PROTOCOL REVIEW

SCIENTIFIC REVIEW PANEL OF THE BENZENE HEALTH RESEARCH PROJECT

Meeting held September 27, 2001
Westin O'Hare Hotel
Chicago, Illinois

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INTRODUCTION

The purpose of the meeting and subsequent activities was to provide a review of the draft protocols for the Benzene Health Research Project, which encompasses three interrelated studies that will be conducted over the next five years in Shanghai, People's Republic of China. This review was performed by the members of the Scientific Review Panel (SRP) that was selected by representatives of the sponsoring organizations. A meeting was held at the Westin O'Hare Hotel in Chicago, Illinois on September 27, 2001 for the purpose of reviewing the comments made on the protocols, and a conference line was established to allow those unable to attend in person to participate. During the meeting, comments were responded to and discussed reviewer by reviewer, beginning with those who were on the telephone from Europe (Dr. Rice and Dr. Cherrie), then proceeding with those on the phone from the U.S. (Dr. Herrick, Dr. Mueller, and Dr. Albertini), and finishing with those present in Chicago (Dr. Li, and Dr. Larson). Comments made by those reviewers who could not participate were addressed as time allowed, and those comments not included in the discussion will be addressed in writing by the researchers and will become a part of the documentation for the protocol review.

Meeting Participants

Name	Affiliation	Status on Review
Otto Wong	Applied Health Sciences	Epidemiologist. PI for the Case-Control Study of AML and NHL.
Tom Armstrong	ExxonMobil Biomedical Sciences, Inc.	Industrial hygienist working on exposure assessments within the studies
Richard Irons	University of Colorado Health Sciences Center	Toxicologist, experimental hematologist and clinical pathologist. PI for the Disease Progression and Molecular Epidemiology Studies
Patrick Beatty	Chevron	Toxicologist with one of the sponsors. Chair of the Technical Committee
Shan Tsai	Shell Chemical Co.	Epidemiologist for one of the sponsors. Sitting in for Vice Chair of the Technical Committee
Guilan Li	Chinese Academy of Preventative Medicine	SRP Member – Genetic Toxicology
Richard Larson	Leukemia Research Program, University of Chicago	SRP Member – Hematologist
John Cherrie (via phone)	University of Aberdeen	SRP Member – Industrial Hygiene
Rob Schnatter	ExxonMobil Biomedical Sciences, Inc.	Epidemiologist working on the DP and ME Studies
Nancy Mueller	Harvard School of Public Health	SRP Member – Epidemiologist. Area of research is viral exposures and lymphoma
Robert Herrick	Harvard School of Public	SRP Member – Industrial Hygiene

Name	Affiliation	Status on Review
	Health	
Richard Albertini	University of Vermont	SRP Member – Genetic Toxicology. Background in biomarkers of genotoxic effect and molecular epidemiology
Jerry Rice	International Agency for Research on Cancer	SRP Member – General Toxicology. Background in chemistry and comparative pathology
George Woodall	American Petroleum Institute	Moderator of meeting. Toxicologist with API providing staff support to the project.

Opening Remarks

Dr. Beatty first gave a brief overview of the steps taken to get to the present protocol review, an explanation of the organization of the consoritum, and some business items for the SRP to consider. The main points of those remarks are outlined below.

SRP Business

- Conference Call. There is a need to have another conference call for the SRP in the near future to address some of the issues in this list.
- Selection of a Chair. The consortium will be contacting individuals of the SRP to identify one who can be the chair.
- Additional positions. The consortium has agreed to fund an additional two positions on the SRP that will be filled at the discretion of the SRP itself. That was done for the SRP to have some guidance over its composition initially and to allow for additional expertise if needed.
- Support staff. The sponsors feel the SRP has to be as independent of the consortium as possible to avoid perceptions of undue influence. Currently, API staff is serving as administrative support as it is on the rest of the consortium activities. API has agreed to provide overall administrative support to the consortium. If the SRP desires and feels its in their interest from a standpoint of independence, the sponsors have agreed that they would be willing to pay for a third party to provide that support.

Consortium Structure

The consortium consists of three committees and two panels. There is an overall business Oversight Committee made up of business representatives from the sponsors that is responsible for budget and overall guidance issues. Reporting to the oversight committee and providing technical support and oversight is the Technical Committee, made up of sponsor members who have appropriate technical backgrounds such as toxicology, epidemiology, industrial hygiene and so forth. The Technical Committee was responsible for identifying and recruiting initial SRP members and making the recommendations to the Oversight Committee. In addition, there is a Communications Committee that oversees communications between the consortium and the outside world. If there is a need at some point in the future to respond to comments or if this endeavor develops media attention, they will be responsible for the interface.

There are also two independent review panels, one of which is the Scientific Review Panel. You have received documents that explain the responsibilities of the SRP members. We will be sending a larger document that outlines the whole consortium structure and the relationships of the panels within it. The SRP is charged with an independent oversight of the scientific aspects of the study involving initial protocol review, protocol review amendments and then review and comment of any manuscripts that will be developed for submission.

In addition, there is an Ethics Review Panel (ERP), which has been active now for over a year and involved in development of the protocols as they raise bioethics issues. What you have seen in the protocols regarding informed consent and questionnaires has been developed with the assistance of the ERP, and that includes a Chinese bioethicist. It is intended that the ERP and SRP interface with each other since protocol issues may have both scientific and ethical implications.

Roles in this Meeting

Consortium representatives present will not take part in the discussion on scientific issues in order to avoid any perception that we have steered the discussions of the SRP. Finally, this is really a forum for dialogue between the reviewers and the investigators. We want to do this by addressing each individual interviewer's comments with each investigator responding to those. Additional SRP members that have comments can comment as they feel appropriate.

There appear to be three classes of comments that will be handled differently today. 1) Scientific issues: the scientific validity or integrity of the protocols in addressing the stated aims of the studies. Those are the comments that are the priority of this meeting. 2) Bioethics issues: most of these comments regarding informed consent or questionnaires have already been discussed with the Ethics Review Panel and, in general, we propose those comments be forwarded to the ERP for additional review. 3) Scope of the study: these are comments suggesting additional endpoints that could be measured, or additional aspects of the study that could be added on. At this point the scope of the study has been developed through a prioritization process by the sponsors. Since this would involve expanding the scope and the cost of the study, scope comments will be considered by the Oversight and Technical Committees.

Deliverable from the Meeting

The deliverable from this meeting will be a report of the SRP, initially drafted by Dr. Woodall, and then distributed to the reviewers and investigators. The intent is to develop a consensus document that will reflect the intentions of the SRP. At the time of the SRP meeting the format of the report has not been finalized. There are a number of legal issues that must be addressed before the exact format can be finalized. This will document all of the relevant comments and the results of the SRP review meeting discussion.

It is anticipated that the protocols will be revised based on agreements reached in this meeting. If there are issues where there is a difference of opinion that cannot be resolved, the report will serve to document the rationale for both positions.

Historical Background – Relationships between Studies

A number of comments from various reviewers indicated some confusion about the way the three protocols of this project were integrated. The project has been an evolutionary process, beginning several years ago when Dr. Wong proposed to do a case-control study in China to address the relationships between acute myeloid leukemia or non-Hodgkin's lymphoma and benzene exposure. As the result of several trips to Shanghai to assess the feasibility of the case-control study it became clear there were a number of other questions that could be addressed with the exposure data and other opportunities that existed. Additional program components were developed that now exist in the form of the disease progression in the molecular epidemiology studies. As these were developed, additional collaborators from a number of different institutions within Shanghai became involved. It has resulted in a three-facet study that is heavily integrated. At this time, due to the relationships developed for each of these program components, and to contractual considerations as well, it would be difficult to restructure this using a NIH program project structure, as was suggested by several reviewers. . However, in large part the projects will operate in much the same way as a program project.

Project Organization and Preliminary Discussion

This discussion was conducted by Dr. Richard Irons. The question and answer session was included participation by all members of the SRP and the researchers.

To begin the discussion, it should be made clear that the Disease Progression and Molecular Epidemiology protocols you have are a version that is dated June 2001. There have been numerous protocol changes made relative to informed consent and other issues that you don't have because it would have been too cumbersome to provide multiple iterations of this document for distribution. Some of the comments refer to typographical errors, some of which have already been identified.

There are three studies that are separate on paper, separate with respect to objectives, but are interrelated with respect to a number of facets including laboratory support, collection of material, interviewing, and exposure assessment. The studies are:

1. A Case-Control study of AML and NHL which is under the direction of Dr. Wong.
2. A Disease Progression study of Aplastic Anemia, mild dysplastic syndrome, AML and benzene poisoning.
3. A Molecular Epidemiology study of benzene-exposed workers.

Central Diagnostic Laboratory

A central laboratory supports all three in terms of diagnosis of diseases, the assessment of outcomes, and endpoints for the epidemiology study. This laboratory has undergone renovation in Shanghai and is now ready for occupancy. It is first and foremost a clinical laboratory. It meets both US and Chinese standards of operation under the guidelines of the College of American Pathologists (CAP). It is functioning as a formal clinical laboratory. It has contractual agreements with the participating hospitals to serve and support the management of patients who are subjects of this study.

The guiding philosophy of the laboratory is first of all, collect everything. For virtually every subject, we are collecting DNA, RNA, bone marrow cells, lymphocytes, and serum. There are some variations depending on subsets of subjects. By collecting,

studying, and storing every possible media, many of the questions regarding scope may go forward under other sponsorship or other support in the future. The first goal is to collect and preserve everything.

As a clinical laboratory, it will be providing clinically relevant reporting of data to treating physicians with respect to all of the subjects studied in this laboratory. The methodology must meet the clinical and regulatory restrictions both here and in China. That places some restrictions on the lab, but also provides more stringent methodologies than are routinely used in research studies. The laboratory has been engineered to meet the College of American Pathologists criteria for lab structure and function. It will operate 24 hours a day, 7 days a week.

The lab will provide centralized methodological support for the clinicians in terms of collection teams, so that hematologists involved in the Disease Progression and Case-Control study will be supported and provided standardized methodological collection and handling of specimens. The interview team, which will be a collaborative effort among the participating institutions, will be standardized and exposure assessment will be standardized. There are several aspects of regulations both in the US and China that pose restrictions, such as volumes of individual samples and laws regarding the exportation and processing of clinical material.

The lab, which is formally titled "Joint Sino-US Clinical Molecular Laboratory," will provide hematology services, differential diagnosis for NHL, MDS, AML and potentially aplastic anemia. It will function primarily under the criteria the WHO classification ICD-0. It will provide morphology, immunophenotyping, molecular and cytogenetics analysis. It will also provide an analysis of disease specific biomarkers of effect as they pertain specifically to the pathogenesis of the diseases in question. It will provide analysis of confounders and other co-factors that relate to the biology of the diseases.

Some limited metabolism analyses will be performed, but these are restricted by the volumes of samples we are receiving and new Chinese law restricting the exportation of material. A license to export material for support of clinical diagnosis is expected but it will strictly prohibit the exportation of material for research purposes. It is hoped that analysis of prognostic factors and potentially polymorphisms will also be provided in the study.

Participating Institutions

There are several institutions participating in this study that have formalized contractual agreements. These include:

- University of Colorado Health Science Center (UCHSC)
- Applied Health Sciences, Inc. (AHS)
- Fudan University, which is a major university in Shanghai
- Shanghai Municipal Centers for Disease Control and Prevention (SMCDCP), which is primarily responsible for the study of occupational health in Shanghai.
- Shanghai Municipal Institute for Public Health Supervision (SMIPHS), which is primarily responsible for enforcement of occupational standards in Shanghai.
- Shanghai Hematology Society and the Shanghai Pathology Society, which more than being academic or professional organizations actually play a major role in setting standards of practice and clinical activities in Shanghai and are major signatories to this study.

- ExxonMobil Biomedical Research, Inc. which is primarily responsible through the good offices of Mr. Armstrong for the exposure assessment and Dr. Schnatter for epidemiology for the Disease Progression and Molecular Epidemiology studies.

Criteria for Diagnosis

The diagnosis and criteria for diagnosis for all of the disease entities and all of the studies will be done in this laboratory and will be standardized according to criteria set forth in the protocol, which is the ICD-0 – the World Health Organization new classification for hematopoietic and lymphoid diseases.

Exposure Assessment

Exposure assessments will be performed collaboratively by Mr. Armstrong of ExxonMobil Biomedical Science, the SMCDC and the SMIPHS. Fudan University will assist in the development and leadership of the exposure assessment teams. Dr. Wong has agreed that the Case-Control study will also use the same protocol for exposure assessment as used in the Disease Progression study.

We no longer have issues with respect to the control of the AML and having separate sets of controls for the Case-Control and the Disease Progression studies with respect to AML. So that is a major change in terms of the protocol that is not reflected in what has been sent out for review.

Relationship with Area Hospitals and Clinics

The major referral hospitals in Shanghai have been contracted to provide coordinators to identify AML and the disease progression study endpoints. The coordinators are senior hematologists to which all of the potential hematopoietic diseases will be referred in the Shanghai region. The hematology coordinators in each of the major hospitals will triage cases for aplastic anemia, MDS and AML. There have already been two training sessions for these coordinators, and there will continue to convene sessions to provide training and orientation with respect to ethical obligations, clinical procedures, and lab procedures.

The diagnostic materials will be collected in standardized fashion. The lab will provide immediate clinical support for diagnosis, which will be reported back to the clinician.

The lab will accept referrals from outside of Shanghai, specifically to find cases of benzene exposure that have resulted in a clinical problem. At present, 14 of the 15 referral hospitals in Shanghai have been included into the referral network. These hospitals see approximately 80-90% of the severe hematopoetic diseases. The one hospital that has declined to participate is the only hospital in Shanghai that has a clinical cytogenetics laboratory.

The Hematology Society is also trying to recruit the central district hospitals, of which there are 23. The central district hospitals are both out-patient and in-patient clinics, however, most would not treat or manage a severe hematologic malignancy. These central district hospitals will see approximately 50% of the NHL cases and probably 50% of what we call mild or out-patient cases of bone-marrow suppression. They also perform lymph node biopsies but do not routinely do bone marrow biopsies.

Capture of Cases

There are two different criteria for capture. 1) For hematologic diseases it's the coordinator, so for these diseases we have very tight control at the level of initial physician referral and diagnosis. 2) For lymphoma and for mild cases, there is a different setup because there is not a control on who is going to see a lymphoma case. It may be an oncologist, an intern, a hematologist or other physician. All of the major departments have agreed to provide for standard triage and evaluation of pathology, which is the primary tool for differential diagnosis of lymphomas.

The pathology departments often overlap with the participating hematology referral centers and the central district hospitals. The organization is provided through the Shanghai Pathology Society, which has agreed to standardizing the triage and processing of all lymph node biopsy material for which a diagnosis of NHL needs to be ruled out.

Cases that involve surgery, surgical resection or biopsy will also be captured through the pathology departments. There is a relatively high proportion of extra-nodal lymphomas in China especially related to naso-pharyngeal and some abdominal lymphomas. These will be captured through surgical pathology. The procedures for capturing and preserving the material will be adopted into the hospital protocols.

Study Databases

The lab will provide appropriate data sets for inclusion into the Case-Control study, the Disease Progression study, and the Molecular Epidemiology study. The databases are going to be strictly controlled by ethical standards and legal criteria. Only the clinical database will have identifiers because it must function in a clinical mode and to support diagnosis and management. Identifiers of individual will be eliminated prior to entry into the study databases. Informed consent is going to be required for each study specifically related to the databases. There are firewall protection standards that are going to be set up and access to the databases will be restricted to a very limited number of specific individuals with clinical responsibility in the lab.

The lab will interface and partner with the SMCDC and with the SMIPHS to provide for the assessment of exposures and access to subjects in the molecular epidemiology study. This database will also be used to improve standards for industrial hygiene and regulation.

Definition of Mild Cases

Mild cases are out-patient cases of MDS, aplastic anemia, or individual cytopenias. It would be impossible to include these in the Case-Control study because they would be so numerous. These cases will be kept under surveillance in the greater Shanghai area as those more likely than the general population to progress into a disease category that would be appropriate for recruitment. The protocol calls for CBC monitoring of these potential cases only, and will not include any exposure analysis. Hopefully, this will provide the earliest indication of progression for any of these cases and then they would immediately become candidates for the Disease Progression study.
[Note: as a result of subsequent discussions we have elected not to monitor mild cases with annual CBC but to enroll outpatient subjects that meet the study criteria for AA and MDS.]

Laboratory Analysis

The lab work scope for NHL cases will include:

- Histopathology,
- Immuno-phenotyping, and analysis of Epstien-Barr virus expression in clonal lesions (tumors). Serology on EBV would have very little biological value since the vast majority of the population is positive.
- Banded chromosome analysis, FISH/PCR, Southern Blot, multiplex FISH, competitive genomic hybridization (CGH)
- Serology will be conducted for HIV I and II, HBV and HCV.
- DNA and RNA extractions will be done routinely.
- Cryostorage –80 degrees and nitrogen freezing.
- Lymphocyte immortalization and cryostorage
- CBC
- LDH

Staging support will be provided for the clinicians for study subjects, not as part of the protocol but as one of the ethical and clinical responsibilities. Bone marrows will not be routinely taken unless physicians order it.

Lab work for aplastic anemia, MDS, and AML cases will include:

- CBC
- Morphology: bone marrow smear, and core biopsy.
- Immuno-phenotyping will be done primarily on the core biopsies, but for instances where specific markers are indicated, the smears may be used.
- Banded chromosome analysis will be conducted on each case. FISH panels will be used along with PCR as a back up along with Southern blot, and competitive genomic hybridization. The secondary FISH panel will be conducted on all normal karyotypes.
- Cryostorage,
- Lymphocyte immortalization and cryostorage
- Bone marrow culture.

The bone marrow culture activities that have been described in this study are supported by a funded NIH grant and are not described in detail; however, they do involve analysis of apoptosis in MDS cases and an examination of cytokine response and differentiation in culture.

Diagnostic Team

Professor Jude Chong Zhang is head of Tumor Pathology for the Shanghai Tumor Hospital, and is an internationally known immuno-pathologist. He will diagnose all of the lymphomas. Du Xinxu from the Shanghai Second Medical University will be evaluating all of the cores. Xi Meirong from Hua Shan Hospital is a hematologic-morphologist who will evaluate the smears. Dr. Irons will moderate this group, which will meet weekly or bi-weekly for the first year.

This team will perform the initial diagnosis and the differential diagnosis for the NHLs, AMLs, and hematopoietic lesions. These will be independently confirmed by John Rider, hemato-pathologist at the University of Colorado. An external pathology

working group will also be established that will meet at least annually and provide consultation on difficult cases, or cases where a conflict needs resolution.

For cytogenetic analysis, primary analysis in the field will be conducted by Liming Bao, who is a board-certified cytogeneticist, and board-certified pathologist in China, also board-certified in medical genetics in the US. Secondary review of cytogenetics will be performed by Lois McGavern at the University of Colorado and the Colorado Genetics Laboratory where she is director.

Technical staff will include individuals trained in the US who are routinely involved in clinical support diagnostic activities at the University of Colorado, as well as Chinese individuals who will be trained at the University of Colorado and will return to assume responsibilities on this project. The laboratory will start off primarily staffed by US personnel, and over the following five years will transfer to Chinese staff. All technical staff will have the same accreditation that the CAP requires and that China requires.

Laboratory Facilities

The laboratory has undergone renovation that meets the engineering standards required by CAP. It has offices, a conference room, reception, molecular biology lab, a BSL 3 high level containment culture facility, equipment room, hematology, histology, and FISH labs, and reading rooms for both FISH and light microscopy.

Cytogenetic Analysis

The primary use of cytogenetics is for clinical testing, and not as a screening tool. The objective is to determine the presence of acquired clonal changes in bone marrow aspirates, core biopsies, and/or blood of symptomatic individuals. This includes patients with aplastic anemia, MDS, mild proliferative disease, AML, NHL, and benzene poisoning. Analyses will include standard cytogenetics of non-stimulated bone marrow cultures, mitotic figures, and G-banded analysis. The data will be recorded as a number of cells per clone and number of normal cells. Studies using non-specific chromosomal breakage or mutations as monitors of mutation, toxicity, or exposure, are not proposed in the current study design. Non-specific chromosomal aberration studies will not be done. Bone marrow aspirate is our primary tool, then peripheral blood if no bone marrow is obtained and analysis to include a FISH panel if no mytotic figures are obtained.

Samples for cytogenetic and FISH studies from NHL cases will include lymph node or mass fresh tissue from surgery or biopsy, touch preps or formalin-fixed, paraffin embedded 4 micron sections as backup, bone marrow for staging if indicated or requested by the physician. The FISH panels that we're going to use will be performed for aplastic anemia, mild dysplasia, AML and benzene poisoning if the standard genetics are not successful. They will also be used on all NHL cases irrespective of banded chromosome analysis.

The panels we're using are listed in the protocol and they differ with respect to each disease and protocol. The data will be expressed as frequencies of positive and negative cells in 200 nuclei examined. The value will be compared with concurrent controls and currently the only controls we have to use are US controls. Positive tests will be defined by comparison with cumulative laboratory clinical control values. That is based on the experience we have at the Colorado Genetics Lab. RT-PCR studies will be used as an adjunct to clinical diagnosis, and will be used if FISH probes are not available. It will be

used to identify FISH positive translocation or rearrangement probes where possible. mFISH and SKY will be used to evaluate complex karyotypes. Competitive genomic hybridization will be used to evaluate gain or loss with respect to gene amplification, which is expected to be rare, but can be done if deemed necessary.

For the molecular epidemiology, banded chromosome analysis will be used to identify or rule out a clonal lesion, we are not doing non-specific chromosomal aberrations. The secondary leukemia screen will be used regardless of the chromosomal analysis. If a negative chromosomal analysis is found, a secondary screen will be done. If banded chromosomal analysis fails a complete FISH screen panel will be used. The reason for comparing peripheral blood to bone marrow is to analyze the validity of blood FISH as being reflective or in concordance with what is observed in bone marrow. Metaphase cytogenetics is being done on unstimulated bone marrow cells that is being compared with FISH analyses of unstimulated peripheral blood mononuclear cells.

Phase 1 is an evaluation of historical data and the identification of factories and the individuals that work in those factories. Phase 2 will follow up on the individual analysis primarily in blood and exposure analysis for those same individuals. A subset of those individuals will be recruited to provide bone marrow and they will be sent to Wei Xein Hospital. It will involve all exposure groups, including no exposure as well as the different exposure categories that are described in the study. A subset of all of the subjects in each of the exposure groups will be sampled for bone marrow and peripheral blood.

Monitoring Databases

The monitoring databases and is one of the reasons Shanghai is such a unique environment for these studies. The Institute for Public Health Supervision has two monitoring databases. One is a benzene poisoning database which is a registry and the other is a monitoring database of factories. I'm going to provide an overview of what is in the monitoring database for factories. The current computerized form was set up in 1986. On an annual basis it monitors about 20,000 facilities. This varies depending upon the market. There is manual data back to 1980 at least, but the computerized data begins in 1986. The data fields that are collected include factory ID, location, ownership (public or private), industry type and activities conducted there, information on the specific chemical hazards and occupational diseases associated with those hazards, the individuals working there, and the locations where air monitoring is done. It also contains information on the number of employees exposed at each of those stations and monitoring, up to quarterly, by site, and remediation info, if any.

The monitoring results are a mix of short term area samples and some personnel samples. There are additional personal monitoring, in terms of time weighted average for a work shift as we understand are in individual facility records. The concern for an industrial hygienist is, do these area samples reasonably represent the personal exposures. The answer is both yes and no. In many cases, the ventilation is for general comfort and ends up being a breathing zone sample in a very well mixed chamber. The workers are in many cases at a fixed workstation for a good portion of the workday. There is still some time activity and task correlations to turn some of these samples into a better estimate of the full shift average.

The other database is a benzene poison registry. It is a legal requirement of the physician to report benzene poisoning cases in China. That database has been linked to this one and it is possible to identify factories with high exposure by identifying excursions in their previous monitoring database and correlating that with the reported cases of benzene poisoning. Our spot measurements using a benzene selected monitor have independent confirmation that those exposures are still present.

Question and Answer Session

The discussion continued with a brief question and answer session. Many of the issues raised by the reviewers in their written comments were answered in this session and from the preceding discussion.

Q: One concern is that you're taking bone marrow from healthy workers and how many healthy workers are willing to give bone marrow. Has this been pilot tested that we know the acceptance of this approach?

A: We don't know the number of individuals that we're going to be able to recruit. The protocol in terms of worker acceptance and ethical and scientific has been developed with participation by a Chinese ethicists and with the participating regulatory agencies. A recommendation that they have made, which is not in the protocol right now, is that we initially examine 2000 workers in phase1, and 1600-2000 in phase 2. They think that will give us a comfortable margin for the recruitment of 200 in phase 2b.

Q: In the 200 that you're going to recruit for phase 2b, are you going to pick these for those that have specific chromosomal aberrations in the blood, to know that you have something to compare to in the bone marrow?

A: No.

Q: Because these are proving to be very, very, infrequent in Martin Smith's studies. So you could end up to 2b then with nothing in the peripheral blood to compare with the bone marrow.

A: The criteria for selection of the 200 is exposure just as it is in the 2000 in 2A. The reason we've called them phases is because they will be going on concurrently, so we will select people on the basis of exposure analysis to recruit 2b and their analysis of peripheral blood is going to go on concurrently. A subset of 2a is 2b, but they're not going to be sampled twice.

Q: So, the question is to say that if you have 1% of people with the specific [marker] in the blood, and you pick 200 people, you might be doing 198 peoples bone marrow and you have nothing in the blood to compare it with.

A: We're going to over-sample the high-exposure group.

Q: That's assuming that you've got the answer to the question you're asking. That is that high exposure increases the frequency, which is not necessarily proving out in the other studies.

A: We know that the most sensitive indicator so far in the HEI study is peripheral blood cytopenias. So we will be looking at that correlation. We are not going to select on the basis of cytogenetic aberrations or FISH, we're going to select on the basis of exposure.

Q: Using bone marrow is somewhat more invasive, which is probably quite justified if you have a reasonable expectation that you're going to find something to compare it with. But, statistically, most of those 200 people will have a bone marrow sample taken for nothing.

A: We are looking at metabolism, we are looking at exposure, we're trying to see if we have an internal, if we can make that leap so we can compare all of the in vitro data that has been done in looking at in vitro cytogenetics and mechanism. The focus is on mechanism and an attempt to capture early events that may relate or predict progression to the disease we're interested in.

Q: Are there pilot data showing that analysis of FISH on peripheral blood samples is useful? Do we know what the likely incidence is in FISH positivity in the peripheral blood lymphocytes of these highly exposed workers?

A: There are two contradictory studies currently available. The Smith study in workers demonstrated a relatively high frequency of numerical changes; however, the numerical changes show a random distribution that would project an aneuploidy rate of 25% in unexposed workers, which is outrageously high. There are some serious issues with that data. The HEI study has preliminary data from David Eastman which does not find a concordance with those findings, and in fact shows no dose response for FISH analysis in peripheral lymphocytes. Our primary goal is to determine whether or not what we see in the peripheral lymphocyte population reflects what we see in the bone marrow.

Q: To avoid wasting effort on bone marrow samples on patients with normal peripheral blood findings, you might structure your study so that you do the peripheral blood FISH first and, at least, some patients who have a positive FISH result before asking them to come back to have the bone marrow done. I think you can work with your statisticians to improve the power of your ability to find a correlation if there were one.

A: The in vitro studies suggest a marked difference in susceptibility between the bone marrow myeloid cells and peripheral lymphocytes. If that is the case, the lymphocytes may be less sensitive and, if that hypothesis is correct, we will see changes in bone marrow before we see changes in lymphocytes. The other issue is if we don't sample the controls then we have a problem with respect to the metabolism studies. There is more than just the FISH issue with respect to bone marrow control. The goal is to test the hypothesis that the pattern and frequency of effects in bone marrow may not be reflected in lymphocytes and, if so, that would provide some conclusion with respect to the representative nature of peripheral lymphocyte studies.

We are not prohibited from sampling the phase 2b individuals for the peripheral effects early. We would have to sample blood twice, which would affect our cost. The other issue is with coordination and recruitment of the individuals for the metabolism

studies. Bone marrow sampling requires we take the patient from a factory to a hospital for bone marrow sampling and subsequent metabolism studies. We were hoping to be able to do it in such a way that the time interval between exposure and marrow samplings was relatively constant. We will have to give that issue some thought.

Comment: The second study (phase 2b) might provide useful information on the validity of the first study (phase 2a). Rather than an expensive extra step, the best thing may be to call the patients who were positive on the first screen back on the day of the bone marrow exam and check blood simultaneously.

A: That would add another dimension to the study, and the cost impact is going to be very significant.

Comment: You might save money by not performing exams on non-informative patients in phase 2b. You might want to calculate how many you would need within the 200 in phase 2b. You may want to use 40 patients who had positive FISH results.

A: I agree. If we get a cytopenia, we would also want to enter that candidate into the Disease Progression Study. We might be able to monitor our cost is we modify the number of bone marrows that we analyze.

As a general guideline, we want to over sample high end exposures relatively early in the study. The extent to which we are able to do this depends on the number of cases that we encounter in any given exposure category and is simply not predictable at this point.

DISCUSSION OF SPECIFIC REVIEWER COMMENTS

As mentioned in the introduction, a discussion of the comments from specific reviewers was performed reviewer by reviewer, with additional comments and questions handled as the discussion progressed. There were a number of questions that were answered in the opening remarks, and others that may have been addressed by more than one reviewer that were answered before the comments of an individual reviewer may have been addressed. The sections below summarize the discussions based on the comments from the reviewers that participated in the meeting. This is not a direct transcript of the discussion. The complete written comments from each reviewer is included in Appendix A. The responses of the researchers to comments of reviewers that were not addressed in the September 27th meeting are summarized in a separate section of this report.

Comments from Dr. Jerry Rice

The introduction was satisfactory to clarify the relationship between each committee and what each participant's roles are. Those comments have been addressed.

Case-Control Study

Q: The protocol as written proposes one control to one case. Is a one-to-one ratio adequate for purposes of this study?

A: Since we have agreed to a common exposure metric and will not be independently selecting controls for the AMLs, it makes sense that we provide the same number of controls for all diseases of interest. The one control was selected as a cost consideration but other aspects [affect] whether these procedures are successful. There may be issues of non-participation in cases and/or controls. If you lack data in a control then essentially you have lost case data as well. Two controls may not be completely adequate either, but it would provide a greater assurance that you would avoid having to throw out a case.

The limitation on the controls is cost. Doubling the controls across the board increases the cost by about $100,000. This would be partially ameliorated by the fact we are not duplicating AML controls. If the number increases to 3 or 4, it becomes a question of how much money do we have to add controls. Is there a number that you would propose for controls? This cost estimate does not include increased analytical and industrial hygiene costs which will probably increase the total impact to about $250,000-$300,000.

Comment: The value to the study would increase at two controls, beyond that I would be more inclined to accept the argument of cost.

Q: How will the controls would be selected. One of the projects says that the controls would include any cancer diagnosis accept for LMP diseases. Why is it so important to select controls with cancer?

A: What was meant was that subjects would not excluded based on cancer status, so controls are open to any diagnosis that gets hospitalized.

Molecular Epidemiology Study

Q: There are two concerns – 1. On the technical details of detection and quantification of benzene metabolites, there is a rather scanty description of the feasibility study for analyzing these endpoints. 2. Focusing on the oxidation products of benzene, there is limited evidence in rodent species and I would interject a note of caution.

A: The pilot study is a very small study. There has been extensive development in analysis of the ability to analyze benzene metabolites in bone marrow and blood of animals. This will use the methodology developed by Lovelace and specifically evaluate the initial derivation for hydroquinone and substitute solid phase extraction methods for liquid extraction methods in order to increase the sensitivity and decrease the variability of the methods. Because this is a small addition to what has been done, we have elected to do it directly in human bone marrow. We are required because of legal and ethical issues to do those on human volunteers in the US, which is where the developmental work will be done. It will be conducted with the Chinese chemist who will also do the work in China. It's a one to two month pilot study to optimize the conditions for extraction and to determine if it is acceptable. Right now I believe we are looking at in animals a minimum limit of detection in nano-moles. If we can reduce detectability by an order of magnitude and increase reliability by solid face extraction, then we would go forward with the studies in humans. If we can't we need to evaluate justification to invest human bone marrow from exposed workers in those studies.

In response to concern number 2, you are entirely correct with respect to the carcinogenic evidence. However hydroquinone has been demonstrated to cause hematotoxicity in vivo and that toxicity is comparable to what is seen following the administration of benzene. The cytogenetic and the stem cell effects of hydroquinone on bone marrow in vitro are comparable to what has been described with benzene in vivo. Hydroquinone is the only established metabolite of Benzene that produces hematotoxicity. The other metabolites can contribute or enhance toxicity but only if hydroquinone is present. The original proposal was to provide a direct link between all in vitro studies that have examined the toxicity of benzene metabolites and actual concentrations of those metabolites in bone marrow. We have a very strict limitation on volume that's going to be available for this so we have had to prioritize what metabolites we would look at. Thus hydroquinone to provide an in vivo link in human bone marrow with all the in vitro studies of cytogentics and stem cell biology. If we have additional material we will also look at catechol. Catechol enhances the effects of hydroquinone . That I think is my response to the second questions.
We do not intend to pursue the analysis of bone marrow metabolites in workers in China until we have established a satisfactory method that approaches the sensitivity needed and that will probably be the first publication coming forth from this project.

Disease Progression Study

Q: What is the relationship of the cases from the Case-Control Study with those in the Disease Progression Study.

A: By and large they would be the same cases. There may be some cases that may refuse to participate in the Disease Progression study but would participate in the Case-Control study. That number will be small. In terms of the difference between the

numbers quoted in the two protocols (500 and 600) I think it is a matter of estimates the Chinese government provided at various times. All the AML cases and NHL cases would be identified from the same lab.

Q: Can you clarify whether your references (page 12 and 13 in the Case-Control study protocol) are to the Case-Control study or the Disease Progression study. This section could benefit from further clarification.

A: The relationship would be we would have access to the AML and NHL cases in the lab database. We would not have access to the Disease Progression study at all because that is an entirely different study. The lab is set up to support all three studies we have access to the data for the AML and NHL patients. The criteria for transfer of data from the clinical databases to the study databases is informed consent.

Q: Concerning the identification of all possible confounders that might be important in your studies, handling of certain confounding conditions (notably HIV) differ between proposals. In the Disease Progression study it is clear that HIV positivity could be considered. It would also raise the ethical issue of from whom it is communicated and I presume that it would be through the patient. I am concerned that the tip of an iceberg is beginning to emerge in China with respect to the prevalence of HIV infection and that could affect your study.

A: The test for HIV is actually a part of the lab analysis, and that is how that information would be collected among the cases. For the Case-Control study, that information is needed for both cases and controls, otherwise there is no comparison. When this issue was brought before the American Ethics panel member, the impression was that that question could not be asked. The question was referred to the Chinese Bioethics expert and he allowed the question, but maintained that how the virus was acquired could not be asked. A further ethical restriction was imposed that any information obtained from the questionnaire could not be used on any shared database.

Both social and legal dichotomies exist here between the US and China. It is important for us to know if the cases are HIV positive or not. There is no restriction on that in China; however, by Chinese law we are required to report any cases of HIV. The Ethics panel has concurred that whenever we have an issue of this nature we refer to Chinese law. It is dealt with in terms of informed consent. The subject is told that we are going to look at that and if it is positive then we are required to report it. This is at the level of the clinical lab and not the studies.

Q: A certain number of cases will turn up positive. What will be done with those? Will you exclude them from your studies? Will you be guided by the numbers and include them as a subset?

A: We will not exclude those cases with confounding factors, but we will characterize them and use them in the progression study. All exposure cases will be included and HIV status will be treated as a confounding factor in the analysis.

Comments from Dr. John Cherrie

Q: There is a need for documentation of Tier 1 exposure Classification - because there is great deal of subjectivity that the procedures should be well recorded at a later stage for any individual study, or simply for quality assurance. It was clear that this would be taken care of, but it wasn't clear who would actually conduct the assurance and how many people would be involved.

A: We have been in the process of defining the professional qualifications for the staff for public health supervision. The initial classification on exposure based on the database is going to be pretty robust. There are a significant number of factories that have been covered in the database and will be used for initial sorting. All facilities not in the database will trigger a site visit to evaluate the presence of Benzene.

Q: So in other words, there will be greater reliance on the database.

A: That is a fair summary, but we are not going to rely only on the database; it will not be the only resource. There are a number of people who have a great deal of experience with the Shanghai area working conditions and will be contributing to the evaluation. Most of the initial work history will be collected at the hospitals, but not necessarily by those who assemble the exposure data. They will interview the participant if no other information sources are available. We have had discussion on ways to summarize the assessors data.

Q: Will each individual have the same training and style of approach?

A: Yes, experienced team members will provide some dual coverage and the degree of redundant coverage can be discussed in more detail. From an organizational standpoint, the collaborating institutions have made commitments to provide the experienced personnel. It has been made clear that it will not be a revolving door of personnel staff. We would like to have continuity through out the course of the study.

Comment: One of the important things is that a record is kept so that the information can be reviewed later by either the team on-site or someone else. The study can then be reviewed by someone external to the study. My only other concern is I just want to make sure the list of confounders is not limited.

Q: Could you clarify the second Tier sampling.

A: The fixed location is within the participants breathing zone. The standard method that has been used in Shanghai for taking samples is to operate the calibrated air sampling pump long enough that they believe they have collected enough of the benzene to provide proper analysis back at the laboratory. So instead of sampling for a fixed time they sampled to a specific level on the tube and recorded the time, which translates to the sample volume.

Q: My concern is the amount of time affects the levels recorded.

A: A great deal of consideration needs to be given in translating the area breathing zone estimations accurately. Some of that will be concurrent sampling by that tradition method with concurrent personal sampling. We do have the luxury of a commitment

of staff at the University on a research level. The researchers will be able to help develop the history of samples and their interpretation for each factory.

Q: What do you mean by breathing level samples? Could you elaborate a bit on that?"
A: The operation I best dug into was at a shoe factory where a few workers sat at the end of an assembly line putting glue on shoe parts with comfort fans blowing. In monitoring, the chemist had the air sampling pump held at breathing level, apparently hand held throughout the sampling.

Q: I have some concern at the choice of process for measuring levels. How are you going to deal with intermittent and daily exposure?
A: There are samples available at facilities to supplement the variability and indications of if this activity was routine or daily. You are right to point out the biological basis needs. The variability throughout the day is probably not that significant. What is needed is the average daily exposure and a measure of any variability within the week.

Q: What is the general approach to dermal exposure?
A: What dermal contact has occurred needs to be recorded. In the shoe factories the contact was minimal but in other industries that may not be the case. It should also be noted that the only respiratory protection equipment mandated in China is a cloth face mask when there is exposure to Benzene. A cloth face mask is not adequate protection but abides by Chinese law.

Comments of Dr. Robert Herrick

Case-Control Study

Q: Wong and Fu criticize the Dosemechi (NCl) exposure estimates as being too low, however, the attached letter from Dr. You-xin Liang notes that the MAC in Chinese workplaces is 12 ppm. Are the majority of Chinese workplaces out of compliance with the exposure limit?
A: The control paradigm in China is somewhat different than in the West. We are used to controlling exposures. The Chinese are basically controlling to disease end points. As long as they are not seeing the benzene poison cases they are not too worried about what is going on as far as exposure. The 12ppm standard is loosely enforced. I won't say that most of the workforce are out of compliance with the standard but there certainly is a significant fraction that are above the standard. In the database without too much effort we identified about a dozen facilities that had exposures over 100mg per cubic meter at their sites. There is no short term exposure limit.

We were able to look at factory records and visited 5 or 6 facilities in two different trips and the high exposures were exceptions. There are a variety of ranges and nondetects in the exposure database. In the epidemiology study we are looking to recruit higher exposed individuals and can easily fill in with the lower exposed individuals.

For the disease progression study, the exposures will be assessed as we go forward. For the molecular epi study, we will be choosing the facilities we want to study.

In addition to the data that we see at the Shangai CDCP, we also have quite a few reports in Chinese occupational journals reporting leukemia and benzene exposure. They also report exposure information among those cases. We are talking about exposure in the range of several hundred ppm.

Q: The discussion of the exposure assessment procedures states that an industrial hygienist will review the occupational histories based upon a list of occupations with potential benzene exposures. This will be the first step in triggering the search for employment records, etc. In order to do a comprehensive exposure assessment it is imperative that this information on employment, job title and job location be obtained for all subjects (cases and controls) not just those on whom the initial determination was that they were benzene-exposed. What becomes of subjects exposed to a potential confounder, but not to benzene? Under the current exposure assessment strategy, it seems that no further information would be obtained for these subjects.

A: The SMCDCP database covers known benzene exposed industries in the Shanghai area. There is information in that database on exposures to other agents in the facilities. We will develop a list of jobs, and if the job didn't involve any benzene exposure, that would be the end of it in terms of exposure assessment of benzene. As far as exposure to confounders, it will need to be thought through a little more as far as relying on the database.

Q: The third question I had was what about people who do not fit into these catagories from the factories. What do you do with them?

A: I think the first question would be what kind of operation and also was the individual always within that profession. All of those not fitting into a category go into a further investigation category.

Q: Do you have any sense of how far back you are going to back into someone's work history?

A: As far as we can. The 15 year latency has nothing to do with how far back we will go in terms of exposure assessment.

<u>Disease Progression Study</u>

Q: Please clarify mild to severe cases. My understanding is the severe case is hospitalized and the mild case has been released and sent home.

A: The answer to your question is yes. We chose the terminology out of convenience. Mild cases are clinical data that does not meet the specifications for inclusion in the Disease Progression study. This would include anything that would approximate benzene poisoning.. Mild cases represent outpatients, who, if they are not older and retired, would be in the workforce.

Q: What if a person with a mild condition, who goes back to work and then turns up again through the current sampling. You might end up running a sampling on someone who already is a case.

A: We have elected not to screen for "mild cases" that do not meet our study definition for AA or MDS. Therefore a "mild case" should already meet the standard definition of benzene poisoning in China and should not be in a workplace environment where they can be exposed to benzene.

If we identify them as meeting criteria for the study, we are going to report them. And they become a followed patient or benzene poisoning case, and we are going to try and recruit them for the benzene study. Mild cases will only be kept under clinical surveillance. We are not doing any exposure assessment of mild cases other than to get a good work history for the patient that has returned to work.

Comment: This only adds to my concern for the database monitoring. We need to monitor changed situations.

A: I agree. Monitoring should indicate if they have changed work environment and what they are doing differently.

Q: What happens to the mild cases? Are they told? Do they just return to work?

A: Since we won't have exposure data on a patient who presents as an outpatient at one of the hospitals, we have no control over what brings them in to the clinic. If they meet the criteria then that will trigger the monitoring, but we will not be in a position to tell them they have been exposed to benzene.

Q: Is there any occupational history taken that would be there routinely?

A: The questionnaire will collect work history data. What we are really trying to do is increase surveillance.

Q: How will this play out in years from now? We knew these people were exposed and is there an ethical violation.

A: We do not know that a person has a progressive condition thus we are not responsible.

Q: How do you define a mild vs. severe case?

A: We have set some criteria with respect to blood analysis.

Q: Do you have an estimate of how many of these mild cases will cross over to the diseases you are actually tracking?

A: We do not have an estimate. We have discussed this with the Ethics committee in reference to a questionnaire. If they don't meet study criteria or benzene poisoning that doesn't trigger any ethical question about them going back to work. At the mild level we don't know what to expect.

Q: The mild cases could be better handled even though not included in the study.

A: The Chinese hematology community feels strongly that there is a fairly large population of walking aplastic anemia cases. Our purpose for including these is to

provide a uniform capture protocol for the cases we are studying. It's not presented with a formal Case-Control study but the intent is to identify the earliest form of progression.

Comment: The inclusion of mild cases confuses the study group. If they have a bone marrow analysis, they should be in the study, but if they don't, then they should not be followed. If they get sicker, then they will come back.

Q: Are there patients that the Chinese call mild cases that may be environmentally-induced that are not diagnosed via bone marrow? If there are, it would make sense to track clinical parameters on those cases to see if you can detect early changes. I think the problem here is nomenclature.

A: Your points are well taken. We might be biting off more than we can chew. We can extend to the local hospitals to any one who has a need. I think you are right it may not be worth the investment.

Molecular Epidemiology Study

Q: The protocol states that the population will be stratified into 5 cells based upon benzene exposure. What is the rationale for the selection of these cutpoints of exposure? It may be more appropriate to specify the cutpoints after more is known about the distribution of exposures for the population.

A: The checkpoints were chosen with the Industrial Hygienists. I think this strategy was chosen to guard against going into a factory where we thought the exposures were high but actually were not to make certain we include enough high exposure cases. This will have to be done very carefully with attention to selection. The idea was to ensure that a range of exposure levels were included.

Q: Concerning the discussion of inclusion criteria, the investigators will select subjects based upon one year experience at a station covered by existing exposure data. Could this introduce a bias in selecting certain types of jobs over others? How have jobs been selected for monitoring? How might this selection process influence distribution of exposure to potential confounders over the selected jobs?

A: The database picks up any facility where benzene is present. Facilities that are not in the database are new and are not necessarily operating under the law. Some of the Regulatory staff are street savvy and are pretty certain what is going on and where.

We are not prohibited for sampling the phase 2B individuals for the peripheral blood effects early, except that we would have to sample blood twice, which would effect our cost.. We would have to consider how much redesign needed. The other issue is with coordination and recruitment of the individuals and the metabolism studies. Our goal was to come up with a reasonably constant paradigm as to when we do the bone marrow sampling. Bone marrow sampling requires we take the patient from a factory to a hospital for bone marrow sampling. We are hoping to be able to do it in such a way that the time interval between exposure and bone marrow samplings was relatively constant.

Comment: The second study (phase 2B) might also provide useful information on the validity of the first study (phase 2A). Rather than an expensive extra step this might

be the best thing to call the patients who were positive on the first screen back on the day of the bone marrow exam and check their blood simultaneously.

A: That would add another dimension to the study. Again the cost impact is going to be very significant.

Comment: You might save money on the exams [not performed] on non-informative patients. You might not want to do it on all patients of phase 2B. Within that population of 200, you could focus on perhaps 40 patients who had positive fish samples.

A: I agree. If we get a cytopenia we would want to enter that candidate into Disease Progressions study. If we find someone who meets any criteria for bone marrow suppresion we are reporting them. We may be able to monitor our cost if we modify the number of bone marrows that we do.

Comments from Dr. Nancy Mueller

Case-Control Study

Identification of AML/NHL Cases and Matched controls

Q: Subjects will be identified quickly and a large network has been developed to capture cases for the NHL Case-Control study. Has this network been checked for completeness of ascertainment compared to the cancer registry?

A: This project will be out in front of the cancer registry with respect to the diseases being studied.

Comment: It may be useful to look back 3-4 years in the cancer registry and note whether all the cases reported were captured in your network of hospitals. This could be a quick check to see what proportion cases in the cancer registry came from each reporting hospital versus the proportion of cases from each institution in your network. You need do make sure you have a good capture ratio.

A: A good suggestion, but the patterns will be changing over time. Although hospitals are required to report cases, they are given an infinite amount of time to do so. The reliability of the cancer registry may still be suspect even after 3 years.

Q: Where are the case and control interviews to be held?

A: All interviews will be held in the hospitals.

Q: In the US, most lymphoma patients are seen on an outpatient basis. Will these cases be followed home for an interview?

A: No, they will be identified through an initial morphological triage, then asked to return to the hospital for treatment and administration of questionnaire. Our follow-up should be as good as that of the clinicians. Obviously the surgical cases will all be in the hospital.

Q: Regarding the timetable, it may take longer to get enough cases.

A: What we have presented is our best estimate.

Q: What is your expected participation rate?
A: We expected around 90%, which would be overly optimistic if the study were done in the US or Europe. A better rate can be expected in China
Comment (Dr. Li): I agree with that characterization. In similar studies in China, participation rates are around 80-90%.

Questionnaire and Accounting for Confounding Factors
Comment: A criticism of the NCI study was that they had good documentation on benzene exposure but not on other potential disease factors (e.g., other chemicals).
A: That is understandable because it would be difficult to do in a cohort study. The reason we chose a case control study approach was to allow collection of information on confounders. The questionnaire will have yes/no questions regarding exposure to other chemicals, (as noted in the protocol) but there will not be any estimates of exposure to these other chemicals.

Another resource is the SMIPHS database which is populated with data for some other known chemical toxicants in the Shanghai area. However, it will not be enough to account for all chemicals in all industries and for all work histories. It will be informative about the nature and magnitude of some of these exposures.

[Request of Dr. Mueller to provide the reference on height and BMI in respect to NHL. It will be added to the questionnaire if it is deemed important.]
Comment: Transfusion history would also be useful information. There is an excellent opportunity to be comprehensive in this type study.

Informed Consent
Comment: The consent statements concerning benzene exposure should be the same for cases and controls. Having two wordings may differently influence the accuracy of response – over and above potential recall bias.
A: Originally, there was only one consent form but the Ethics Review Panel (ERP) requested that separate forms be developed for cases and controls. Other than a couple of sentences, the wording is identical between forms.
Conclusion: This needs to be referred back to the ERP for clarification as to whether benzene needs to be specifically mentioned, or if "chemical exposure" might be a useful surrogate term that is less leading.

Disease Progression Study

Specific Aims
Comment: It would be useful to clarify for each aim the number of subjects, length of follow-up if any, and the statistical approach. It may be useful to layout a grid and fill it with numbers needed for each aim. It is a complex study that will try to reconstruct retrospectively what might have been a preceding condition. You will be in a position to follow-up with cases and do a case-case comparison. I am also worried about the power of your study. You should pick-up strong effects but be limited in others.
A: This is a concern because many of these diseases are extremely rare and many markers are rarer still.

Q: Partial follow-up may be helpful. Will you be able to look at prior medical records?
A: Yes, at the clinical level but there is no guarantee beyond the clinic. Some work places have their own clinics and this may be another source of information.

Strategy for Matching Controls to Cases
Comment: This relates to cases that come in from other sources (from outside the province)
A: We will not do any matching of controls for those cases, nor would any exposure estimates be done.
Comment: This is okay and will still give valuable information

Selection of Controls
Comment: Withdraw this comment

Clinical Assessment
Comment: HBV is not an accepted risk factor of NHL. What is established is that HCV is a possible risk factor, and both HIV and EBV (for some subtypes) is established. HIV screening is always a problem but you should determine for yourselves that the prevalence – at least in the cases is low enough that you can ignore. You should do that blindly on batched samples from your first 100 cases.
A: FDA frequently requires HBV in clinical trials and it was included here for that reason. We would be in favor of eliminating it in favor of HHV8.
Comment: HCV,HIV and EBV should be included. HTLV should also not show any relation, and I do not recommend doing HPV.
Note: HPV will be removed and replaced by HHV8.

Analysis of Confounders
Comment: You may also be interested in the effect of the modifying factors. The prevalence of infection with EBV will be universally high and is not worth measuring itself serologically. Basically, you need to control for modifiers, such as gender, but this is a small point.

Statistical Analysis
Comment: The case series can be analyzed quite validly as case control data. Matching may not affect your risk estimate and you may want to go to an unconditional analysis stratifying for major matching factors. Look at both the matched and unmatched risk estimate and if they are essentially the same you can do a stratified analysis.

Inclusion Criteria
Comment: Suggest you use 18 years of age as minimum – to be consistent with the larger case control study and to avoid to problem of getting parental approval.
A: This has been done

Q: What about the upper limit? Why choose 75?

A: The upper age limit may be somewhat arbitrary. CLL and small non-cleared lymphomas (CD-5t) are age related diseases in the US but are believed to be rare in China. This may be an artifact of how these diseases were diagnosed in the past in China.

Conclusion: Do not apply an upper age limit.

General Considerations on NHL

Comment: For testing of tumor for EBV, you also should doLMP-1. HTLV-I is rarely positive for cutaneous T-cell and China has extremely low prevalence of the infection. I'd only test any ATLL. I wouldn't screen for HBV, HCV is non-integrating – you would instead test sera. Hodgkins Lymphoma will also be differentiated. Only about 10-15% of NHL tumors will be EBV positive.

A: This could also be looked at retrospectively.

Q: Is CLL included as an NHL? Are these all lympho proliferative disorders?

A: Following the WHO guidelines all lymphoid leukemia will be classified as NHL including Multiple Myeloma. Hodgkins will be excluded from the study but will be identified and diagnosed in order to differentiate it from other disease states.

Q: There is an overlap between Hodgkins disease and large B–cell lymphomas that are hard to distinguish. Wouldn't it be easier to include them than to draw a vague line between them? Do we know that Hodgkins disease is not benzene-related?

A: There is no evidence to support it [Hodgkins disease] but will need input from colleagues on the ramifications of including Hodgkins Lymphomas. On differentiatal diagnosis, every case will be reviewed by Dr. Ryder at USHSC. The expert work group panel of pathologists will review difficult cases or discrepancies in diagnosis.

NOTE: from a practical standpoint, the only limitation to including Hodgkin Disease cases is the added cost of providing controls and exposure analysis. This would probably escalate the cost increase estimated above by about $60,000.

Molecular Epidemiology Study

Polymorphins and Susceptibility to Benzene Toxicity

Q: Regarding GST-T1, negative studies were validly designed and executed, you would have strong evidence that the gene is not going to be an important marker.

A: The field of polymorphisms in relation to benzene is not well developed. This line of research is desired by the study sponsors but may not bear much fruit. The analysis in this study is limited but the development of a data set may allow for outside funding of satellite studies.

Note: The criticism is well taken, we have solid epidemiologic data to rule out a role for GST-T1 and it should be eliminated from the protocol.

Experimental Design

Q: Power is an issue in the design. In the protocol it mentions "200 exposed workers will be selected." What will be the criteria to determine that?

A: As mentioned in the protocols, there will be over-sampling in the exposure group.

Comments from Dr. Richard Albertini

General Comments

- Potentially rich source of information from these people with multiple endpoints that are becoming critical for early warning of disease and mechanisms of disease.
- Encouraged by the fact that most material will be cryo-preserved and should include red blood cells.
- Could become an international resource in the future, with additional funding, for looking at other endpoints.
- Cost does enter as a factor in what can be done in the present studies; however, cryo-preservation does ameliorate this somewhat.

Disease Progression Study

Comment: Phase 1, Phase 2a & Phase 2b: the effect biomarker will be FISH analysis for clonal expansions in blood or bone marrow. The research questions are:

1. What are the sensitivities specific to changes in bone marrow cells in non-disease patients and what is the dose-response?
2. How does the comparison of the sensitivity relate to the sensitivity in peripheral blood lymphocytes?

Suggestion for a conditional approach: If FISH changes in blood are not to be used to set up phase 2b, then what if you reverse these and do the 200 subjects looking at FISH changes in blood and bone marrow? You then get one of four scenarios;

1. Nothing or very little in either blood or bone marrow, which makes the question in 2a go away.
2. A lot in bone marrow and nothing blood which answers your bone marrow question but makes 2a another question.
3. A lot in both, or
4. More in blood, then 2a becomes important.

This could focus the question in 2A better from results in 2B, which would improve your power calculations. If 2A becomes irrelevant, there would be substantial cost savings to the study, or do work on the cryo-preserved samples. These comments are based on results from other studies on peripheral blood lymphocytes using FISH or RT-PCR which have been very disappointing in finding anything. Most have shown the greatest effect related to age where time has allowed for clonal expansion to express themselves.

A: Another issue that is not addressed in that suggestion is the analysis of cytopenias. (Phase 1 does not include any invasive procedures but only is used to identify factories and subject groups for inclusion in 2a & 2b.)

In vitro data we have reported is very provocative. There is a marked difference in the frequency of selective cytogenetic abnormalities following exposure to benzene metabolites between bone marrow and lymphocytes.

Comment: You are already applying an iterative approach to exposure assessment, you could do the same here with molecular analysis. Benzene poisoning cases are also a valuable resource and cryopreserving is a important step for all cases with high benzene exposure to allow a later look for early biomarkers. If you do 200-300 people on 2b first and found no translocations in the peripheral blood, you may not want to do another 1000 with no effect in 2a.

A: There may be ramifications to issues of recruitment. (The volume of bone marrow being collected is less than 10ml., maybe 20ml for BP cases.)

Comment:Suggestion to look for translocations first. There also may be age effects you'll want to stratify for, which will affect your power calculations. Glycophorin is a marker for genotoxicity in marrow.

Conclusion: Suggest having off-line discussion on protocol and recruiting issues raised.

Comments from Dr. Guilan Li

Comment: Concern with case-control study, that most workers exposed to benzene at high levels, only do so for short durations.

A: We do not know what the exposure pattern will be at this point in time. There may be some with lower-level exposure for longer duration.

Q: New cases may mostly be associated with the high-exposure, short duration scenario. Not many cases may be coming from the lower-level exposure group.

A: We should be able to capture both with our prospective study design. The development of the lab in Shanghai allows for a more rigorous study that could not be done retrospectively. The suggestion in your comments to include a retrospective component to increase the number of cases could not be done with the same level of diagnostic rigor. The diagnostic criteria between how things were done previously in Shanghai and within this study are not compatible and could not be compared. The study should be able to capture cases from a wide variety of exposures. In the US, such a design would be expected to capture AML cases related to longer-term, moderate-level exposures and we expect to be able to do the same in China. The comment to increase sample size by including retrospective cases is well taken, but the over-riding issue is diagnostic accuracy.

Comment: Suggest you should document in the protocols that the addition of retrospective cases was not pursued and explain why.

Q: Also a concern with collecting bone marrow from healthy workers may be difficult and that workers may be alienated by the invasive procedure. That may result in low participation. Another concern with ethical issues, perception of the use of Chinese population as an experimental animal.

A: Based on these concerns and on the other comments today, we should oversample the exposed population and undersample the unexposed population. From Dr. Xu's perspective (Chinese bioethicist), the participation of workers in this study will contribute to the greater good of all workers in China and is, therefore, well ground from an ethical standpoint. There is more concern from the regulatory bodies

(SMIPHS) in getting an adequate number of subjects than on ethical issues related to sampling.

Comment: The differences between this study and other Chinese experience is likely due to the present study being conducted in hospitals versus in the field.

Q: How do you ensure quality control on the bone marrow collection?

A: All bone marrow samples will be drawn by the same study coordinators at the Hua Shan Hospital. Sample preparation and bone marrow aspirate will be processed by the same team.

Q: Will any individuals be used as quality control subjects with multiple bone marrow drawings to ensure repeatability?

A: We will not sample bone marrow more than once from any individual, except in clinical follow-ups. The inter-individual variation is too great for this approach to have much value.

Comments of Dr. Richard Larson

Q: Concern with inclusion and exclusion criteria for cases and controls. Mild cases, cases of MDS and/or identified in the central lab, will they all become cases in the case-control study or only those that meet the 20% blast rule to meet the definition of AML.

A: By using them all, we can segregate in the analysis later.

Comment: This would include refractory anemia with excess blasts; refractory anemia with multi-lineage dysplasia; refractory cytopenia with multi-lineage dysplasia; and other advanced subsets of MDS which are clearly in evolution to an acute myeloid leukemia. This is also important if cytogenetics will also be done on these materials.

Q: Will cases of aplastic anemia be included in the case-control study?

A: Not in the case-control study of AML and NHL. In the Disease Progression Study, the aim is to follow progression to other disease states and aplastic anemia cases would be included. All cases meeting the legal definition of benzene poisoning will be included, but that will not be the diagnosis. They will be diagnosed based on the clinical analysis. Controls will be matched following the diagnosis only. If the benzene poisoning case is not an in-patient, they would not be matched with a control.

Q: Concern with finding patients on the disease progression study that are at the terminal end of the disease state, e.g., AML. There is also not a follow-up for survival. Two projects in the Benzene Poisoning Disease Progression Study: 1) Characterization of AML at presentation (these are also subjects on the Case-Control Study); and 2) a benzene poisoning study of other pre-leukemia states.

A: By definition, AML is the terminal end point. However, there will be follow-up as far as the clinical database. It will be possible for the Chinese to also collect treatment data, which can be used in later analysis outside this study.

Q: If benzene-related leukemia is anything like therapy-related leukemia in North America, perhaps only 5% of AML cases will be benzene-related. Therefore, 95% of what you see will not be informative to your study. Is there a reason for excluding a priori people with a heritable condition?

A: There are two: 1) presumptively, these would not be related to benzene exposure, and 2) it is also a cost issue that the system would be overwhelmed by the numbers. However, if they also have AML or other diseases, they would still be included (this may need to corrected in the protocols to clarify this point). What is intended is to exclude anemias with these etiologies but not if they present one of the subject diseases also. Cancer that has metastasized to bone marrow will also be excluded.

Comments of Dr. Mark Minden

Dr. Minden was not present at the meeting, but it was determined that discussion of some of the issues he raised in his comments would be beneficial with the panel members present. Not all of the issues raised by Dr. Minden were addressed in this discussion. Summaries of a comment were first stated, then discussed.

Summary: Dr. Minden proposes us of HUMARA as an indicator of clonality independent of the markers for FISH. This raises the concern that the interpretation of what is and is not clonal in benzene poisoning may be controversial without verification in FISH. Would the other reviewers comment or provide feedback.

Comment:Gary Gilliland had some data about patients recovering following autologous bone marrow transplant suggesting there was skewing of hematopoiesis and he used the HUMARA assay. It looked interesting at the time but have not heard any follow-up. He may have run-up on some of the same limitations mentioned, and he may be a good resource to consult. This may be a good objective to add to the study: to see if HUMARA correlates at all with the selection of a clonal stem cell population following exposure to a hematotoxin like benzene.

A: This also becomes a cost issue. We would have to do lymphoid lyphocyte controls in every case.

Summary: Suggestion to detect Flt-3 and MLL duplications, which should be easy to add.

A: There may be a misunderstanding with respect to t(15;17) in that we don't believe it is completely secondary. It is included in the panel because it has been hypothesized [as being associated].

Note: we propose to add FLT-3. MLL is already in the protocol. The cost impact will be investigated.

Comment: Bimolane should be included as a confounder. It is a psoriasis drug that was not used in North America, but has been associated with therapy-related AML in China and Great Britain.

A: Good suggestion.

Comment:You may want to include an LDH as part of the hematologic work-up and 2-microglobulin as regards the lympho-proliterative disorders. They figure into staging classifications for lymphomas.

Note: LDH will be added.

Summary: Issue of bone marrow sampling, the collection of a core sample and aspirate from the same puncture site.

A: Based on previous discussion today, we will likely be collecting from separate sites. If we have unsuccessful aspirate, we will likely take two cores to allow adequate material collection.

Comment: It may be useful to document some of the discussions regarding sample collection in the protocol.

RESPONSES TO COMMENTS NOT ADDRESSED DURING THE PROTOCOL REVIEW MEETING

Several reviewers were not able to attend the meeting held in Chicago on September 27, 2001. The investigators provided written responses to those comments following the meeting, which have been incorporated into this report. Those comments that were responded to during the course of the meeting are not repeated here, nor are comments relating to typographical or editorial changes.

Replies to Comments from Dr. Rockette

Case-Control Study

Q. The difference in terminology (AML and ANLL) for the two studies is confusing and should be made consistent.

A. AML and ANLL are used almost interchangeably in the literature. We started with AML, but, based on Dr. Brody's (the bioethicist) recommendation, we changed it to ANLL, because ANLL was the term used in the NCI-CAPM investigation. We will use the same terminology in our case-control studies as the one used by Richard Irons in his disease progression study.

Q. Given that one of the criticisms of the CAPN-NCI study is the inadequacy of the exposure assessment, is there any information on the completeness and adequacy of such information in the proposed study, i.e. given 50 randomly selected individuals from hospital records, for how many can adequate exposure histories be obtained?

A. The exposure database at Shanghai Center for Disease Prevention and Control (CDPC) is very comprehensive and complete. One of the major differences between our case-control studies and the NCI-CAPM cohort study is that we can spend more time and effort on exposure characterization per subject, because of the much smaller sample size. In addition, we will use an individualized approach. That is, unlike the NCI-CAPM study, we will not be using broad categories or general models of exposure classification.

Q. The sample size estimates given for the case control study should include the percentage assumed to be exposed. Not only does the statistical power depend on this number but the estimated percentage exposed is of some interest since in doing a hospital-based case-control study of an occupational exposure, there is always a concern as to whether there are adequate numbers of individuals in the occupational groups that are included in the study.

A. The prevalence of benzene exposure in residents in Shanghai was based on previous studies published in Chinese occupational medical journals (including some papers by Dr. Lin who is an hematologist and one of our collaborators in Shanghai, which were cited in our protocol). The reported prevalence ranged from 5% to 10%. Based on this prevalence range, a sample size of approximately 500 cases will have adequate power (a=0.05 and b=0.20) to detect a minimum relative risk between 1.5 and 2.0.

Replies to Comment's from Dr. Checkoway

Case-Control Study

Q. There are, however, some limitations to the proposal that will need to be addressed. First, there should be a stronger justification for a hospital-based case-control study, other than this design would be the most convenient way to identify large case and control groups. In particular, it is not at all clear that past exposures to benzene would be sufficiently common in a sample of cases and controls drawn from hospitals to ensure reasonable statistical power to detect an association. The investigators mention that preliminary pilot studies have been conducted in Shanghai, yet they offer no estimates of the expected frequency of benzene exposure. The statistical power for specific disease subtypes (e.g., NHL categories) may be very low.

A. Please see Section 2.5 on page 7 of the draft Case-Control Study protocol and the reply to Dr. Rockette's third comment, above. The statistical power for specific disease subtypes may be much lower. There is not much that can be done about it, unless we want to extend (in time) or expand (in geographical locations) the investigation.

Q. Hospital-based studies of occupational cancers are typically poorly suited for estimating associations with specific chemical or physical agents, let alone quantification of dose-response gradients. Even the Montreal study conducted by Siemiatycki and colleagues (J. Siemiatycki, "Risk Factors for Cancer in the Workplace", CRC Press, 1991), which is widely considered the most thorough of such epidemiologic studies, has not been successful at quantifying agent-specific risks. The situation in Shanghai is no doubt more advantageous than most for conducting a hospital-based study because of access to historical data from the SMCDC. However, the SMCDC data will undoubtedly have gaps, especially for early years (pre-1980) which are probably most relevant etiologically. In general, the plans for exposure assessment are presented in a very perfunctory manner. Perhaps the detailed methods outlined for the Disease Progression study will be followed, although this is not stated explicitly.

A. We agree with Dr. Checkoway that in general exposure information in population-based case-control studies conducted in the US or other western countries is not very reliable or specific. In China (Shanghai in particular), we have a very unique advantage. Through Shanghai CDPC, we will have access to records at factories where the cases and controls worked (employment records, industrial hygiene data and other relevant documents) as well as the large database of industrial hygiene measurements maintained at Shanghai CDPC. In essence, our proposed hospital-based case-control studies in Shanghai are equivalent to nested cohort-based case-control studies in the US. Dr. Checkoway is right concerning the potential difference in the exposure assessment approaches between the studies. After the review meeting in Chicago, we have decided to make a substantial commitment to harmonize our exposure assessment approach with that used in the disease progression study.

Q. The selection of controls deserves more careful consideration. The plan is to select hospital controls free of the index or related diseases, which is clearly logical.

However, potentially eligible controls might be over-represented with some conditions that may be positively or negatively associated with exposure potential. For example, patients with diabetes or chronic cardiovascular diseases may not have been permitted to work in certain industries, which could bias the study results.

A. Dr. Checkoway is theoretically correct in that certain diseases may potentially bias the selection of controls in a hospital-based case-control study. However, we don't have any evidence that employment in some specific industries (particularly those with potential benzene exposure) is related to diabetes or chronic cardiovascular diseases. Furthermore, controls will come from a variety of diagnostic categories, which will minimize such potential bias. Nevertheless, we welcome any suggestions that Dr. Checkoway may have to guard against such potential bias in the selection of controls.

Q. Various logistical aspects of study conduct need to be explained in more detail. In particular, it is not stated where subject interviews will take place or who will conduct interviews. Will proxy respondents be approached for subjects who are too ill or are otherwise incapable of answering questions? A sample of the questionnaire should have been provided, and ideally results of pilot testing of the questionnaire should be available (these data would also give some indication of the frequency of employment in benzene-exposed jobs).

A. We agree with Dr. Checkoway that the various logistical aspects of the studies have not been worked out in detail. Hopefully, we will work out the details in the next few months. In particular, we will pre-test the questionnaire in a small number of patients, as suggested by Dr. Checkoway.

Q. The plans for data analysis seem generic—more specification of how exposures and potential confounders will be handled in the various statistical models should be given.

A. We agree with Dr. Checkoway that our proposed analyses are "generic," if by that he meant "traditional" or "commonly accepted." In our protocol, we have listed the basic analyses that we anticipate to run at this time. Undoubtedly we will propose some additional analyses once we have developed and familiarized ourselves with the exposure data. We are sure that members of the Scientific Review Panel will be able to make some suggestions to us when the time comes.

Replies to Comments from Dr. Minden

Case-Control Study

Q. 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 2-3 times 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?

A. Yes, the incidence of leukemia in China is much lower than that in North America. All cases for the CC and DP studies will be hospital based (exception will be out of area BP cases in the DP study which not be controlled but collected as a case series). The incidence of AML is about 2-3 times less than an American city, AA's are about 5 times higher. We have 15 of the 16 major referral hospitals signed to contracts to use the lab as a clinical resource and to recruit cases to our studies. To date, we have added 2 central district hospitals (of a total of 23) that typically see outpatients, referring serious hematology cases to the 16 referral hospitals. We are attempting to enlarge Central District Hospital participation to capture more outpatient cases. For serious cases we have been told to expect 80-90% case participation.

Q. Will cooking style, etc be included in the history?
A. No, we don't plan to include cooking style in the questionnaire for the Case-Control Study. For the DP study we are amenable to collecting information on diet if someone can clearly enunciate what we should be looking for. Is there a connection between cooking style and ANLL or NHL?

Q. 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.
A. When we first develop the questionnaire, our Chinese colleagues told us not to include a question on hepatitis because some may consider the question sensitive. However, we do see the scientific value of such a question. We will discuss this issue with Dr. Xu (the Chinese member of our Ethics Review Panel). If Dr. Xu agrees, we will add the question. Subjects evaluated by the laboratory will be routinely tested for HBV.

Disease Progression Study

Q. 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.
A. The language of informed consent forms is being tailored to meet the requirements and suggestions of Chinese ethicists and physicians. These issues have been the subject of discussion by the ethics panel and the Shanghai medical community. They will also be reviewed by IRB's in the US and China. At this point in time our Chinese colleagues have indicated that individual molecular tests do not have to be

individually approved in China as long as the study goals and objectives are clearly outlined. Studies that are not part of the diagnostic paradigm are listed in the informed consent

Q. 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.

A. A 20 ml blood draw is only indicated for BP (DP) or Molecular Epidemiology subjects. All others are 10 ml. This information is already stipulated in the informed consent forms. We do not believe a table is necessary.

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

A. We do not understand this question. Mild case monitoring, which has now been eliminated from the study design, called for a complete CBC. All other sampling paradigms in CC and DP call for CBC, bone marrow core biopsy and aspirate or tissue biopsy (NHL). Sampling paradigms for workers in the ME study call for CBC at a minimum. Cell lines will be established from tumor cells or EBV infected peripheral lymphocytes.

Q. 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.

A. The clinical database must maintain full patient identifiers in order to provide continuing diagnostic support for referring physicians. Normal clinical laboratory procedures call for maintaining patient information in a confidential manner but do not require blinding patient identity which could be very hazardous. On the otherhand the study databases will use identifier numbers cleansed of identifying personal information.

Q. 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.

A. We do not understand this question. APL t(15;17) has been found to occur as a *de novo* disease and in a subset subjects previously treated with topoisomerase inhibitors. We have no intention of classifying cases presenting with t(15;17), *apriori* as secondary cases. The WHO classification requires all of the items listed as records, and, independently these items are all included in our protocols as described.

Q. 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.

A. Secondary AML's have traditionally demonstrated a high prevalence for deletions of chromosomes 5 and or 7 (65-90%). Translocations involving chromosome 8 are equally represented in both de novo and secondary AML's (8-12%). The only study demonstrating an increased prevalence of numerical changes in 8 in benzene workers is the Smith study, which also indicates an extremely high background or numerical changes in 8 in unexposed controls.

We have no reason to assume t(8;21) is any more indicative of one or the other.

We are functioning as both a clinical and a research laboratory. We will routinely evaluate clonal cytogenetic abnormalities using banded chromosome analysis. We will also employ either a standard leukemia or lymphoma panel of FISH probes, or in the ME study, an abbreviated panel of FISH probes commonly found in secondary MDS/AML cases. We will add Flt-3 to the panel. We have SKY available but do not currently have plans to employ it routinely. SKY on normal karyotypes will have low yield. Usually, the problem with normal metaphase chromosomes is that the normal cells are proliferating and are scored, while the leukemic cells are not dividing, or their morphology is too poor attempt analysis (SKY would have limited use here, and the results would not be clear cut.) Also, this would be prohibitively expensive, and is not currently budgeted.

The workscope of this study utilizes cytogenetics and molecular genetics to characterize and detect clonal lesions in AA, MDS, AML, BP cases and benzene exposed workers in the ME study. We will routinely use criteria for analysis that are standard of practice in clinical cytogenetics laboratories in the US. These routinely exceed the methodologic rigor typically employed in previous epidemiology studies. We do not intend to perform analyses of non-specific chromosome aberrations (SCE, aberration frequency, etc.) which are beyond the scope of our budgetary constraints.

Molecular Epidemiology Study

Q. 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.

A. The charge of the sponsors for the design of this study was to focus specifically on molecular and genetic endpoints that are known or thought to be involved in the pathogenesis of the specific diseases in question and not to evaluate non-specific biomarkers of exposure or effect. The rationale for this is one of prioritization and is primarily governed by budgetary considerations.

The polymorphism studies outlined in the proposal are initially intended to provide a minimal examination of susceptibility genes. It is our opinion that the literature in this area is insufficient to invest in a major evaluation of genetic polymophisms at the outset. We recognize that previous data on GST-T1 provides a reasonably sound rationale for not including it in this study. Therefore, we intend to eliminate from our initial analyses. Similarly, studies on the phenotype of CYP2E1 can be criticized for being marginally significant in previous studies and, in any case, not specific for CYP 2E1. Therefore, we are considering eliminating chlorozoxazone hydroxylation, which presents strategic difficulties in implementation in the field as well. At present, NQO1 oxido-reductase appears to be the most important polymorphism to study. Our primary goal is to capture material that can be used to support a more cogent polymorphism study at a later date.

We have serious reservations about routinely measuring HUMARA in female patients. First of all, we do not believe that general clonality in bone marrow of female patients will be specific enough to describe a neoplastic process. Because of issues related to heterozygosity resulting from discontinuities in clonal succession in hematopoietic stem cells, the actual distribution of heterogeneity in HUMARA that corresponds to an abnormal result appears to us to be controversial. From this standpoint, we are afraid it would probably be less sensitive than specific clonal lesions identifiable by FISH. If the purpose is to determine what level of HUMARA heterogeneity actually does correspond to a clonal abnormality it would be beyond the scope of this study, and we would have to seek outside funding.

We are collecting DNA as well as cryopreserving cells. We will also cryopreserve erythrocytes for potential analysis of glycophorin A mutations at sometime in the future. The same can be said for changes in methylation or mismatched repair genes which are interesting questions but for which no strong rationale can be made up front for their involvement in benzene-induced leukemogenesis.

Q. 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?

A. In the modern era one has ever characterized the cytogenetic abnormalities in an

unequivocal benzene-induced leukemia case so we don't know. Hopefully, we can address this question.

Q. 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.

A. The cell culture experiments referred to are part of a funded ongoing NIH leukogenesis grant which has been independently reviewed and therefore was not described in detail. Human bone marrow cells exposed to alkylating chemotherapeutic agents typically exhibit "clonal hypersensitivity" to GM-CSF. [Irons, R.D.et al (1992) PNAS 89:3691-3695; Irons, R.D. and Stillman, W.S. (1993) Stem Cells, 11:235-242; Irons, R.D. and Stillman, W.S. (1996) European J. Haematol., 57: 119-124; Irons, R.D. and Stillman, W.S. (1996) Environmental Health Perspectives, 104 (6) :1247-1250.]

Q. 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.

A. HPRT is not known to be a specific benzene-induced lesion nor one demonstrated to be associated with the pathogenesis of the diseases under study. Therefore, these experiments are beyond the scope of the present study.

Q. 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?

A. We do not know what a q3mon CBC is.

To: George Woodall <Woodallg@api.org>
From: Dr. Baruch Brody <bbrody@bcm.tmc.edu>
Cc: 'BenzConsort-CC@listserve.api.org' <BenzConsort-CC@listserve.api.org>; Patrick Beatty (E-mail) <PWBE@chevrontexaco.com>; Donald Burnett (E-mail) <burnetdm@bp.com>; Patsy Clegg (E-mail) <pmclegg@shellus.com>; Stuart Cagen Ph.D. (E-mail) <szcagen@shellus.com>
Bcc:
Received Date: 2001-11-27 04:27:02 GMT
Subject: Re: SRP Meeting Summary

George

Here are my comments:

p.2-- it needs to be clarified that the communicatioon committee has no control over the content of the scientific data and its release at the end of the project

p. 3- we still need to clarify (see below) the final membership of the ERP, its responsibilities, and the nature of its interface with the SRP

p.5-- there is a need for clarification that the lab will remain in place after the study is completed with some limited support

p. 15--the pilot is okay but it will need its own consent form clarifying its special purpose

p. 16-- the agreement on HIV is as follows: (a) the HIV information comes from clinical testing, not from the research questionnaire; (b) the resulting information will appear in the research data base without identifiers; (c) all this will be explained in the consent form. I don't myself see how that gives us HIV information about the case controls, unless they are being tested clinically for other reasons.

p.20- I'm not troubled because the clinical significance of being a mild case is unclear. But we never discussed whether or not they would be given the information.

p.23-- we definitely need separate forms for reasons given. I would prefer the more specific word 'benzene' because it is more infdormative

p.27--response needs to further emphasize that this study meetsShanghai public health need

p. 31-yes

p. 34- good point. The ERB has nevber seen the full set of revcised consent forms and needs to see them.

p. 35--on sample identifiers is correct

BARUCH

P.S. We do need to talk about the issues raised in connection with p 3 and the ERB needs to see the full set of consents.

At 09:37 AM 11/12/01 -0500, George Woodall wrote:

Dr. Brody,

I apologize for not getting this to you sooner. Attached is the draft summary of the Scientific Review Panel meeting that took place on September 27. I have highlighted in yellow those items that deal with ethical issues. A few of those highlighted sections have questions for the Ethics Review Panel (ERP) to discuss and respond to. I do not have an e-mail address for Dr. Xu, and would very much appreciate if you could send me that e-mail address if you have it, then forward this to him. If you do not have it, I will try to get it through Otto or Rich. If you could review and respond to the issues raised by November 27 I should be able to incorporate them into the final Consensus Report that I hope to have drafted by November 30. If there are issues that need to be discussed via conference call with the investigators, I can set up the call. If you would rather communicate with them via e-mail, please do so but please cc: me on those exchanges so I can keep the discussions open and a part of the official record.

We should also discuss having a meeting or conference call of the ERP, and work toward completing the panel. My understanding from the last communications we had were that we wanted to get a European representative, or at least a U.S.-based person that had dealings in Europe/globally. I have also attached the last e-mail I sent on some of these issues, since they have not changed appreciably.

Thank you,

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Message-ID:
From: George Woodall
To: "'Baruch Brody (E-mail)'"
Subject: Update on Benzene consortium
Date: Thu, 2 Aug 2001 09:36:35 -0500
MIME-Version: 1.0
X-Mailer: Internet Mail Service (5.5.2650.21)
Content-Type: multipart/alternative;
boundary="----_=_NextPart_003_01C16B87.850C0900"

Baruch,

I have been remiss in not informing you on the decision to go forward on the Benzene Health Research Project in China. I have been so buried in the details of getting the consortium committees up and running and scheduling a meeting of a Scientific Review on the protocols that I had not thought to contact you. Among the things that we will need to discuss sometime soon:

* Members of the Ethical Review Panel (ERP)
* Expectations for the ERP
* Scheduling an organizational meeting of the ERP

You can be brought up to speed fairly easily by visiting the consortium web site and reviewing the minutes from the Oversight Committee and Technical Committee meetings that took place July 26 and 23, respectively. The particulars for accessing the web site are as follows:

URL: http://www.api.org/benzene-consortium
Username: china

Password: bz2glue

Best Regards,
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