From: George Woodall <Woodallg@api.org>
Sent: Wednesday, September 26, 2001 11:48 AM (GMT)
To: Richard Irons (E-mail) <richard.irons@uchsc.edu>; Otto Wong (E-mail) <OttoWong@aol.com>; Robert Schnatter Ph. D. (E-mail) <arschna@fpe.erenj.com>; Thomas Armstrong (E-mail) <twarmst@erenj.com>
Cc: Patrick Beatty (E-mail) <pwbe@chevron.com>; Shan Tsai (E-mail) <sptsai@shellus.com>
Subject: FW: benzene meeting
Attach: sept 01 comments.doc; minden.vcf

-----Original Message-----
From: Dr. Mark Minden [mailto:minden@uhnres.utoronto.ca]
Sent: Wednesday, September 26, 2001 10:43 AM
To: George Woodall
Subject: benzene meeting
George
Linda was away at a retreat so I did not catch up with her.
Appended you will find my comments on the proposal. As you will see, I think the cytogenetic stuff needs some work. A lot of reliance is being put on FISH, however there is actually a fairly high background rate to this technology that has to be considered. Molecular tests in general are more sensitive and specific.
I look forward to hearing about the outcome of the meeting and the follow up meeting.
Sincerely yours
Mark Minden

Comments re Benzene Studies

Sept 25, 2001

Mark Minden

Comments relating to the draft 6-26-01

1. in the overview of the participating hospitals, there is no indication that "cases" will be referred from other hospitals in the Shanghai area. For the population of Shanghai the incidence of leukemia appears to be about 2-3X less than an equivalent North American city. Is this the case? Do the individuals at risk, live near and typically go to the indicated hospitals? What fraction of cases will be referred on to a participating hospital? Do individuals who are sick, return to their home towns/villages? Will these cases be missed?

Will cooking style, etc be included in the history?

In the draft it is mentioned that questions about hepatitis will be avoided, while this is clearly tested for in the protocols. Given the association of hepatitis with NHL and AA, this needs to be included.

Comments relating to Protocol I

I found the consent written at a relatively high education level. They need to be written at a grade 8 level. In the consent for cases, there is no mention as to the disease the patient has. This should be included. There is no indication in the consent as to the scope of testing, or ownership of samples. I do not know where things are going in China but in North America and Europe, this is a very sticky issue. For example in Quebec if a patient has not been consented for a specific genetic test, their banked sample cannot be tested. It is probably worth adding something to the effect that the samples will be tested for genetic changes related to the development of leukemia, and or genetic polymorphisms that may be associated with susceptibility to the development of leukemia/lymphoma/BP/AA. With regards to ownership, it should be clearly stated that the cells etc belong to Fudan. Also it should be indicated that the individual can request at any time that the stored samples pertaining to them should be destroyed.

The size of samples taken varies from place to place. In some cases it says 10 ml will be taken, and in others 20 ml. This should be clarified in a flow diagram.

Is a blood slide enough for the BP, AA studies? This will not be adequate for establishing cell lines.

Sample identification. At one point it is stated that samples will have full patient identifiers so that the lab personnel can be sure of what sample they have, while later it is stated that only the unique identifier will be used. I prefer the latter labeling procedure.

page 60 pH should be Ph.

With regards to disease classification I note that the WHO is being used for AML. I agree with going to 20% for the definition of disease, and applaud the statement that if a certain cytogenetic abnormality is present, even if blasts ,20% that this is AML. I do have some concerns with the WHO classification, as a case of t(15;17), arising as a result of chemotherapy is coded as a secondary leukemia and not t(15;17). I would think that in addition to the WHO classification it is necessary, and this seems to be being done, to keep separate records of 1) morphology using FAB, 2) flow results, 3) cytogenetics, 4) drug exposure 5) history of antecendent hematologic disorder.

Heparin used in the tubes should be SODIUM heparin, and not lithium heparin

When drawing the 10 ml of bone marrow, is this a single pull, or is the needle repositioned through out this. If it is a single pull it is possible that there will be dilution of the sample. Our local cytogenetics group would be unhappy with this, as they feel it leads to reduced yield.

In the nice flow sheet showing disposition of the BM samples it is indicated that the EDTA sample is for molecular studies. However, in the culture studies it states that the lab wants an EDTA tube. Either the flow sheet should be changed to indicate this, or the documentation in Appendix H changed to indicate heparin.

On page C21-it should be indicated that this is a -20C freezer. Could -70C be used?

Page H9-RLT buffer is not defined. The supplier and purchase number for this should be provided.

Page H13-the tests described are RT-PCR tests with RNA as starting material, not DNA.
The primers for these tests should be defined.
Will Flt-3 and 11q23 duplications be assessed?

Page L1. Here and in other protocols, impossible volumes are indicated eg 16.66 ml. Here it should simply be equal volumes of the two solutions. In other places the numbers should be rounded to what is possible with the equipment available.

Page L11. Does 10% FCS give optimal storage? Many protocols use 40-90% FCS.

Comments regarding proposed studies.

As an overall comment, I agree with Dr. Larson, when he states that the proposed correlative studies should be put forward much like an RO1 or PO1. I have specific comments on the cytogenetic, molecular and tissue culture studies that are proposed.

1. Cytogenetics. The proposed studies will look at standard cytogenetic abnormalities using a variety of probes. Is nothing already known about the spectrum of genetic changes seen in this group of patients. If, for example t(8;21) is a rare event, what is the point of monitoring peripheral blood or bone marrow for this? If normal cytogenetics are obtained, what will be done to resolve this? We have data that the addition of Flt-3 ligand will improve yields. Will SKY be carried out,

for normal cytogenetic cases? How often will cytogenetics be carried out? How many metaphases will be looked at? Could other methods such as sister chromatid exchange be looked at? It must be remembered that cytogenetics is a relatively insensitive technique. A brief review of the literature indicates that trisomy 8 is common in benzene induced disease. I have read over the paper by Smith Ca Res 58:2176, 1998. It is unfortunate in this paper that no metaphases are shown for review, nor the frequency of such metaphases in lymphocytes of the positive patients. When I was in China several years ago and visiting the cancer hospital at Tianjin?, it was my impression that t(8;21) made up 20-25% of AML in China. If this is the case it may indicate either a different susceptibility in this population, or some environmental factor. It would be of value to determine the incidence of t(8;21) in all AML, and those thought to be associated with benzene.

2. Molecular studies. The proposed studies will look at a few genes felt to be important in the metabolism of benzene. The BP group of patients provides some outstanding opportunity for study. What fraction go on to AA or MDS? It would be useful to look at skewing of hematopoiesis in this group, using HUMARA in female patients. With progression, what early changes are seen? Can one detect changes in expression of DNA repair or mis-match repair genes? changes in methylation? There are quite sensitive RT-PCR methods for these studies, and they might provide insight into some of the earlier genetic changes in this disease state.

Is the MDS seen in benzene exposed patients similar to that seen in other patients? Is apoptosis prominent in some patients? It would be useful to determine whether the types of disease following benzene exposure mimic the full spectrum of MDS and AML, or show a restricted pattern.

In addition to the genes being studied, duplication of Flt-3 ligand and 11q23 are recurrent abnormalities gaining in interest. Are these abnormalities present in the benzene exposed patients?

4. Cell culture studies. What is the hypothesis being tested here? Is the point to show hypersensitivity to GM-CSF? Could this be of use for other studies? Is hypersensetivity a stable state? Is there enough of a difference between the hypersensitive cells and normal cells, that one could use this as a "separation" technique, that would then allow more molecular studies, aimed at identifying the nature of genetic changes in these "progressed" cells? For example FISH should be coupled with the culture studies to look for trsiomy 8 or monosomy 7.

Dr. Albertini, suggested looking at the HPRT. I assume he meant in the presence of 6TG. This would be a very useful experiment. One could use either bone marrow cells or lymphocytes to identify the frequency of HPRT mutants. Sequencing of the HPRT mutations could give some insight into the type of genetic changes that are occurring in cells. By looking at different cell populations, would could get an idea of tissue specific sensitivity to benzene.

Other thoughts

In doctor Albertini's write up he asks if AML can develop without prior MDS or AA. As the cases will only be identified when they are sick, unless there is routine CBCs being done for benzene workers, it will not be possible to do this. Would it be possible to do q3mon CBC on a cohort of individuals working in a benzene plant?