

UNOFFICIAL* PROGRESS REPORT
June, 2003

**I. ANALYSIS OF DISEASE PROGRESSION FOR APLASTIC ANEMIA,
MYELO-DYSPLASTIC SYNDROME, ACUTE MYELOGENOUS LEUKEMIA
AND BENZENE POISONING IN SHANGHAI, CHINA**

**II. MOLECULAR EPIDEMIOLOGY OF BENZENE-EXPOSED WORKERS IN
SHANGHAI, CHINA**

A MULTICENTER INTERNATIONAL STUDY

**Molecular Toxicology and Environmental Health Sciences Program
Dept. of Pharmaceutical Sciences, School of Pharmacy
Department of Pathology, School of Medicine
University of Colorado Health Sciences Center, Denver, CO.**

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**School of Public Health, Hua Shan Hospital, Cancer Hospital,
Fudan University Medical Center, Shanghai, China**

I INTRODUCTION

A. Executive Summary

1. Overall Status of Budget

*The University accounting office cannot provide “official” budget numbers until later in August after I return to China. Therefore, we are providing this as an “unofficial” summary in order to provide you with information in anticipation of the annual meeting.

Comparison of actuals to budget projections cited in this report is derived from the updated budget projections provided to API in September 2002. At the time of this writing JCML is transitioning from the preparatory phase to start-up of actual laboratory operations. During the first half of 2003, JCML was in the initial phase of this transition which resulted in numerous changes in the details of operation and management. As a consequence, over the first half of 2003 and continuing through the present, there is a great deal of cost shifting taking place from one budget category to another. Therefore, variances over the last 6 months between originally budgeted costs and expenditures for individual categories can be misleading and should not be presumed to represent changes in the overall budget. At the present, accurate numbers are the “project-to-date variances” which represent our “bottom line” status going into clinical operation.

The overall status of the budget for the first half of this calendar year is as follows: We spent \$1,721,162 as of June 30th compared to a projected budget of \$1,230,711, resulting in an apparent increase of \$490,451 in funds expensed during this period (Table 1). However, for the entire project to date, we carry forward a net surplus of \$878,245 compared to our total budgeted expenditures (Table 2). The negative variances for the first half of 2003 are more than accounted for by \$372,225 that was budgeted for EMBSI in 2002 but not scheduled for payment until 2003, and \$217,867 in equipment that was listed in the January 2003 report for 2002 but not paid until 2003.

Table 1. Budget Summary (Reporting Period)

	Budgeted ¹	Expenditures	Variance
Personnel	\$331,316	\$309,703	\$21,613
Operating Expenses	\$198,373	\$158,850	\$39,523
Subcontracts	\$549,014	\$845,860	(\$296,846)
Travel	\$11,340	\$9,339	\$2,001
Equipment	\$0	\$274,508	(\$274,508)
Indirect	\$140,668	\$122,902	\$17,766
TOTALS	\$1,230,711	\$1,721,162	(\$490,451)

¹The budgeted amounts are the sum of each category from Jan-03 through Jun-03.

Table 2. Budget Summary (Entire Project)

	Budgeted ²	Expenditures	Variance
Personnel	\$1,076,616	\$1,020,491	\$56,125
Operating Expenses	\$589,966	\$464,272	\$125,694
Subcontracts	\$2,467,673	\$1,871,232	\$596,441
Travel	\$35,287	\$17,986	\$17,301
Equipment	\$1,210,213	\$1,174,150	\$36,063
Indirect	\$468,486	\$421,865	\$46,621
TOTALS	\$5,848,241	\$4,969,996	\$878,245

²The budgeted amounts are the sum of each category from Dec-01 through Jun-03.

II EQUIPMENT

A. Overview

Equipment necessary for startup of JCML laboratory operations has been received, installed and is functional. As forecast in the January 2003 report, additional automated clinical chemistry equipment that was not included in the original budget (e.g. COBAS Integra 400) was purchased and installed. This capability is required by Chinese authorities for JCML to serve as an official laboratory for occupational benzene examinations which we have agreed to do as part of the ME study. In addition, \$453,874 in what was originally budgeted as laboratory start-up costs was expensed in different categories, (i.e. \$272,673 in equipment, \$88,867 in operating expenses, and \$92,334 in subcontracts) (Table 3).

B. Budget

Table 3. Equipment and Start-Up Supplies Purchased

DATE	VENDOR	ITEM	COST
3-Jan-03	Thermo Life	Cryo Plus Storage Container	\$ 7,795
3-Jan-03	Thermo Life	SpeedVac System	\$ 29,551
3-Jan-03	Thermo Life	Freezer/Storage Rack/Incubator	\$ 44,354
3-Jan-03	Thermo Life	Biosafety Cabinet/Freezer	\$ 45,751
3-Jan-03	Thermo Life	Upright Freezers	\$ 45,122
13-Jan-03	Gene Company	Thermo Cycler	\$ 6,500
31-Jan-03	Beckman	TO Prep	\$ 33,600
16-Apr-03	Roche	COBAS Integra 400	\$ 60,000
		Subtotal Equipment	\$ 272,673
18-Feb-03	Gene Company	Reagents	\$ 11,565
27-Feb-03	TKO OPTO	Microscope	\$ 4,600

implementation and “debugging” of individual system features is underway and will proceed until further notice.¹

IV BENZENE METABOLITE PILOT STUDY

We have conducted a pilot study to develop and evaluate a sensitive method for the analysis of benzene metabolites in human blood and bone marrow. After initial developmental work, we ultimately have settled on a liquid-liquid extraction method coupled with electron capture GCMS. Using this method we have successfully quantitated phenol, catechol and hydroquinone in blood and bone marrow of human subjects not occupationally exposed to benzene. We have standardized and validated the procedure with cold labeled isotopes and established standard curves for each analyte in small volumes of blood and bone marrow. Precision, accuracy and sample stability have also been determined. A “shakedown” evaluation of the method for quantitating the phenolic metabolites of benzene was conducted in blood of participating subjects as part of the pilot factory ME study conducted in June (See V below). Normally, these analyses will only be performed in Phase 2b of the ME study. Some problems with background measurements were encountered in this evaluation that appear to be instrument related and are being resolved at the present time. Methods of analysis for PMA and tt-MA have also been developed using the same extraction procedure and are being implemented in the field.

We have revised our initial plans to publish these as separate methods in favor of a single paper that outlines the methodology and validation for all 5 compounds and that optimally provides an analysis of background blood and bone marrow concentrations of these metabolites in workers with no demonstrable occupational exposure to benzene. We believe that this will necessitate the accrual of approximately 10-15 subjects in the control exposure group from Phase 2b prior to publication. The methods characterization is essentially complete.

V EXPOSURE ASSESSMENT

A. Molecular Epidemiology Study

A pilot exposure assessment project began 20 June and will end 4 July*. This involved a shoe factory with approximately 200 employees, in minimal benzene exposure tasks. The Exposure Assessment Team collected personal samples, IPHS-type area samples and UltraRAE benzene detector samples for five work groups in this factory, over the course of 10 normal production workdays. The benzene physical exam and blood collection took place July 2 and 3. Exhaled breath samples were collected 3 July, for method familiarization and field test purposes (exhaled breath measurements are planned only for the Phase 2b set of 200 workers.) Our team is also conducting ventilation system evaluations. The ventilation data may be used in evaluating a regression relationship between the area samples and the personal exposure samples. This relationship will be of use in the retrospective assessment aspects of the project.

We are also determining the composition of the current glues and solvents and are seeking information on the previously used materials. This information is also of value in developing the project's retrospective assessment methods.

The next two factories, that currently have benzene exposure, are selected and scheduled. Their evaluations will begin in late July.

*At the time of this writing, this pilot study has been concluded. The industrial hygiene measurements, informed consent, subject sampling procedures and laboratory operations worked very well. Additional minor modifications in procedures are being made to streamline exposure assessment, laboratory sample handling and reporting of clinical results to CDC. The results of benzene analyses await evaluation. As a result, we plan that the factory evaluations, beginning in July, will not be pilot experiments but will begin formal ME case accrual.

B. Disease Progression and Case Control Studies

Pilot questionnaire data have been entered into the JCML database for evaluation purposes. The entry is not finished as of 1 July, but dedicated data entry clerks will soon be on board and trained.

Four Questionnaire Administrators have conducted the pilot interviews and the initial case/control interviews. Three Exposure Assessment Team members (currently doing field work for the ME Pilot) are ready to do work site inspections associated with subject work histories. Additional personnel have been recruited for questionnaire interviewing and additional time-effort committed for exposure assessment in support of all protocols.

A Chinese coding system for factory and job classification is in use and is being translated to English. These codes, with the bilingual database, will give English language information on the industries and jobs held by the study subjects.

Additional review of selected pilot study files and initial study subjects will be conducted by the Exposure Assessment Committee. This review is to develop further understanding and provide guidance to the Exposure Assessment Team's investigation work. The review is also to complete the exposure summaries for the pilot subjects and refine the day-to-day aspects of the subject's exposure assessment and exposure summaries.

VI EPIDEMIOLOGY

We have recruited an additional 5 hospitals to the DP/CC studies bringing the total number of participating hospitals to 21. Training of clinical coordinators with respect to selection and recruitment of subjects and controls is largely completed, but additional sessions and individual feedback will continue to be provided as more hospitals refer patients and individual issues are raised.

We are collaborating with Professor Ni of Yang Pu District Hospital, who is President of the Occupational Medicine wing of the Chinese Academy of Medicine, to collect and review medical

records of previous acute benzene exposures in Shanghai and to identify factories that have had benzene poisoning (BP) cases. The clinical records are thought to involve up to 200 cases for which detailed medical records, including test data, and some slide materials are available. In addition, Professor Ni is organizing the review of occupational physical examination records from all Shanghai hospitals (~38) in order to abstract physical examination records and to identify factories with previous BP cases. These will be cross-referenced against the IPHS database. The physical examination information will be used to support ME Phase 1 and BP case factory identification will be used in conjunction with IPHS records for the selection of factories in Phase 2a. The Factory Selection Committee is presently selecting factories for Phase 2a & 2b studies which begin to accrue cases in August.

VII REGULATORY HARMONIZATION AND LICENSING

Fudan University and JCML have obtained all necessary approvals and licensing for operation in China. Last minute modifications in IC for all study protocols have been approved in the US and China.