PROTOCOL NO.: 02-113

Full Protocol

China Project: Metabolism Feasibility Study

Revision date March 29, 2002

I. Background

A research collaboration has been established between the University of Colorado Health Sciences Center, Denver, Colorado and Fudan University, the Shanghai Hematology Society, the Institute of Public Health Supervision, the Center for Disease Control and Prevention of the Shanghai Municipal Health Bureau, and 15 major Shanghai hospitals.

The results of these studies will provide a basis to answer the question of whether there is a practical threshold for the dose of benzene producing hematologic and molecular changes in bone marrow cells that are involved in the pathogenesis of AA, MDS or AML. Moreover, comparison of the molecular, cellular and cytogenetic events directly occurring in bone marrow with those observed in peripheral blood cells will provide validation of peripheral biomarkers of exposure and effect. Further, analysis of polymorphisms between benzene unexposed workers, benzene exposed workers with no bone marrow toxicity and benzene-exposed workers with bone marrow toxicity will serve to confirm and extend previous studies designed to identify susceptible populations. Finally, analysis of benzene metabolite concentrations in bone marrow will link in vivo benzene exposure with previously characterized in vitro effects of benzene metabolites on hematopoietic and lymphoid target cell populations.

II. Study Objectives

The objective of this study is to establish detection limits and limitations of measuring hydroquinone, catechol, s-phenylmercapturic acid, and trans, trans, muconic acid in blood and bone marrow. These procedures will then be exported to China for use in the Molecular Epidemiology of Benzene-Exposed Workers in Shanghai, China study.

III. Methods

A. Recruitment methods

Subjects who are needed to give bone marrow are recruited by a study coordinator assigned to our project by the University of Colorado Cancer Center Clinical Investigations Core (CIC). Subjects who are needed to donate blood are recruited by Wayne Stillman, who has 10 years of experience coordinating studies with human subjects and has completed COMIRB 101. COMIRB approved campus postings will be used to recruit normal donors as necessary.

B. Consent procedure

The subjects needed to give bone marrow are interviewed by telephone or in person by the CIC study coordinator to see if they meet study inclusion and exclusion criteria (see attached questionnaire). The subjects needed to give blood are interviewed in person by Wayne Stillman to see if they meet study inclusion and exclusion criteria (see attached questionnaire). For both blood and bone marrow, if all criteria are met, then the patient's

rights and the consent form are discussed with the subject, and a signature obtained. The subject is given a copy of the signed consent form. Both Mr. Stillman and the CIC coordinator have completed COMIRB 101.

C. Treatment, intervention or observation

Bone marrow is taken and the patient is observed to see if he/she is adequately recovered to leave the clinic. When blood is taken from normals the patient if observed to see that he/she is not disoriented or bleeding. The bone marrow donors are instructed to call Dr. Lori Maness if there are signs of infection or other concerns about the site(s) of donation as outlined on the consent forms. The blood donors are instructed to call Richard Irons Ph.D. if there are signs of infection or other concerns about the site(s) of donation as outlined on the consent forms.

D. Inclusion and exclusion criteria

Bone marrow and/or blood will be taken from healthy volunteers between the ages of 21 and 65. We use current blood bank exclusion criteria to identify individuals who may have a problem with the donation, are carriers of transmissible agents, or may not have normal bone marrow. People excluded as having a potential problem with the donation include subjects that are allergic to Xylocaine or have had a stroke, heart problems, hip surgery, diabetes, etc. Those subjects that have HIV, hepatitis, malaria, Lyme's disease, transfusions, etc. may be carriers of transmissible agents. Subjects that may have altered bone marrow cells includes those that have had or are taking drugs such as chemotherapeutic agents, growth hormones, glucocorticoids, etc. Additional exclusion criteria include pregnancy, an abnormal blood count, or the volunteer is feeling ill. No special classes of subjects will be used.

E. Selection of study population

The subjects will be taken, first come first serve. Children are not recruited to these studies because the methods developed in this study will be exclusively used on adult populations in China. There is no reason to expect differences between men, women, or ethnic groups. No one will be excluded on the basis of gender or ethnic group.

F. Patient accrual

We anticipate taking a total of 10 bone marrows and blood from up to 30 normal individuals for this study.

G. Estimated duration of study

Donating blood will take about 5 minutes, however, the entire visit will take 15-20 minutes. Donating bone marrow will take 10-15 minutes, however, the entire visit will take 1 hour. The entire study will take up to 2 years.

H. Examinations, laboratory tests, procedures, and follow-up visits

The subject will give blood by venipuncture and/or donate bone marrow. The pretesting of blood for normal bone marrow donation includes a complete blood count, a test for HIV, hepatitis B and C, and □-HCG on female donors to verify that they are not pregnant. The procedure for bone marrow donation is as follows: The posterior iliac crest area is first cleansed with Betadyne. The skin and subcutaneous area posterior to the iliac spine as well as the periosteum in this area is anesthetized using 1% Xylocaine. A number 11 needle is inserted into the marrow cavity in a standard fashion. Using forceful aspiration, 50-60 ml of bone marrow is removed into a heparinized syringe. Subjects who donate only blood will have it taken by venipuncture and their blood may be tested for HIV, and hepatitis B and C. The blood and bone marrow will be used in a series of experiments that will determine the sensitivity of Electron Capture Ionization-Gas Chromatography Mass Spectrometry (ECI-GCMS) and Liquid Chromatography Tandem Mass Spectrometry (LCMSMS) to detect hydroquinone, catechol, s-phenylmercapturic acid, and trans, trans, muconic acid in these samples. The experiments will attempt to maximize sensitivity by developing and optimizing procedures involved with tissue sampling and preservation, isolation and purification of the analytes, and the GCMS/LCMSMS analysis. Titered concentrations of the benzene metabolites listed above will be added as necessary in order to establish and validate the procedures under development.

I. Drugs (and doses), devices or instruments to be used - N.A.

J. Data analysis

1. There are no data (except gender, age, race, etc) being taken on the normal subjects since they supply the blood or bone marrow for study and are not the subject of the study. Significant differences between *in vitro* chemical treated and control groups of cells will be determined using appropriate statistical procedures (e.g. ANOVA, Students t test). Individual *in vitro* experiments will be internally controlled. Variability between and within individual experiments (including sample variation) will be analyzed by ANOVA. The data analysis will be performed by Dr. Mark Duncan.

2. If there is pain, symptoms of an infection, or there are concerns about the site of the needle sticks resulting from the blood or bone marrow donations in the days following these procedures, the subjects are asked to call Dr. Lori Maness. The subjects that donate blood only are asked to call Richard Irons Ph.D. if the above symptoms occur as a result of the blood donation.

3. Data will be analyzed following each donation/experiment, since this forms the basis for the experimental design of the subsequent experiments.

4. There will be no data safety monitoring board. Dr. Irons will conduct ongoing monitoring of this study.

5. The CIC clinical coordinator and Mr. Stillman will monitor study conduct.

6. The P.I. and laboratory personnel will treat the identity of the volunteer and all records with professional standards of confidentiality. Subjects and their blood/bone marrow samples will be identified by date and time of aspiration during the study. Although information relating to this study may be published in the scientific literature, the identity of the volunteer will not be revealed. Hard copy files will be kept under lock and key to prevent unauthorized access to study data. The computer database established to track subjects/results will be password protected to prevent unauthorized access to research information in the unlikely event of computer access by unauthorized parties.

K. Safety Management

If there is pain, symptoms of an infection, or there are concerns about the site of the needle sticks resulting from the blood or bone marrow donations in the days following these procedures, the subjects are asked to call Dr. Lori Maness. The subjects that donate blood only are asked to call Richard Irons Ph.D. if the above symptoms occur as a result of the blood donation. It is unlikely that there will be any adverse events. If so the study will be modified in order to prevent any further adverse events.

IV. Risks

A. Subjects

For the bone marrow donation the risks include the discomfort associated with the needle stick and there is the possibility of an allergic reaction to the anesthetic (risk of approximately 1 to 10,000), bleeding or an infection. These risks are extremely low. This study may involve unforeseeable risks.

B. Investigators/Institutions

There is a risk of laboratory personnel contracting a human pathogen from the donor.

V. Benefits

There are no benefits to the subject except normal subjects will receive $210 monetary compensation for bone marrow/blood donation and $15 for each blood donation.

VI. Funding

This project is funded by the Benzene Health Consortium, which is an international consortium for the study of the health effects of benzene in Shanghai, China. Funding members of this consortium includes: Exxon/Mobil Corporation, British Petroleum Corporation, Royal Dutch Shell Corporation, Chevron Corporation and Conoco. All research procedures are paid for and the subject is not responsible for any costs.

VII. Special consent issues

There is potential for coercion to participate among employees of the investigator. No employee will be contacted or asked to participate in the study. However, employees may participate in the research if the employee initiates participation.

Table 3. ANLL and NHL Case-control Studies
Budget Estimate forFudan University Medical Center

1/8/2001

Labor (time)

Category	Annual salary* in 2001 US$	Year 1 Time	Year 1 Amount	Year 2 Time	Year 2 Amount	Year 3 Time	Year 3 Amount	Year 4 Time	Year 4 Amount	Year 5 Time	Year 5 Amount	Ye Time
Physician epidemiologist (FU)	$16,900	20%	$3,380	0	$2,535	15%	$2,535	15%	$2,535	25%	$4,225	10%
Graduate research assistant 1	$6,240	50%	$3,120	1	$3,120	50%	$3,120	50%	$3,120	50%	$3,120	
Graduate research assistant 2	$6,240	50%	$3,120	1	$3,120	50%	$3,120	50%	$3,120	50%	$3,120	
Total for labor			$9,620		$8,775		$8,775		$8,775		$10,465	

[* includes salary, bonus and benefits.]

Expenses

	Unit cost in 2001 US$	Year 1 No.	Year 1 Amount	Year 2 No.	Year 2 Amount	Year 3 No.	Year 3 Amount	Year 4 No.	Year 4 Amount	Year 5 No.	Year 5 Amount	Ye No.
Fee for Shanghai CDC staff			$6,000		$6,000		$6,000		$6,000		$3,000	
Fee for AML patients	$30	60	$1,800	120	$3,600	120	$3,600	120	$3,600	60	$1,800	
Fee for NHL patients	$30	60	$1,800	120	$3,600	120	$3,600	120	$3,600	60	$1,800	
Fee for controls	$30	240	$7,200	480	$14,400	480	$14,400	480	$14,400	240	$7,200	
Long transportation			$300		$300		$300		$300		$300	
Copying			$200		$200		$200		$200		$200	
Postage and Express Mail			$300		$300		$300		$300		$300	
Total expenses			$17,600		$28,400		$28,400		$28,400		$14,600	
SUB TOTAL (IN 2001 US$)			$27,220		$37,175		$37,175		$37,175		$25,065	
Overhead Charges (15%)			$4,083		$5,576		$5,576		$5,576		$3,760	
GRAND TOTALS (IN 2001 US$)			**$31,303**		**$42,751**		**$42,751**		**$42,751**		**$28,825**	

Quarterly Break-outs	4	2001	$7,826	2001	$7,826
	1	2002	$7,826		
	2	2002	$7,826		
	3	2002	$7,826		
	4	2002	$10,688	2002	$34,165
	1	2003	$10,688		

Table 3. ANLL and NHL Case-control Studies
Budget Estimate forFudan University Medical Center

1/8/2001

ear 6

Amount	6-Year Total
$1,690	$16,901
	$15,602
	$15,602
$1,690	$48,100

ear 6

Amount	6-Year Total
	$27,000
	$14,820
	$14,820
	$59,280
	$1,500
$100	$1,100
$100	$1,600
$200	$117,600
$1,890	$165,700
$284	$24,855
$2,174	**$190,555**

2	2003	$10,688			
3	2003	$10,688			
4	2003	$10,688	2003	$42,751	
1	2004	$10,688			
2	2004	$10,688			
3	2004	$10,688			
4	2004	$10,688	2004	$42,751	
1	2005	$10,688			
2	2005	$10,688			
3	2005	$10,688			
4	2005	$7,206	2005	$39,270	
1	2006	$7,206			
2	2006	$7,206			
3	2006	$7,206			
4	2006	$2,174	2006	$23,792	
			Total	$190,555	$0