become persistent, I can't tell you; but it certainly is my hypothesis that that will be the case.

Q. In trying to follow that, then, the long-term culture, when you use that terminology in this context, that's something that you would like to be able to do, but you can't do it yet?

A. We can do cultures after four or five weeks. The state of the art for long-term culture of human cells -- human bone marrow cells to date is on the order of four to five weeks and still maintain what is called

long-term repopulating.
Q. In this context, are you talking about for long-term culture, starting off with pluripotential stem cell component cells and maintaining them for four weeks?
A. Yes, with significant repopulation of all the different compartments, including the pluripotent compartment, not just an exhaustion of pluripotent cells.
Q. And if you start off with pluripotent stem cells, is that the beginning phase? I mean, are all the cells that you start off with when you attempt this, long-term culture pluripotential stem cells, or do you have a mix of pluripotential, myeloid, and lymphoid that you're culturing up simultaneously?
A. Most of the types of experiments that we have either planned or done are the second variety; however, we now have the technology to separate out very early cells. So I don't think we're far from being able to have at least a highly enriched population of long-term repopulating cells. I could tell you a lot more next year than I can right now.
MR. BELFOUR: Excuse me, Herschel. Would this be a good time for a break? We've been going for about two hours.

MR. HOBSON: It's up to you guys, whatever.
(Break was taken.)
Q. (BY MR. HOBSON) Is it possible, when you look at this four weeks or so, that you can culture cells that start. off as pluripotential stem cells, that you can grow them long enough that they do fully differentiate into the lymphoid or myeloid compartment cells?
A. Oh, yes. That's not a -- yes. That's the answer to that one.
Q. And you can culture the pluripotential stem cells, expose those to the metabolites of benzene, and then let them culture for four weeks or so before you do any evaluation?
A. That is our ultimate -- that's one of the ultimate types of experiments we hope to perform. We are, as I said, trying to take it in stages, and we're looking at both sides, the cytotoxic and cytogenetics, and the altered-growth regulation side, and hope to bring those together in the same context that you're talking about.
Q. But you're not there yet?
A. No.
Q. Can you even go so far as to start off with a cell from the pluripotential stem cell compartment,

expose that cell to benzene metabolites, have it differentiate, and then do the cytogenetic analysis after it's differentiated to a myeloid or lymphoid cell?
A. Yes.
Q. Have you done that?
A. We are in the process of doing those studies now. I don't have any results.
I do have results looking at prototype -- as I said, prototype cell lines, but I don't have results on cytogenetic analyses in human stem cells at the present time; but I could have -- I mean, we're talking right now, that type of experiment is underway now. So within a matter of months, if we're successful, we may have some information.
Q. I think we're planning on going to trial here in about two or three weeks. Anyway, that would be ready by then?
A. I'm sorry; that's a little bit- too challenging a time frame.
Q. Is your ultimate goal or -- I should ask it a different way.
Is a goal of your work to be able to take cells from the pluripotential stem cell compartment, expose those to benzene metabolites, let them culture and then analyze the -- do cytogenetic analysis on all

of the cells that grow from those pluripotential stem cells?
A. I am not sure I understand precisely - whether I really understand what you are asking me.
Q. Let me do it again, then.
You take pluripotential stem cells, expose those to benzene metabolites, culture them for a period of time, you will still have, I would expect, some of those differentiate into myeloid line cells and some into lymphoid line cells. Do you plan on doing cytogenetic analysis of both lineages?
A. Eventually, yes.
Q. How far away do you think you would be before you could do that?
A. Right now we're looking at myeloid. If we go into what are called a bioreactor type of system, we could do both simultaneously, but that increases the complexity of the system by orders of magnitude; so we probably are better off at present trying to do these as separate types of experiments.
Q. So months or years, at least?
A. Ultimately years, but early events we may be much -- we may be much closer than that. We may be able to say a lot more about dosimetry and early events, both myeloid arid lymphoid, within the span of, say, for

instance, a year or so. We already clearly have differences in susceptibility of populations with respect to seeing any observable response.
Q. I'm sorry, I didn't hear you.
A. Seeing any observable response at all, and there the myeloid cells are much more acceptable. They have the metabolic machinery to activate the metabolites; lymphoid cells don't. So you are looking at a totally different dosimetric to begin with, as well as very clear-cut differences in cytokine response.
Q. The case that we're here on is a multiple myeloma case. Multiple myeloma comes out of the lymphoid stem cell compartment. Would that be accurate?
A. I would quibble with that. Because of the nature of B-cell development, we can say a lot about the ultimate clone origin of multiple myelomas, and the cell of origin with considerable degree of confidence. I think it's fair to say that people that are looking-specializing in looking at the pathogenesis of multiple myeloma are in general agreement that the cell of origin is an early B cell. It's not a stem cell; it's not a clonogenic cell in the context of colony formation. It's a cell that has received its walking papers. Now, the transformation may be at the point at which it receives its graduation certificate as a B

cell, but it is at that level, the level of an early B.
Q. Where would you put an early B cell if we started off talking about a pluripotential stem cell?
A. We are not even -- we're even past the committed progenitor cell stage. We are further down - we are -- that cell has no clonogenic potential for colony formation. So it's not -- that's more -- the last stage of B-cell differentiation that falls into the colony-forming-unit type of progenitor is probably the pre-B. And there are changes that occur, including the loss of CD34, that are commensurate with the maturation process, and that's a functional marker. Unlike many of them, that is consistent with clonogenic potential or colony formation. That's lost before gene rearrangements lead to ultimate commitment as a B cell to the actual epitope-specific B cell. So it has lost its potential to form colonies before it has that capability; and multiple myeloma cells have that capability, so transformation occurs either there or slightly later.
Q. And "there" is where again?
A. Post -- these cells have lost their ability - they're not CFU. It's not a pre-B; it's an early B.
Q. Even earlier than pre-B?
A. No. It's early B. It's post pre-B and

early B. We have an advantage there because of the gene rearrangements that happen in immunoglobulin gene rearrangement and the cell committing to the - responding to a specific epitope, producing a specific immunoglobulin molecule. Those events are very -- they are at a fixed point in time when they occur, and they're consistent with the phenotype of that cell as a B cell.
Q. Let's see if I have got this close to right, then. Multiple myeloma occurs as plasma cells or a disease of plasma cells?
A. Plasma cell is -- if you recall when we were talking about leukemic cells, I differentiated between the circulating abnormal leukemic cells, which also may divide, and the cell of origin, which was a progenitor cell in the bone marrow. This is a rough analogy here. A cell of origin is an early B cell. The phenotype of the tumor cell is a plasma cell. So that cell differentiates to become an antibody-producing cell that specializes in antibody production, not proliferation, and that's a plasma cell. That's the hallmark of multiple myeloma.
So you get a single clone -- expanding clone of plasma cells, they're all the same, they're all a knockoff on each other. And they are the phenotypic

manifestation of the tumor. The origin of the cell is a B cell.
Q. A B cell that should have gone to being a plasma cell gets, somewhere in the process, defective, just like we were talking about for a leukemic cell, that ultimately leads to the development of multiple myeloma?
A. B cells have a number of facilities -- a normal B c,21I. It can proliferate; it can respond to stimuli and proliferate and also differentiate; and if it differentiates, it differentiates into a plasma cell, and basically becomes an immunoglobulin factory.
In multiple myeloma, the transforming cell does that selectively; and so you get -- you get proliferation and terminal differentiation to a plasma cell, and you get all these plasma cells producing a single type of immunoglobulin molecule. You don't have the normal panoply of the responses the cell can undergo and it doesn't need any.
So it's no longer an antigen process at all, and those cells continue to produce -- they don't divide rapidly, so they are not a highly proliferative tumor type, but they're highly destructive, because they produce immunoglobulin molecules and they influence the pattern of cytokine release, so they cause lytic bone

lesions and a variety of other symptom=.
Q. What would we -- what could we call this cell that becomes the originator of the multiple myeloma disease?
A. Well, certainly, the major descriptor that is used by people in the field are early B cell.
Q. Is it your view that it is biologically implausible that multiple myeloma can be caused by a chemical agent? Can it be secondary to a chemical agent?
A. We're touching on semantics here, and I don't want to -- I don't want to play games. In terms of transformation of lymphocytes, the basic patterns that we see tend to be consistent with things that promote proliferation, antigen stimulation, retroviral or viral-mediated processes. They factor very strongly into lymphoid peripheral diseases that Pre associated with environmental exposure, and I am using "environment" here not to mean -- I mean it in a very generic sense.
For instance, the only analogy -- and this is not a close analogy that I know of -- are lymphomas that are associated with exposure to immunosuppressive drugs, not alkylating agents, per se, but agents that are potent immunosuppressive agents. It's seen in organ

transplantation, it's seen in conditions where patients are treated with immunosuppressive agents chronically. Virtually all of those involve -- that I have seen involve Epstein-Barr virus -- virtually every one I have seen has molecular involvement of Epstein-Barr in the tumor.
It is fair to say that there is no precedence in that model system for a process like we see in AML, because alkylating agents don't specifically do that. Some patients with alkylating therapy may develop lymphomas, but it's invariably because it's also associated with a potent immunosuppressive component process. We don't have anything comparable for multiple myeloma, even to that level.
When we get into the epidemiologic data, there are patterns that are seen. There is a whole lot of inconsistency in the epidemiologic literature associated with multiple myeloma.
I don't know of a plausible molecular mechanism ire the lymphoid system to use by way of analogy to say that it's biologically plausible that you can derive. a lymphoid tumor via comparable process. All evidence suggests it's a different process, certainly in humans.
Q. is it your opinion that it is biologically

implausible that benzene is a cause of multiple myeluma?
A. Based on my knowledge today and my knowledge of the literature, I see no basis upon which to conclude that benzene produces multiple myeloma. I cannot, in my -- as I review the nature of the pathonenesis of the disease, I cannot come up with a paradigm -- biological paradigm for the production of multiple myeloma that is consistent with what I know about the disease when we're talking about benzene or other chemical exposure.
Q. So is the answer, then, it is not biologically plausible, or is it that you just haven't been able to figure one out?
A. Well, I think plausibility, there may be degrees of plausibility. I do not mean to rule out abjectly that there is no conceivable mechanism that a chemical can cause multiple myeloma.
By the same token, I don't see any paradigm that I could hypothesize that I would think is consistent with what the data on either the pathogenesis of the disease or the epidemiology will support. And also by analogy, I cannot describe for you an animal model that is at all satisfactory for predicting the human condition with respect to the development of multiple myeloma.
Q. Multiple myeloma, as a disease for rats or

mice, they're not there, are they?
A. I don't think there is any model for multiple myeloma as we know it. There are one or two spontaneous B-cell neoplasms that have been defined in the balb/c mouse. There is a polyclonal lesion that can be produced in mouse, not by alkylating agents or agents that produce leukemia in the mouse or even T-cell leukemia in the mouse, but only agents that induce chronic granulomatis inflammation and are highly irritative adjuvants -- molecules that are characteristically used as adjuvants.
And even in that model system, it's a polyclonal lesion, to begin with, and it involves again endogenous retroviruses, although agents that classically cause leukemia in other mouse strains don't induce plasmacytomas in that one model. It's, I think, a horrifically lousy model for predicting anything.
Some agents are not associated with the development of lymphomas and plasmacytomas, and as I said, these is a retroviral confounder, the fact that it readily appears to be a polyclonal response to granulomatis inflammation.
Q. I take it that you have been informed at least about some of what Dr. Goldstein has said about multiple myeloma/benzene exposure.

A. Yes, I reviewed a letter that he recently wrote to Mr. Baggett.
Q. Is it your appreciation of what Dr. Goldstein has said that Dr. Goldstein believes that there is biological plausibility for benzene causing multiple myeloma?
A. Is it my appreciation that Dr. Goldstein thinks this is biologically plausible?
Q. Yes, sir.
A. I think that's a given, based upon his letter.
Q. Have you seen any of the basis of his statement of biological plausibility enough so that you can be instructive as to criticisms of it?
A. Yes, I think so. The criticisms I have of the letter are more -- are the same criticisms in general that I have with his original opinion piece, which he published a few years ago, and I think are more specific with respect to his letter. He has, by his own admission, made an incomplete survey of the literature associated with the development of multiple myeloma. He has focused on certain studies that he is aware of and that support his opinion, but he has not made an exhaustive critical review of the epidemiologic literature or the mechanistic literature associated with the development of multiple myeloma. Prior to 1990,

'91, '92, I could put mice in the same context, where I had selective knowledge of what was out there, and I had some concerns.
Having made a critical review of that literature, I have formulated a formal opinion -- that is, that there is no evidence to support that benzene is associated with the development of multiple myeloma.
It's based upon that critical evaluation.
So, in general, I think an incomplete evaluation of the literature is not useful because it's the overall pattern that one sees in the literature as a whole that I think is important, not focusing on individual studies and in microcosm.
Having said that, there are a couple of points that Dr. Goldstein makes that I think are critically flawed with respect to his analysis. The most important deals with his critical review of the Rinsky study, which he places a great deal of weight on with respect to his opinion.
The Rinsky analysis -- first of all, the assignment the cohort that was done in the '87 analysis that found four multiple myelomas basically provided no critical criteria for exposure duration. One day's worth was sufficient to be included in the cohort. So within the original four individuals, we

have one who was exposed -- or had the opportunity of exposure for four days. We had one who had the opportunity of exposure for ten months. We then have varying other durations of exposure higher than that. If you accept that all of those are exposed workers in the context of the definition for the cohort, I have some problems with that. But let's take on the assumption, simply for purposes of this discussion, that they're all members of an exposed cohort.
Dr. Goldstein, taking that assumption, then says that the fact that the risk decreases with follow-up is most probably due to a dilution of the exposure-dependent dynamic versus outcome within that cohort, and that with time, you have a dilution phenomenon; that's why it disappears.
I think that's a critical flaw, and the reason is, the follow-ups -- first of all, the follow-ups that demonstrate no significant association -- the reduction in association are looking at an additional six-year follow-up, are not looking at several decades. So we're not looking at a huge increase in the latency, number one.
Number two, the exposure dynamic, which is - predicated on a high-dose-exposure scenario, is the same for all neoplasms in that cohort. At the same time, the

risk of multiple myeloma has decreased in that population with follow-up.
There has been a 50 percent increase in the risk of acute myelogenous leukemia, same exposure paradigm, same exposure dynamic. We are not seeing a dilution that is related to the exposure and the nature of the exposure relative to latency of the disease.
AML is going up with respect to the risk associated with it. We have seen an increase in collection of AMLs in that cohort. We have lost the significance of the multiple myeloma, but his explanation for why that study does not support his opinion, I think, is critically flawed, because we are not seeing a dilution of that exposure dynamic in terms of the outcome. We certainly aren't seeing it for AML.
If he was correct in his evaluation or critique of the follow-up studies, we should be seeing a decrease in the risk of AML with time, and we're not. We have seen an increase.
Q. You mentioned that he has been incomplete in the mechanistic literature review. Which pieces from the literature do you find he has not relied upon or not included that he should have?
A. Well, he certainly has not taken the time to provide certainly me with enough confidence that he is

talking the same language. He talks about the fact that benzene targets B cells as being commensurate with a potential role in multiple myeloma. Well, the data on B cells relates to experimental data and some clinical data that they're suppressed, and much of that work was conducted in my own laboratory, and we're looking at suppression of proliferation at noncytotoxic doses associated with exposure of benzene or metabolites to circulating B cells. That's going to reduce the number of B cells that are circulating, but it is decreasing proliferation. It's not a cytogenetic event, it's not a cytotoxic event.

So I cannot rationally see the connection between that and the potential pathogenesis of multiple myeloma. They are not to me -- an analogy would be if water is overflowing the bucket, there must be a hole in the bottom of it. They're not related with respect to cause and effect. I don't see how that directly follows. He refers to that as though it's a direct association.

Basically I don't think he has taken the time to completely evaluate the literature, and I would say that that generically is the same with the early opinion that he had where he did not review the entire epidemiologic and biologic literature. He has an

opinion based, I think largely, on gestalt, and he has selected -- to a certain extent, I think he is guilty of selecting explanations to support that without having to deal with and wrestle with the entire literature that is out there.
Q. But which mechanistic literature or citations would you have included that he did not?
A. He didn't include any. He didn't include any. He simply refers to the fact that benzene, quote, targets B cells.
Q. Which mechanistic literature should he have included or considered, in your view?
A. Certainly, what is known about the pathogenesis of multiple myeloma. I have brought several of those references here. Certainly, what is known about the effects of benzene and/or metabolites directly on lymphocyte proliferation. Certainly, distinguishing particularly -- well, the inconsistent, and in some cases, particularly unimpressive data with respect to the effects of benzene exposure and proliferating lymphocyte cytogenetics in humans are B cell, they clearly are not B cells. All the models are looking at T-cell proliferation-dependent processes. None of that is addressed, and albeit where he does talk about -- he does admit to the fact he hasn't reviewed

the entire literature and he does admit to the fact that some of the findings are inconsistent, some support - he thinks support his opinion; others he thinks are contrary to it. I would say that that's consistent with my perception of the literature as a whole. It tends to be very inconsistent.
Q. Are you finished?
A. Yes.
Q. I'm sorry. You said "uh," and I thought you were waiting for me there. I had a couple of things I wanted to ask you about. I have what I think is a chapter from a book that you wrote, "Benzene and Other Hemotoxins."
A. Yes.
Q. I'm not sure when that was published. I didn't bring that particular page, but I guess it was sometime in the -
A. 1991 is when it came out.
Q. You had a statement in here that I wanted to talk to you about. You may have that chapter with you.
A. I do someplace.
Q. It might help to look at the same page, then.
A. Okay.
Q. Page 719 in that chapter.
A. Here we are.

Q. Page 719 of the chapter. The paragraph on "Study Design," the first paragraph, you make the statement that "a major weakness is that a reliable exposure history is exceedingly difficult to obtain," and you go on there, "rarely, if ever, is chemical exposure documented even remotely as well as the diagnosis."
Do you still feel that way?
A. Are you talking about case reports?
Q. Yes, sir.
A. In case reports, certainly retrospective case reports, yes, that chemical exposure is rarely - individuals publishing individual case reports rarely provide any amount of information that would allow you to quantitatively assess what the exposure or characterization is.
Q. How would you characterize exposure history in a case report as compared to exposure history that you would find in the usual medical practitioner's files?
A. I don't understand. What is your question?
Q. A person becomes ill, goes to the physician, perhaps they have leukemia, multiple myeloma, some other blood disease, and they go to see a usual oncologist/hematologist, and the oncologist/hematologist, as most physicians do, takes a history.

Do you have any opinion as to how a reliable exposure history that hematologist/oncologist is going to get, compared to the exposure history you find in case reports?
A. I am not sure I understand the question, but I would -- if I understand what you are asking, you are asking -- are you asking me how they differ -- are you asking how they differ?
Q. Well, let me ask it this way. If you find that exposure histories are unreliable in case-control studies, wouldn't you expect that exposure histories as found in a general practitioner's or a hematologist;' oncologist's patient files would be even less reliable for exposure history?
A. I would say that they are ostensibly comparable.
Q. Both unreliable?
A. Well, it's degrees of unreliability. It depends what the purpose is with respect to use of the information. I am here making some very serious criticisms with respect to the use -- the use of case reports. That doesn't mean they aren't useful in the aggregate to provide impetus to do quantitative studies, either prospective or retrospective or what have you; but as individual studies, they are very unreliable with

respect to providing an adequate characterization of exposure for use and causation, for assigning causation or rendering a testable hypothesis.
The problems associated with a particular history that that practitioner takes are comparable to the same problems with a case report. The only thing different is for reasons either associated with previous case reports, bias, hunch, lightning, whatever, the practitioner has elected to publish it; but in general, the amount of emphasis that is put on exposure characterization is very, very low in case reports. It's understandable in the context in which the practitioner is operating. He's either getting self-selected information from the individual, or if he is doing a case-control study, it may be by questionnaire. I am simply saying that it varies dramatically in terms of how reliable that information is.
If it is someone who has been in an occupation, that may be useful as a qualitative indicator of the person's previous exposure history, still not very useful for quantitative purposes.
MR. BELFOUR: Herschel, let me object. I think your question was vague, because you asked him a question about quantifiable. Then you asked him a

question about taking a history of exposure, which could mean any exposure, not whether that exposure was quantifiable. With that objection, please proceed.
Q. (BY MR. HOBSON) Let me set up a hypothetical, if I might.
A hematologist/oncologist has an office in town, and the hematologist/oncologist has been in practice for, let's say 30 years, and so when the hematologist/oncologist around the 1960 time period set up his practice, he believed that benzene and AML were the only cause of these relationships from benzene. So far okay?
A. I understand it.
Q. A patient comes in who has a disease, be it multiple myeloma, leukemia, lymphoma, various different variants of leukemia.
The practitioner's usual history-taking goes, if he has acute myelogenous leukemia, I might ask him about, Have you ever been exposed to benzene? But if he doesn't have acute myelogenous leukemia, I don't take a history of benzene exposure, and that's gone on for 30 years. Follow me so far?
A. Okay.
Q. Now, you go to that hematologist/oncologist in town and say, Do you believe that benzene is causative

of anything other than AML after 30 years of practice? And the benzene -- I mean, the oncologist/hematologist says to you, I have never seen in the histories that I have taken any association between benzene and any of these other blood, lymph, bone cancers that I have ever seen. Does anything about that scenario strike you as being less than reliable?
MR. BELFOUR: Objection, vague.
A. I definitely don't understand the question. I understand the scenario that you just described. I don't understand the question. I can try to answer what I think -- let me try to restate it.
If what you are asking me is, Is that a likely scenario, it's one possibility. There are several other hypotheses within the context of the whole limitation associated with the issue of case reports and that process. It represents a hypothesis.
Some physicians might respond to a history of benzene or reputed benzene exposure and another disease as being novel and worthy of publication. Somebody else might ignore it. They all suffer from the same self-selection process, so I would -- you could be looking at a case of omission; or a case of case histories, as I said, can also represent a bias that is not supported by fact or what subsequent study proves to

be real. There is no denominator.
So even in the case that you have presented, there is no way of determining whether or not what the appropriate denominator is in the context of publication of a case history.
Q. We may not have been communicating on the question. I will try it again a different way.
If we're talking about this hypothetical oncologist who sees patients who have various cancers, of the blood and bone marrow, lymphomas, leukemias, non-Hodgkin's -- lymphoma, the whole combination in his practice, and he believes for the 30 years he's been in practice that the only disease that he regularly sees that is associated with benzene exposure is acute myelogenous leukemia, so the only time he ever inquires of a patient and their history about benzene exposure is when he sees an AML. And then the question is presented to that physician, Do you see any evidence from your practice that benzene could ever cause any of these other kinds of disease, other than AML? Would you think his ability to answer that question would be rather unreliable?
MR. BELFOUR: Again, objection. I don't think you have given him the entire hypothetical. Herschel, I think I understand what you're asking, but the

deposition that you took lasted a lot more than a couple of questions, but with that, go ahead.
A. It depends on a number of real intangibles.
If he's a practicing physician for 30 years, he may be in an environment where he has seen individuals who have been occupationally exposed to benzene and he has seen AMLs he thinks are related to that. He may very well have other cues that would be appropriate surrogates for benzene exposure.
If an individual is employed, for instance, in an environment where there is opportunity for exposure to benzene, he may not -- if he sees a pattern there, that could very well be -- in fact, that's used in quantitative studies in some circumstances as a surrogate for exposure, as you well know.
So I think a whole lot depends on the individual, whether or not he -- what he basically uses as a cue to tip him off to the prospect that benzene could be the epidemiological agent.
I think, given the same limitations that we are talking; about with respect to case histories, they're all part and parcel of the same process. Whether he asks for a benzene history may depend upon whether he thinks that the individual's history is one that is related to or could conceivably be associated

with it. In the case of AML, it may be something he specifically does. In the case of anything else, it may just be part of the way he takes the history.
That's another one of the problems with respect to an evaluation of case reports, is you're talking about an art form here, not a quantitative science; and it's that practice of the art that makes one case history maybe worth reading and another not, and you don't have a clue.
Q. (BY MR. HOBSON) Let me ask it a different way, because I'm not sure yet that I have gotten my question across to you.
If I started out, and this is my experiment, my question is, What diseases of the blood, the lymph and the bone marrow can benzene cause? That's the question I am going to start out to answer.
And so what I am going to do then is for 30 years every time I come across someone who has one of these diseases, I am going to take certain steps, and those steps are along this line: If the person has acute myelogenous leukemia, I am going to ask them about their exposure to benzene and learn as much about it as I can. If they have any other disease, I am not going to inquire at all about their history of exposure to benzene or in their work history enough to know if they

ever had exposure to benzene.
Would my method be flawed? It's pretty
obvious it would be, wouldn't it?
A. It depends -- I don't think it's that clear
s cut, and the reason I don't think it's that clear-cut is
there are other cues that physicians use.
For example, did -- well, let's discuss the
total futile exam. If the only patients that individual
sees come from a specific occupation, a specific
environment, you will never know what a denominator is,
ever. All he does is see cases. All he can do is tell
you whether he's seen a lot or a little vis-a-vis that
one universe, so he has no denominator. There is no way
he can possibly relate that.
If, however, he sees a cross-section of the
community and he sees the diseases that appear as a
cross-section of the community, and he doesn't have a
cue with respect to an association with an occupational
setting that is consistent in his own experience with
benzene exposure, then he may not feel that that's an
area worth looking at, vis-a-vis the others.
If he sees an AML, he may have a totally
different criteria based upon the fact he has seen an
association.
All I am saying is that if he is seeing a

cross-section of a community, there are a number of other surrogates or other behavioral characteristic that may provide cues with respect to the probability of an exposure related to benzene that are not necessarily going to be lost in that process.
Q. Would you agree that before a physician should express an opinion about whether or not benzene causes any particular disease or not, based on what he has seen in his clinical practice and learned from the histories that he has taken, that he should first know where the potential for benzene exposure is?
A. I think the responsible answer to that question lies in the degree. It's not an all-or-none, yes-or-no question.
One would hope that there was some knowledge with respect to potential levels of exposure, but at what point that becomes critical, I can't tell you.
Q. The physician might not have to know each and every potential exposure known to mankind, but ought to at least have a good grasp of what is generally known about benzene exposure; is that what you are saying?
A. Some concept of what the -- in very crude terms, where the opportunities for exposure are.
Q. Like there is benzene in gasoline, for instance, if a person is a service station attendant

A. Well, now we're getting -- I disagree with that. I disagree, based upon my knowledge of the benzene content in gasoline, whether that constitutes a health risk with respect to benzene exposure.
Q. I wasn't asking about health risk. I'm asking about whether or not -- shouldn't a physician at least have an appreciation that there is some benzene in gasoline?
A. Well, I personally do not feel that the amount of benzene that is present in gasoline constitutes a risk with respect to benzene exposure. I think that chronic exposure to gasoline provides many others. If you're talking cumulative exposure, then gasoline -- if you assume that cumulative exposure to low levels of benzene plays a role in benzene leukemogenicity, then gasoline, like smoking and everything else, constitutes a potential source. I do not believe that the amount of benzene present in gasoline constitutes a health risk with respect to benzene exposure. So I would disagree with you on the premise that the amount of benzene that is present in gasoline constitutes something that a physician should be necessarily aware of.
Q. Where would you think that a physician should necessarily be aware of benzene being out there in the

world for workers to be exposed to it? Where are those, then, that you would expect a physician to know about, if he is going to give an opinion, based on his clinical practice, that benzene is or is not causative of anything? What should he know?
A. Well, without talking specifics, certainly historically, there is no, I think, argument that opportunities for exposure to benzene have been found in the petroleum industry, and have been found in refinery situations. Certainly, in this country, those are major potential sources or opportunities for exposure to benzene.
Q. Any others?
A. It depends. I mean, we get into the highly specific. I have seen examples of individuals exposed to benzene in a number of scenarios that are not generic; they're highly specific -- using it to clear, metal parts that are hot -- very bizarre types of scenarios where benzene is employed.
Historically -- certainly not today, but historically -- I mean, certainly in the '50s and into the '60s, and perhaps through the '60s -- painting was a major source of benzene exposure. In China that constitutes probably the heaviest source of benzene exposure, even given the other horrendous exposures they

have.
Do we have to go back and invoke shoe workers in Europe? I mean, using benzene as a glue is a highly hazardous practice.
Q. What about using benzene in paint removers?
(Mr. Tillson left the deposition room.)
A. Consistent with benzene contamination of other solvents and aromatics into the '60s, that is a logical potential source. With the efforts to remove benzene from most aromatic stocks that are used as solvents in the '70s and certainly up through the present, I would say that that's a highly improbable source today.
Q. What about up to the mid-1970s, are you aware that -
A. Any aromatic distillate fraction that contained 10, 15 percent benzene is a potential source of benzene exposure, and at those levels the presence of substantive benzene isn't going to afford effective protection. At lower levels, if you're below 5 percent, then I think you have to get into the issue of what the potential exposure is and whether or not there is a tremendous excess of toluene or xylene present to evaluate the potential hazard associated with benzene.
Q. In your chapter here on page 724, you say that gasoline currently ranges from 0.5 to 2 percent benzene

concentration. Any knowledge at all of what historically gasoline has had in it as far as benzene content?
A. Certainly 3, 4, 5 percent. I don't know if there were higher proportions in the '50s or '60s. There has been an effort -- historically, I am aware that there has been an effort to reduce benzene certainly below those levels, even prior to the general consensus with respect to the potential hazards of benzene, because of the economic issues. You don't want to throw benzene in gasoline; you want to market benzene as benzene.
Q. Would you know, one way or the other, if any of the refiners who had the ability to extract benzene at their refinery would ever blend benzene back into their gasoline stocks when the market for benzene was soft?
A. I have absolutely no general or specific knowledge about that.
Q. Would that matter to you in knowing about tile benzene content in motor gasolines?
A. Again, it doesn't -- that would not tell me what the levels were. To me, what is important with respect to potential for hazard is going to be the amount of benzene that is present.

Q. Have you seen reports of motor gasolines containing 8 to 10 percent benzene?
A. No, I have not.
Q. That would surprise you, then?
A. It would surprise me today. I am not sure it would surprise me back in the '50s.
Q. How about in the 1970s, through 1975?
A. I would be dumbfounded after 1975 to hear that.
Q. I would be dumbfounded after '78.
MR. BELFOUR: Herschel, why don't you show him the papers you have, if we're thinking of the same thing, and just ask his opinion. In all fairness, if you have got something in a document, why don't you just show him it.
Q. (BY MR. HOBSON) Basically what you're saying, Dr. Irons, is that up until the benzene ETS was proposed, you wouldn't be terribly surprised to find high concentrations of benzene in many petroleum products; is that fair or not?
A. With the caveat that "high" could mean many things, that's fair.
Q. 10 to 15 percent or more.
A. It would depend again on what it is. I would be kind of surprised to find 10 percent in gasoline.

Q. Continuing there on page 724 -- as a matter of fact, in the same paragraph under "Environmental Exposure," you lay out two scenarios: one is no threshold, and the other is that there is a threshold. for benzene exposure. And what I assume you're talking about there is cancer risk; would that be accurate?
A. Yes.
Q. I take it that you are of the opinion that there is a threshold concentration?
A. Yes, and I -- yes.
Q. I didn't mean to cut you off.
A. I think the more we know about the process of carcinogenesis, the more it becomes clear that even carcinogens have practical thresholds. And certainly, in terms of mechanisms of benzene toxicity and early events associated with the cytogenetic aberrations we've been talking about, I think, both practically and theoretically, there is a threshold for those types of effects.
Q. I was curious about your choice of words here, which having come to know you as I do, I think you usually choose your words very carefully.
Your scenario for a threshold, you say here, "a threshold concentration below which benzene is not toxic"; you go on though, and you say, "and that

intermittel-it exposures to high concentrations present a much greater risk than low-level exposure."
Did you, in fact, choose your words carefully there? Do you still agree that that's what you should have put in this context?
A. I need to find this.
Q. It's in the paragraph "Environment Exposure," the last sentence on page 724.
A. Could you repeat the question. Did I choose my words carefully?
Q. Yes.
A. I think so.
Q. I am curious, then, to know why you put "intermittent."
MR. BELFOUR: I'm sorry. Are we reading the same sentence, where the sentence begins, "If, on the other hand, one assumes"?
MR. HOBSON: I believe we are.
A. Yes.
Q. (BY MR. HOBSON) Isn't the word "intermittent" there on your copy?
A. What I am saying there -- what I think I am saying and was saying -- and I still agree with -- is I am separating what I think is the biologically plausible mechanism for benzene toxicity and leukemogenesis, a

risk hazard, from the low-level cumulative paradigm that has been the default policy in many risk assessments in regulatory context for benzene. And what I am saying is that if you -- if you assume there is a threshold and that the risk is associated with high-level exposure, even intermittent, but high-level exposure, then the overall impact associated with environmental levels, ambient levels, is negligible. That is to say, low-level exposure to eggs and woods, potentially even cigarette smoke, may, in terms of benzene-risk health effects associated with benzene, be negligible. If you assume cumulative exposure is important, then all of those have to be added in.
So all I am saying there is that ambient exposure, the environmental low levels that we encounter in daily life, are negligible if you assume there is a threshold, and that the risk is associated with exposure to high levels.
Q. So you're comfortable with leaving in the word "intermittent" in this sentence?
A. I think virtually all high level -- I mean, there are exceptions; but I think virtually all high-levels exposures are intermittent exposures. Certainly, the Chinese experience is in that context. People are not uniformly exposed.

I mean, this obviously doesn't apply to the Turkish shoe workers, but people are not historically uniformly exposed to 100 parts per million. They're exposed to one -- to a few hundred parts per million for some period of time, interspersed by periods of time when they're not exposed to -- when they're basically exposed to negligible levels.
Q. Where would you then classify your use of "intermittent" here? Are we talking about an exposure that occurs and then there are many days that pass -- is that intermittent -- before the next exposure?
A. Could be. It would be -- that's consistent. By "intermittent," my use in this sentence is meant to represent a dynamic, as opposed to a specific, scenario. And I am simply saying that maybe I should have chosen the word "transient" as opposed to "intermittent," and that probably would be more precise, because I do place -- certainly today, based upon the work I have done, I think "intermittent" represents something a little bit more specific than this.
But what I am basically -- transient or intermittent, I am simply saying that if you assume there is a risk associated with exposure to benzene, that is associated with a temporal exposure to high levels -- and which I think is the majority of cases

that would be described today -- and from most situations, even in the past, that's where the risk is. If you want to talk about specific intermittency, we can talk about that, but that would be outside this context. I mean, it could be included; it's just that this doesn't refer to a specific regimen
Q. Is there a threshold quantitative value that you can give me for benzene?
A. For acute or chronic?
Q. Exposures?
A. Yes.
Q. Chronic. "Chronic" meaning over a period of years, as opposed to one or two short-term exposures.
A. We have been down this road before. I will, for completeness, go through it, and then I will hopefully provide you with something to clarify the issue.
None of us are going to argue abut exposure to 100 parts per million. We can talk about duration, but that's trivial, I think, in the context of most situations. If you're exposed to 200 parts per million routinely, you are going to be exposed for durations of time that are going to be significant.
I personally am concerned about exposures the order of 50 parts per million with respect to

potential for bone marrow toxicity and for leukemogenesis. I don't think the data is as strong as it is for 100. There are people who will argue with me about 50. 1 am on the conservative side with respect to 50. You can start going below there in terms of the actual exposure levels, and you run out of reliable data very quickly.
At the level of below 25 parts per million, certainly below 10 parts per million -- there is no evidence that exposure to 10 parts per million is, in my opinion, associated with a leukemogenic risk.
Now, that is separate and distinct, a time-weighted average. Because within the context of the broadest excursions possible, a 10 ppm time-weighted average can include very significant excursions to 1-, even 400 parts per million.
So within the context of a time-weighted average of 10 ppm, you can have a scenario that I think is a potential leukemogenic risk, but I don't believe exposure to 10 ppm is associated with that. I don't think there is evidence to support that.
Q. Let me see if I can clarify this a little bit.
i~ I have got a job that requires me, say, twice a day to be exposed to benzene that is on the order of 100 to 200 parts per million, for 30 minutes,

and I do that job for five years, is that a significant risk factor for developing AML, in your view?
MR. BELFOUR: Excuse me, Herschel. You said every day for five years?
MR. HOBSON: Every workday, five days a week, that's part of my job. I might get some vacation.
A. Well, you have picked a duration that is problematic, in that if you had said 15 or 20 minute=, I would question the significance of it with respect to actual delivery of metabolites to the target organ, the sufficient concentrations to produce damage. There is time required for that.
But having said that, I think it is unrealistic to assume that someone is going to have an exposure like that. If they are going to be exposed to 100 parts per million, I would imagine, day in/day out, they are going to be exposed for longer periods of time; as you go above 30 minutes, we don't have a disagreement or a question. I think, to any great extent, exposure certainly on the order of an hour or two are going to be sufficient to produce a health risk.
Q. (BY MR. HOBSON) Let me give you a hypothetical and get your thoughts about it.
Assume for a moment that I am a worker on a benzene unit where benzene is being manufactured, and my

job as an operator -- I am an outside operator, and I go out, and I routinely go through the unit and do my check; but part of what I do is I have to catch quality-control samples, which each sample may take me, say, five minutes. And I am just like a little honeybee, I go from this point to that point, and I have got a rack of quart bottles.
When I get to my point to take my sample, I open up a valve and I bleed benzene on the ground for a while, a concrete pad, until I make sure I have got fresh product. And then I stick a quart bottle underneath the tap, and then I fill it up, turn it off, put the screw tap on it, then I go to the next one. And I collect this tray of benzene samples in this manner. Benzene is hot to the touch; doesn't burn my skin; I don't get third degree burns; but it is hot to the touch.
I take the benzene then to a small building that is maybe 4 feet by 4 feet with no ventilation, other than in the summertime; I open up the door, and maybe I can crack the windows, and I do freeze points where I take the benzene, and I pour it into a small beaker with a thermometer, and put that little beaker in a bed of ice, and I sit there, and I shake it until I see frozen benzene forming on the thermometer, and I

note the temperature, checking the purity of the benzene in this manner.
Now, all the time I am catching my sample-. all the time I am doing my work in the freeze-point shack, I smell benzene; and I do this at least twice a day, and it takes me approximately an hour total to do all of this.
Is that a significant risk exposure for leukemia? That's my job. I do it five days a week, eight-hour shifts. Sometimes I get doubled over, so I do it 16 hours a day. Is that anything that you ought to be worried about?
MR. BELFOUR: Just objection; vague. I am not sure you have enough specifics there to form a quantification of exposure, but subject to that objection, you're free to answer the question.
A. There are certain specifics that I would like to know before giving you a precise definitive response to that.
For instance, whether or not the individual is standing right over it; how far away they were from it. Smell is only useful in the context, to me, qualitatively, because individuals can detect benzene at concentrations that I think are lower than those that pose a health risk.

Given those reservations -
Q. (BY MR. HOBSON) The person is at arm's length. I mean, he has the bottle in his hand.
A. Given those reservations, the scenario you're painting is one that I think is certainly consistent with the opportunity of exposure to benzene at concentrations over periods of time that would pose a health risk with respect to the development of acute myelogenous leukemia.
Q. Have you ever heard that exposure scenario before or anything close to it?
A. I have heard descriptions comparable, I think, in the context of washing tools, using benzene as a cleaning solvent, both on bodies, as well as tools, that type of activity, to the degree that they are reliable descriptions of an exposure scenario that constitute a serious health risk.
Q. In your book chapter, I notice that -- at least it seemed to me, that you did a fairly good job of referencing many of the statements that you make in the bibliography; but there were a few that I didn't see referenced, and I wanted to ask you about at least one of those.
On the same page 724 -
A. You didn't like this page, I can tell.

Q. No. As a matter of fact, I found it very interesting. I did kind of like it.
On the right-hand column, about halfway down the first paragraph, after reference 98, it starts o»t, "On the other hand, there is considerable evidence to suggest that there is a threshold concentration for benzene leukemogenesis." You didn't give a reference.
A. I am referring to both mechanistic, as well. as epidemiologic, studies; and I think it is fair to sp that certainly the most recent evaluations of the epidemiology associated with benzene provide increasing evidence of threshold, and a further clarification of the exposure dynamic that is associated with leukemogenesis.
At this particular point in time when I write this, we didn't have the benefit of the most recent updates, but I think, still in terms of the availability of the existing data, what we just were talking about. earlier, with respect to the reliability of the exposure assessment, suggests that there is a threshold.
In theoretical terms, there are practical thresholds that are beginning to be accepted by many, even with respect to known genotoxic carcinogens, such as aflatoxins. There is a practical threshold for aflatoxin carcinogenesis that you can see in this

country vis-a-vis southeast Asia, for example.
Mechanistically, in terms of what we know
about leukemogenesis, the mechanisms of benzene
leukemogenesis involve aneuploidy; they involve
nondisjunctional events. It's clear that they play a
major role in the process, and there is increasing
acceptance of that as being a major paradigm in
leukemogenesis as opposed to assuming a single-hit type
of mechuriism, and in that context we're dealing clearly
with threshold phenomenon.
Q. Going to page 727.
MR. BELFOUR: Excuse me, Herschel. Are we
going to stop in a few minutes? I need to make one
phone call. Take a break in a little bit.
MR. HOBSON: That would be fine.
Q. (BY MR. HOBSON) The bottom sentence, the last
sentence on the left-hand column, starts out,
"Consistent with," you say, "interrupted exposure
regimen, i.e., multiple-exposure intervals interrupted
by several gays, are more effective in producing bone
marrow supdression than repeated daily administration."
Now, is that still your view?
A. We have seen that at very high levels -- very
high levels of exposure in mice, 300 parts per million,
100 parts per million. Certainly, in the experimental

data, it was much more difficult to see a difference between continuous and interrupted exposure when you get below 100 parts per million. By 25, you can't see anything.
I would say it's at interrupted exposure, :and the interval is important because of the cycle-specific issues; but given the right dynamic with respect to regimen, I think that interrupted exposure is at least as potentially effective as continuous exposure.
Q. And, if I understand what you are saying here, is probably more important?
A. That's my bias. I would say the experimental data at least supports at least comparable.
The problem with this, to be perfectly honest, is the most definitive data that is associated with administration of metabolites -- it gets washed out, to a certain degree, when you are looking at benzene exposure in vivo; but at high doses it is -- certainly the regimen dependence is also dose-dependent.
High-dose regimen-dependence appears to be more of an influence than at lower doses, but the duration makes a difference. You are not talking months apart. We're talking a few days apart. We're talking within the context of certainly a week and not more than that.
Q. Are we talking exposures of 1- to 200 parts

per million for what duration? When you have the intermittent exposure?
A. Metabolism -- primary and secondary metabolism are probably going to be saturated for about three hours. So three hours would be optimal. Obviously, I don't think you need three hours to produce toxicity, but there is a -- there is more to it than simply saying what the cumulative exposure is, because you have got differences -- certainly, this is opinion at this point in time. But we're looking at, at least two types of processes that we discussed here today that appear to be very important in leukemogenesis for AML, in general; and what also benzene metabolites are capable of doing, but they have totally different dose dynamics. Some are low-dose effects; some are high-dose effects.
So you're probably look -- it's probably the rate of rise and the duration of exposure that determines whether or not you have precisely the optimal conditions to produce toxicity.
So the dynamic with respect to regimen probably plays an important role in the process; it's not just what that cumulative exposure is. So somewhere between 20 minutes and 3 hours you are going to be looking at a paradigm that is important with respect to toxicity.

Q. In this road, from beginning of exposure saturation, is it nonlinear?
MR. BELFOUR: I'm sorry. I object as vague.
I don't understand what you are asking.
Q. (BY MR. HOBSON) The saturation curve or the curve that goes to saturation, concentration of benzene metabolite in the compartment.
A. Once you impose upon that, the issue and the dynamics of alterations in stem cell progression proliferation, it is a stochastic process; it is not linear. I am not a mathematician, but it is hard for me to perceive of a linear model for that.
Q. If we were to graph out what you believe to be the response or the concentration for dose in the compartment from beginning of exposure to saturation., does it tend to -- does the dose -- or the exposure, I guess would be more proper -- does the exposure tend to go up steeply and then flatten off, you believe, as you get closer to saturation?
A. I am not sure I can honestly say one way or the other. I would suspect that, without taking it to an absurd extent, probably the most risky scenario does not involve the steep ramp, because somewhere in the middle and somewhere, depending upon the dynamics of the exposure, you are going to alter stem cell regulation;

and that's probably more important than cytotoxicity because you are increasing the number of cells at risk. And that's far more important than just doing the dirty deed in terms of -
Q. Saturation?
A. -- saturation. So I think that, you know, if you are exposed to 1000 parts per million, like now, for 20 minutes, that probably isn't going to do anything; whereas, if you rank -- depending upon what the dynamics are, and whether you were exposed yesterday -- for all I know, exposure to 25 or 30 parts per million yesterday may pose a risk with respect to exposure to 1000 parts per million today.
So I think, you know, saying that, yes, a steep curve is the most potentially damaging -- I don't really think there is a basis to conclude that. You might actually be able to come up with speculation that a more complex exposure dynamic would be more damaging.
MR. HOBSON: Let's take a break.
(Break was taken.)
MR. BELFOUR: Herschel, am I correct in assuming that when we talk about the risk of injury, we're talking about AML, because you haven't always said "AML" when we talked about the disease, and I don't want the record to reflect that you have asked a

question without saying "AML," and the doctor has responded about health risks assuming you were asking about AML.
MR. HOBSON: Well, I presume that Dr. Irons is always answering "AML" since he said that's all he thinks benzene causes. It may not be what I wanted to ask, but I think that's what was his response.
Q. (BY MR. HOBSON) Do you agree that multiply: myeloma is a tumor of plasma cells?
A. Given all the discussion we have had today, yes, within the context of what we discussed today.
Q. Do you agree that plasma cells are in the body cells derived from B lymphocytes which are located primarily in the bone marrow?
A. Yes. All the evidence points to a bone ma,--row origin for multiple myeloma.
Q. Do you agree that multiple myeloma in almost all cases begins with a somatic mutation in a single cell?
A. It certainly is a clonally derived lesion. What the initial events are, we really don't know. Multiple myeloma is not that well understood.
Q. Do you agree that it arises in a single cell, though?
A. Yes, I do.

Q. Are you familiar with a monoclonal antibody that is demonstrated on a serum protein electrophoreses?
MR. BELFOUR: I'm sorry?
MR. HOBSON: That doesn't make any sense to me. Never mind.
MR. BELFOUR: Herschel, may I interrupt for a moment. Did you ask whether the cells that divide and become plasma cells are derived from B lymphocytes or B lymphocyte lineage?
MR. HOBSON: I don't remember.
THE DEPONENT: I think he said "B lymphocytes."
MR. BELFOUR: Okay. Thank you.
Q. (BY MR. HOBSON) Would you agree that a definitive diagnosis of multiple myeloma depends on the presence of an increased number of plasma cells, sometimes with abnormal morphology in the bone marrow?
MR. BELFOUR: Excuse me. I object to that as vague. Hs one component diagnostic criteria? As the definitive diagnostic criteria?
MR. HOBSON: Definitive.
A. I don't want to get caught up in semantics here. The clinical presentation of multiple myeloma should involve -- the clinical diagnosis, definitive diagnosis, should involve bone marrow examination.

There are other aspects that are consistent with the diagnosis of multiple myeloma that can be found in individuals who don't have multiple myeloma, such as -she presence of a monoclonal gammopathy or presence of M protein, even though they are very -- they are components of the diagnosis.
Lytic lesions, certainly on X ray, can always get you there, but ultimately the histopathologic diagnosis of the bone marrow provides you with a definitive conclusion.
Q. (BY MR. HOBSON) In some individuals with the benign monoclonal gammopathy, they will occasionally progress to multiple myeloma, will they not?
A. Yes. I mean, this is one of the biggest problems in determining what the nature of the clonal progression is for multiple myeloma. I think 1 percent of individuals -- there is a certain -- I think it ice. a very low percentage, but a certain percent of individuals are walking around with monoclonal gammopathies; 3 percent of people over 50 have actually M proteins present in their serum. Obviously the incidence of multiple myeloma doesn't come anywhere close to that.
So there are aspects of disregulation of B cells associated with plasma-cell differentiation that

occur in clinically normal individuals and that have no consequence with respect to predicting the probability of the onset of multiple myeloma.
On the other hand, there are people with M protein levels that obviously go on to develop multiple myeloma. It's a clonal lesion at the point at which you have got M protein. So there is a clonal gammopathy present.
"What differentiates that individual from somebody who develops multiple myeloma" is a -- is the question.
Q. You do agree that benzene causes aplastic anemia?
A. Yes, with the caveat that the presentation is not what most purists think of as aplastic anemia, necessarily.
The difference is the very high-portion preponderance of myeloid proliferative abnormalities in the bone marrow of individuals who develop pancytopenia associated with benzene. A real strict definition of aplastic anemia does not include a hyperplastic bone marrow. I am not parsing hairs.
Experts in aplastic bone will distinguish a difference between induced aplastic from hyperplastic on the basis of the fact that if you have got a

hyperplastic bone marrow, it is not really aplastic anemia, but it is certainly a refractory anemia, and it certainly has all the clinical significance of aplastic anemia.
Q. If I recall earlier testimony of yours correctly, you have said that a person can have AML that is benzene-induced and you would expect to find a prior effect upon the blood, but it may not necessarily be clinically evident from the usual blood tests that run, such as white blood counts and complete blood counts. Am I right?
A. That's a reasonable characterization, yes. I think that the majority of people who develop AML, secondary to benzene or anything else, the literature suggests they are going to have some preexisting evidence. That doesn't mean everybody presents that way. It's probably on the order of -- it's certain1y greater than 50 percent.
But one could -- I think it's conceivable that someone could have bone marrow injury and have subclinical presentation.
Q. And still develop an AML to be benzene induced?
A. I think the majority will have some evidence -- preexisting evidence, but that you cannot

exclude that possibility.
Q. And certainly you have opined that benzene is a known cause of acute myelogenous leukemia. We just went through that.
A. That is not an issue here.
Q. What about acute myelocytic leukemia; is that a different term for the same disease?
A. The subtypes of AML within the FAB clarification, with some exceptions, most -- not all - most of the subtypes are associated with secondary AML and benzene. There are some exceptions. Myelocytic is the same; myeloblastic is the same as AML.
Q. What about for erythroid leukemia?
A. M6 is a fairly -- M6 is relatively rare and has a very high predisposition toward secondaries in general and benzene in particular.
Q. So you would agree that benzene is a cause of erythroid leukemias?
A. Yes.
Q. So it would be a correct characterization that benzene is a known human leukemogen, although you would say only for the acute myelogenous?
A. Yes, I am not going to quibble with you over that leukemia.
Q. Would you agree that the literature has shown,

based primarily on case reports, that benzene has also been causally related to a variety of other hematological neoplasms, including CML and non-Hodgkin’s lymphoma, as well as multiple myeloma, even though you don't agree that the literature shows it's causative?
A. That's an oxymoron. You asked me would I agree that primarily on the basis of case reports, that benzene is causally related, et cetera.
Q. No, sir.
A. But not causally related?
Q. No, I'm not asking you that. I'm asking if you agree that the literature has said that.
MR. BELFOUR: Excuse me, Herschel. May I interpose an objection? That any literature, that 10 percent, that half? That they show multiple myelomas in deficits, in excesses? They show other diseases in deficits, in excesses? I think it's overly broad, but if the doctor can answer it, I would certainly allow him to.
Q. (BY MR. HOBSON) Well, the question is, Do you agree that, based primarily on case reports, that benzene has been causally related to a variety of other hematological neoplasms, including chronic myelogenc us leukemia, non-Hodgkin's lymphoma, as well as multiple myeloma? I'm talking about what the literature has

reported.
A. I have a problem with the way you worded that question, because you're saying primarily on the basis of case reports, causally related; and I don't believe a case report constitutes evidence of causation.
If you are asking me, are there case reports that hypothesize an association between benzene exposure and the diseases you mentioned, there is probably a case report out there to hypothesize everything that ever was. So if you are saying, are there case reports that propose benzene is associated with those diseases, yes.
Q. Do you agree that when we talk about benzene causing acute myelogenous leukemia, and looking at the literature historically, that that connection was originally made through case reports and not epidemiologically?
A. That is an overly -- I think that's an overly simplistic characterization of the history of benzene literature. By the time -- if we take from the beginning of the century up through Hunter in the 1930s, '40s, to say, let's say 1960, one can find on the order of 250 cases of benzene poisoning in the literature. Depending upon what you call a leukemia and what you don't, there were between 5 and 12 case reports of leukemia in that literature vis-a-vis a background of

literally hundreds, between 200 and 300 cases of individual bone marrow suppression, aplastic anemia, pancytopenia, thrombopenia, lymphocytopenia, et cetera, et cetera. That, in a few minds, constituted evidence that benzene probably caused leukemia. Hunter was sure of that in 1939. No one else was.

Between 1960 and 1970 we now see the collections of Vigliani and Aksoy, which were case reports; but they were collections, so they're not quantitative, but they certainly constituted a much richer set of concentrated examples of benzene toxicity than we had anywhere else. And by the end of the we now have -- probably 25 percent of the cases that have been reported in the literature are leukemias. That obviously provided a foundation for the epidemiologic studies that occurred during the '70s and which led to a general consensus with respect to the relationship between bone marrow toxicity and leukemogenesis.

That also was superimposed on our knowledge that chemotherapeutic agents that cause bone marrow suppression could lead to leukemogenesis, which wasn't appreciated by hematologists in the '5os; and it wasn't certainly appreciated in the 30s and '40s.

So there was no connection that bone marrow

suppression could be associated with an increase in the number of cells, which is what you see in a leukemia. All of that came about between the late '60s and the late '70s.
Case reports I don't think provided the definitive evidence necessary to provide -- that led to the realization of that relationship; certainly not the early ones. There were very few, and if you added them up, there were really few.
I do think that the collections that were provided to us primarily by the Italians and the Turkish studies provided reasonable evidence of some relationship there before the retrospective epidemiologic studies.
The fact of the matter is, if all we had was the Pliofilm study and nothing else, it might not have had the impact it did. It's much more of a milestone than it is a great epidemiology study. It simply punctuated the cumulative knowledge that we had with respect to benzene and leukemogenesis at that point in time.
Q. Have you ever seen a document from the 1950s where a representative of the American Petroleum Institute and a representative of the Chemical Manufacturers Association, or in those days called the

Manufacturer Chemists Association, signed off on the proposition that the relationship between benzene and cancer of the blood had been established?
A. I have seen something certainly similar to that, and I don't think it's reliable. I think, based upon the -- certainly not authoritative. Based upon the information that was available to them at the time, that certainly was not a general consensus on the part of the medical community.
Q. What are you thinking about?
MR. BELFOUR: Excuse me, Herschel. It might be better if you start with what were you asking about, and maybe your question was objectionable, because it was overly broad and vague.
Q. (BY MR. HOBSON) You said that there was a document that came to mind, or something that came to mind. I was just curious what it was that came to mind.
A. I do not remember the specifics, but I have seen a letter written by a representative of, I believe it was the American Petroleum Institute or a petroleum company employee that said, in essence, what you are saying. I have seen something like that.
Q. Would you agree that animal studies have demonstrated that benzene is a carcinogen producing a variety of solid tumors and lymphatic tumors in animals?

A. In animal -- in various animal models, specifically the mouse, benzene causes a variety of different tumors -- also in the rat. Some in glands we don't even have.
I would like to extend that to indicate that even the solid-tissue tumors that are seen in rodents have a very similar characteristic in that they all have enzyme profiles that are typical of those found in human bone marrow with respect to the ability to induce peroxidated metabolism of the secondary metabolites.
Q. Is it correct that, among the early effects of benzene on laboratory animals and in occupationally exposed groups, there is a decrease in lymphocyte count?
A. It's been reported -- absolute decreases in lymphocyte counts have been seen in one occupational setting priluarily, and that was in the rotogravure workers, pressmen from the Goldwater studies.
MR. BELFOUR: Excuse me, Herschel. I'm sorry, Doctor. Did you ask "laboratory animals"?
THE DEPONENT: He mentioned both.
MR. BELFOUR: I'm sorry. I just didn't understand the question.
A. Animals are much more susceptible to those changes than humans are. Rodents are particularly susceptible to those changes, and rodent lymphocytes

tend to be very susceptible to conditions that are associated with benzene.
Q. (BY MR. HOBSON) And for the laboratory animals exposed to benzene, the effects would be on both the B and the T lymphocytes?
A. There have been -- we presented some real early data on B cells in rabbits, but the methodology, quite frankly, was byzantine compared to what we have today.
In the mouse model, I don't think it's clear what we're talking about in terms of cytopenia. It is clear with respect to the types of tumors that are seen in the -- in most mouse strains, it's a T cell lymphoma. High-dose benzene exposure -- and by "high dose," I mean injections of very high amounts -- will cause immunosuppression, including B cell.
Q. Would you agree that the basic cell type of plasma cells, the B lymphocyte, is affected by benzene, and it's probably in the circ -- it's probably the circulating lymphocyte cell found to have been -- found -- let me start over. I can't even see it here.
MR. BELFOUR: Herschel, is that one of my questions I butchered?
MR. HOBSON: It could be. I have done a better job of butchering it than you ever would, though.

Q. (BY MR. HOBSON) Let's see if I can say it accurately this time.
Would you agree that the B lymphocyte is affected by benzene and is probably the circulating lymphocyte cell found to have benzene-induced cytogenetic abnormalities?
A. No, absolutely not. It's a compound question. The answer was to the last part.
At some concentrations I wouldn't be surprised to see some effects on B lymphocytes, just like I described qualitatively, maybe similar, to what is seen in rodents with respect to B-cell effects, but those are very high doses.
All the cytogenetic studies that I know that have been done, both those that I think provide some useful data and those that are flawed, virtually all the human studies are looking at cytogenetic aberrations in cells and culture after induction with a T-cell mitogen. So some of the ones looking at peripheral lymphocytes in humans have directly examined B cells.
Q. What about laboratory animals?
A. Very few have done that. I think LPS has probably been used as a mitogen in one or two studies; but then again, the induction of B-cell mitogen involves very artificial concentrations of very toxic substances,

so it's a very unreliable system for evaluation. That's why in the human studies the vast majority of those things are done with PHA, which is a T-cell mitogen. It's stimulating proliferation under conditions that are certainly not normal, but at least they aren't killing off half the cells in the culture.
The use of endotoxins and lipopolysaccharides to simulate B cells is a very artificial situation arid it creates a lot of damage just in the process.
Q. So if someone said that probably the circulating lymphocyte cell is a cell that is found to have benzene-induced cytogenetic abnormalities, that person would just be wrong?
A. Based on the techniques that have been used, whether you're talking about Pacciano or Yardley-Jones, to look at peripheral cytogenetic aberrations in workers potentially exposed to benzene, and looking at T cell, they're not looking at B cells, by definition, based on the methodologies that were used. I'm simply saying they can't say anything with respect to B cells, because they're looking at T cells.
Q. Does radiation exposure cause multiple myeloma?
A. You know, that has certainly been a presumed paradigm for many years, but some of the more recent

studies of survivors of the atomic bomb blast in Japan suggest that multiple myeloma has not increased. I would say that whereas I have assumed that radiation was a potential cause in the past, I think there is some question about that now.
Q. Is it enough of a question that you say radiation is not more likely than not a cause of multiple myeloma?
A. Up until recently I would have said it's more likely than not. As of now, I am not sure. I certainly don't see it as a major outcome of radiation therapy, per se. Most of the data is concentrated in the atomic bomb survivors, and that's come under question.
Q. Do you agree that the Chinese studies confirm that DNA damage is discernible in human lymphocytes following benzene exposure?
A. If you want to include, within the broader context of DNA damage, cytogenetic aberrations and/or evidence of deletions, that type of thing, yes. If you want to refer to adductology, no.
Q. Is a human fetus more susceptible to benzene-induced leukemia than a developed individual?
A. There is actually no basis upon which to render an opinion on that. There is no evidence for it or against it, that I am aware of. I know of no

leukemia model that involves either -- exposure that would be associated with benzene, either experimental or any evidence in humans.
Q. Are you aware of anybody ever looking?
A. Certainly reproductive studies have looked at multigenerational studies in rodents. I don't know of any evidence associated with the lymphoma or the leukemic model in those studies associated with exposure in utero.
The predominant leukemia type in young children is an acute lymphocytic leukemia. It has not been associated with exposures to anything that I am aware of, certainly not benzene, and it doesn't fit paradigm that is consistent with secondary leukemogenesis in adults.
Q. Did I understand earlier that benzene metabolites are much more likely to affect rapidly dividing cells?
A. In terms of cytogenetic or cytotoxic effects, yes.
Q. Wouldn't the cells in a fetus be more rapidly developing -- I'm sorry, rapidly dividing than you would have in an infant that has been born?
A. On a theoretical basis, certainly they're more rapidly dividing, but at the same time you have also got

comparable differentiation-dependent protective mechanisms at work during fetal development as well. It's a very complex issue. Certainly, replicating cells constitute a greater -- a more susceptible target with respect to the cytogenetic effects. Whether those cells, in fact, are potential origin -- constitute a potential origin, or cell of origin for leukemia, is another question. I know of no paradigm or model that would provide me with a basis for saying that would be anything but speculation.
Q. And the same would be true: You know of no paradigm or model that would disprove or show that there is no effect on a fetus? Would that be true?
A. Well, no. As I said, there are reproductive studies which don't demonstrate persistent hematological problems in the offspring of animals exposed to benzene.
Q. But not humans?
A. I don't know anybody that has done a study of in utero exposure in benzene.
Q. Or if not even in in utero, of blood or bone marrow that comes from a fetus?
A. We're doing that.
Q. A human fetus?
A. We're doing core-blood studies and comparing

core blood with adult bone marrow. Core blood constitutes a source for fetal blood, fetal bone marrow.
Q. And you're exposing that core-blood component to benzene metabolites?
A. Yes. We're looking for differences in stem, cell regulation and increased susceptibility or decreased susceptibility.
We see major differences in early stem cell hierarchical differentiation between mice and humans that certainly play a role in the susceptibility of the mice to T-cell neoplasms, and we are investigating whether those differences are species-related or age-dependent. And some effects appear to be very definitely species-related, and some effects are age-dependent.
So far I don't have any evidence of differences associated with exposure to benzene metabolite.
Q. Have you published any of that?
A. No, not yet.
Q. Do you still believe that there is more evidence that benzene causes multiple myeloma than lymphoid leukemias?
A. I think you asked me once before, and it was -- I think my answer to that question was related to

degrees of implausibility. You're asking me, Is it less plausible that benzene causes lymphoid leukemia than multiple myeloma?
Q. I am asking you, Do you still believe that there is more evidence that benzene causes multiple myeloma than lymphoid leukemias?
MR. BELFOUR: Excuse me, Herschel. Do you have a reference to where that came from?
MR. HOBSON: Page 55 of his prior deposition taken in the Ellis case.
A. I'm sorry. Could you ask me the question once again?
Q. (BY MR. HOBSON) Do you still believe there is more evidence that benzene causes multiple myeloma than lymphoid leukemia?
MR. BELFOUR: And that deposition in Ellis was taken in approximately 1991 or so?
MR. HOBSON: Late '91, I think, without being held to it.
A. I don't think, as we sit here today, that I could distinguish between the levels of evidence for each. They don't meet any test of consistency for either, and I don't know of a plausible mechanism that would explain a difference in the potential for either.
Q. (BY MR. HOBSON) So today you would say

they're holding their own, being equally bad?
A. My -- I think the evidence against benzene being associated with multiple myeloma has accrued significantly in the last several years. And so what. certainly was my opinion in Ellis is much stronger today. And from that standpoint, I would say that there is not more evidence that benzene causes multiple myeloma than lymphocytic leukemia.
Q. Have you seen the most recent epidemiological studies for butadiene from coal and diesel?
A. Yes, I have.
Q. Do you believe that butadiene is a human carcinogen?
A. I believe that that study raises some very important questions; but based upon my evaluation of that study, in the form I have seen it in, which is probably not going to be its final form, it raises a number of biological plausibility issues with respect to their classification of leukemias.
I think it is probably -- it is evident from those studies that exposure to butadiene and/or something in the polymerization process is influencing the development of leukemias. The question is, Which ones and how? And right now, I think there are some major tests of biological plausibility that are going to

have to be made before I have any idea what that means.
Q. What form was the study in when you saw it?
A. I peer-reviewed an original -- the original rough draft, and it has undergone review, and I have no idea what the final is going to look like.
There are a number of problems that were raised, that I certainly found, with respect to the characterization and analysis, and I have no idea what the final form is going to look like.
Q. Your view of the conclusions, then, from that study, are different than the authors'?
A. Not necessarily. I think it's dependent; I think it raises some very important issues with respect to what is seen. I think there are questions with respect to the exposure -- the relationship between exposure and the -- and leukemia, in general, and there are questions with respect to the classifications of the leukemias. I think many of those concerns of mine are shared by authors. I don't know how they are going to wrestle with those in terms of publication.
MR. HOBSON: I would like to attach all the stuff you have brought today, and I am done.
MR. BELFOUR: You want to read and sign, I believe.
THE DEPONENT: Definitely.

(The deposition concluded at 4:10 p.m.)