Executive Summary

Scientific Review Panel Protocol Review: Analysis Of Disease Progression For Aplastic Anemia, Myelodysplastic Syndrome, Acute Myelogenous Leukemia And Benzene Poisoning In Shanghai, China

The purpose of this document is to summarize the detailed discussions with the independent Scientific Review Panel (SRP) that have taken place regarding the subject study. There have been two reviews of this protocol leading up to submission for IRB review: one by the independent Ethical Review Panel (ERP) and a separate review by the SRP. The summary of the ERP review is provided in a separate report.

The review process began with the selection of the SRP membership; the roster for the SRP is attached. Next, the draft protocol was forwarded to the SRP for review and a meeting was scheduled for September 27, 2001. Following that meeting, the minutes of the meeting were developed, and reviewed for accuracy. Several revisions to the protocols were enacted, as suggested by the SRP. Below is a synopsis of the overall project description, the issues raised in the review and the disposition of the comments made by the reviewers.

Project Description

This study is a five-year descriptive analysis of cases of neoplastic and non-neoplastic diseases of the bone marrow, using a case-control design to perform a quantitative analysis of the effect of benzene on producing the diseases in question. Extensive morphological, cytogenetic and molecular analyses will determine the categories and subcategories of diseases to be related to benzene exposure. These analyses will be conducted at a joint clinical and molecular laboratory (JCML) that will be developed in Shanghai for this purpose.

The AA, MDS and BP patients will be followed at approximately six-month intervals with complete evaluations including laboratory analysis to identify current health status and progression of disease. Progression will be characterized as to rate of progression from these various sub-disease categories and clinical cytogenetic or molecular features of the progression. It will be determined if AML secondary to benzene exposure can develop in humans in the absence of preceding AA or MDS. This will also be determined for non-benzene related AML.

Where possible, unique features of benzene related diseases will be identified to distinguish benzene related from non-benzene related hematological disease. Prognosis for all of the diseases of concern will be defined according to cytogenetic and molecular subtypes and benzene exposure. In addition to serving the purposes of this study, the diagnostic capabilities of the JCML will be used to further clinical management of these patients.

Benzene exposure assessment for all study subjects will be made by retrospective analyses of work and other records. The diseases of interest will be characterized as benzene related or non-benzene related based on this exposure assessment. This will primarily be accomplished by the case control study design that is incorporated within and will serve as a foundation for the overall disease progression study. For this case control study, each hospital-based case of BP, AA, MDS and AML will be enrolled as

cases. Two hospital-based control individuals, matched for age and sex and other factors, will be enrolled for each study subject. Benzene exposure assessment will be as rigorous in the controls as in the cases.

Critique and Responses

A major concern expressed in both the discussions of the SRP and those of the ERP have been the possible perception that Chinese workers are being treated with less care than would be accorded to similar workers/subjects in clinical studies performed in the US. Additionally, there was concern in the collection of bone marrow aspirates from the controls, and that this may be difficult in the context of Chinese culture. Currently, the University of Colorado Health Sciences Center has several approved IRB protocols for doing bone marrow extractions from controls, including an NIH grant that has been funded for 9 years. Many similar studies are being performed by colleagues across the country that do likewise. In discussions with Chinese colleagues there has always been an open question as to whether we can successfully recruit large numbers of individuals to donate bone marrow.

Concern was raised over the possibility that the case-control studies might have underestimated the incidence of AML in China. However, Chinese hematology authorities report a higher incidence rate for AML than in the US, and that the expected accrual of 150 cases of AML per year is considered realistic by Shanghai hematologists.

In the original protocol, one control for each case was proposed. The revised protocol now matches two hospital-based controls for each study case. It was also clarified that benzene poisoning cases referred from outside the hospital study area will be treated as a separate case series; therefore, hospital-based matched controls will not be selected for these cases.

It is widely accepted that material collected from this study population will constitute a truly unique resource for the study of health effects of benzene as well as the pathogenesis of lympho-hematopoietic diseases. It is agreed that every effort should be made to save and preserve tissue material collected from these studies. In addition to blood, bone marrow, tumor cells, and peripheral lymphocytes, serum will be collected and frozen for future analysis for exposure to viruses that may be found to be important in the etiology of leukemia. These may include HHV6 and HHV8. It was clarified that all study cases will be screened for antibodies against HIV.

It was agreed that cytogenetics should be performed on bone marrow aspirate material and that the aspirate should NOT be obtained through the biopsy core hole but rather from a fresh puncture site. A core biopsy should only be used for cytogenetics if patients are inaspirable. Under routine circumstances both a bone marrow aspirate and a biopsy will be obtained via separate bore holes.

It was agreed that recruiting "mild cases" of cytopenias is potentially too monumental a task and that "mild" case recruitment should be limited to outpatients who present with formal criteria for diagnosis of AA or MDS. This also provides practical limitation on the potential ethical problem of following cases of "benzene poisoning" who continue to be exposed, because subjects diagnosed with AA or MDS who are found to work in a benzene-exposure environment will be reported to authorities, and according to Chinese regulations should be prevented from working in such an environement. A

number of other potential ethical issues were also raised that are under deliberation by the study ethics panel and were referred to that group for resolution and approval.

It was clarified that the laboratory will serve in a clinical as well as a research capacity. While follow-up of AA , BP and MDS cases will be conducted every 6 months as a part of the protocol design, complete bone marrow exams and cytgoenetic analysis will be performed at anytime there is a clinical change in a subject's condition (e.g. 3 months). In addition, AML cases will be followed for support of patient management and for documentation of clinical outcome and treatment survival. All subjects will be followed until death or the end of the study.

In addition to the specific genes outlined in the study, Flt-3 will be added to the secondary/treatment-related battery.

A great strength of the prospective case-control design is that it provides an opportunity to develop individualized exposure assessments. It was agreed to expand the discussion of the exposure assessment procedures to include strategies for searching employment records for information on employment, job title and job location, and that, if possible, this information should be obtained for all subjects including those potentially exposed to confounders. Also, a detailed discussion of strategies of for cases and controls who are not employed in factories will also be provided.

Certain issues were raised by review panel members that were viewed by the investigators as beyond the scope of the project. For example, while it is intended to use Spectral Karyotyping (SKY) in support of some cases, it is not planned to routinely use SKY on normal karyotypes because it would be expected to have a low yield, the results would not be clear cut in leukemic cells that are not dividing, and it would be prohibitively expensive. In addition, it is not planned to use HUMARA as a standard measure of clonality in female patients because it is not thought to be specific enough. Further, ascertaining what should be considered positive is controversial because of variability in clonal selection in normal bone marrow, and it is not clear would be gained in sensitivity over and above the FISH studies planned. Finally, it is not intended to follow the frequency of non-specific cytogenetic aberrations or mutations because they have been conducted before in benzene exposed populations, are expensive and provide no specific information on the pathogenesis of the diseases in question.

Conclusion

The protocol for this project has been developed sufficiently to proceed to IRB review. The unresolved issues will be revisited during annual updates of progress on the study by the PI to the SRP and the study sponsors.