From:	Bruce Jarnot <jarnotb@api.org>
Sent:	Thursday, March 4, 2004 5:24 PM (GMT)
To:	benzconsort-tc@listserve.api.org
Cc:	Matt Todd <ToddM@api.org>
Subject:	BHRC-TC... DP & ME (Dr Irons') Dec 2003 Progress Report
Attach:	DP ME Progress + Expense Rpt 12-03.doc; DP ME Progress Expense Rpt 12-03.pdf

Benzene Health Research Consortium (BHRC) Technical Committee (TC) -

Attached is Richard Iron's progress and expense report from the Disease Progression (DP) and Molecular Epidemiology (ME) portions of the Shanghai Health Study, presenting activities from the second-half, July - December, of 2003. The report is attached in both Microsoft Word (doc) and Adobe Acrobat (pdf) formats.

An abbreviated copy of this report, lacking only the front-end UCHSC expense information, was distributed to both the SRP & ERP expert panels.

By copy to Matt Todd, would you please add this report to the SHS website for Consortium Committee access... thanks!

Best Regards - Bruce.

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PROGRESS REPORT
December 2003

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

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Richard D. Irons

A MULTICENTER INTERNATIONAL STUDY

Molecular Toxicology and Environmental Health Sciences Program
Dept. of Pharmaceutical Sciences, School of Pharmacy
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–

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Comparison of actual to budget projections cited in this report were derived from the updated budget projections provided to API in September 2002 (Appendix A). JCML initiated clinical laboratory functions in 3rd quarter, with the case referral rate increasing progressively for a total of 225 during the last 5 months of 2003. Consequently, we are undergoing a transition in the budgetary tracking process and are continually evaluating resource allocation based on study contingencies.

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Clinical Service Statistics

An important measure of clinical laboratory service performance is the time from sample collection to the availability of test results (i.e. turn-around times (TAT)). The TAT for routine hematology in support of the 23 referring hospitals is normally under 2 hours with no excursions in routine clinical cases beyond 4 hours as of 12/31/03. Routine analyses of bone marrow aspirates average 3-7 days. For acute cases, preliminary aspirate analyses are available within 1-3 days. Flow cytometric analysis of bone marrow or lymph node preparations are completed within 1-4 days. The TAT for cytogenetics analyses averages 6-10 days with 70-80% being completed within 7 days. FISH analyses are 90% completed within less than 7 days. For acute cases, cytogenetics are completed within 5 days and FISH analyses within 2-3 days. The AML abnormal frequency rate is 57.14% for cytogenetics and 60% combined for cytogenetics and FISH. For the lymphoma cases, the abnormal cytogenetics rate is close to 80%. These statistics exceed the performance of most US clinical laboratories. The TAT for bone marrow or lymph node histology is 5-7 days. At present, the TAT for bone marrow or lymph node histology is 5-7 days and case-specific availability of control slides is spotty. This is not an acceptable standard of performance, and efforts are underway to correct the situation.

DNA and RNA are routinely isolated from samples received the same day, with all samples processed within 24 hours. Random RNA samples are subjected to electrophoresis, with visual confirmation of the 18S and 28S fractions demonstrating quality RNA. Additional quality control for DNA samples is provided by the amplification of the FLT-3 gene, performed on all samples from a given week on the first day of the following week. At present, routine molecular analyses performed in the laboratory include analysis of FLT-3 internal tandem duplication by PCR and FLT-3

amino acid substitution at D835 using RFLP, BCR/ABL confirmation by rtPCR, and TCR beta or gamma chain gene rearrangements by PCR and hetero/homoduplex analysis. Additional study mandated molecular analyses (e.g. HHV8, NQ01) are not time sensitive and will be processed as time allows.

Initially, MNC from all blood and bone marrow samples coming into JCML were preserved in two aliquots each using 3-4 ml of starting material. This volume of starting material is sub optimal. However, randomly selected samples demonstrate greater than 90% viability upon thawing. Initial EBV viral lots harvested from cells obtained in Shanghai have failed to transform both patient samples and test samples. Therefore, EBV supernatant was prepared in Colorado and shipped to Shanghai. This material has been used to successfully immortalize test cultures. Efforts are underway to increase the transformation efficiency of the culturing process and increase the starting blood sample volume. Patient cultures are now in the process of being immortalized using MNC that have been cryopreserved for subsequent immortalization. Sample thawing viability for December was determined to be 99.4%.

Case Control (CC) /Disease Progression (DP) Case Accrual

By December 31st approximately 190 cases were referred to JCML for diagnosis as part of the clinical operation, and, as of October 31, 106 cases have successfully undergone secondary pathology review. The case contact rate has not reached a steady state but continues to increase with over 270 cases accrued by the end of 6 months. Diagnoses qualifying for inclusion in the CC or DP studies represented 83% of the total case contacts with 17% of cases being diagnosed as non-qualifying or excluded conditions. Individual disease diagnoses included: AML 42, NHL 66, AA 17, and MDS 26. Again, these numbers should be interpreted with caution because JCML has not yet reached a steady state with respect to rate of clinical case accrual. Whether this reflects a progressively increasing case accrual or fluctuating admissions is not understood at this time.

Exposure Assessment

A variety of problems associated with procedure de-bugging, coordination and implementation led to an initial lag in exposure assessment operations relative to JCML case accrual. Early on, these issues were magnified by the high case accrual rate. However, we have re-organized the exposure assessment teams, which have undergone additional training and are now operational. By mid-January over 460 subjects had been entered into the exposure assessment program. The first stage assignment (exposed, unexposed, uncertain) produced a rapid initial sorting. From that stage, 12 subjects were classified as "exposed" for benzene, and are undergoing a more thorough quantitative assessment. The "uncertain" category included 67 subjects for which further information is needed (e.g. review of IPHS database work site inspections). Many of these uncertain ratings are expected to be transferred into the "benzene exposed" category. An additional 35 "other exposures" (i.e. other workplace hazards of concern besides benzene) have

been identified and classified and another 270 subjects were classified as having no exposure or "unexposed".

Independently, the Exposure Assessment (EA) team has compiled the Chinese literature for information on benzene exposure. District IPHS written records (not in the central database) and specific factory records are also being located. A Fudan team member has also initiated quality assurance overviews for the IPHS database searching process.

Control Selection for CC/DP

We have periodically reviewed the demographic characteristics and diagnoses for controls in order to ensure that procedures outlined in the protocol are being implemented correctly in the field. These periodic audits have shown that the matching criteria are being followed (i.e. controls are being successfully matched according to age and gender). However, we have determined that, on occasion, the protocol is not uniformly applied in all hospitals. Specifically, the selection of a control subject with the closest time of admission to that of a study subject is not always adhered to. Therefore, we met with clinical coordinators and reemphasized the control selection procedure with regard to time of admission/diagnosis. Some hospitals do not have a central admissions log, making it impossible to ensure that the control with the closest time of diagnosis is identified. In lieu of this, we are currently reviewing the procedures in every hospital separately, to assess whether there is any systematic bias in selecting controls.

Molecular Epidemiology (ME) Study

Phase 1 activities. The longitudinal exposure analysis of workers is progressing well. We have been successful in identifying several factories from which benzene workers were examined by hospitals over the past several years, and have correlated these with benzene monitoring data in the IPHS database. Professor Ni has identified a subset of factories that historically may have experienced high concentrations of benzene exposure. These are being investigated on a factory-by-factory basis. To date, we have identified a small number of facilities that have both good monitoring data and good medical records. A review and abstraction of medical records for workers in these facilities is underway. In addition, we also have located the medical records for 194 individual cases of benzene poisoning that presented at local hospitals over approximately the past 5 years. Analysis of this data set has begun, and medical record retrieval and abstraction is underway for all of these activities.

Phase 2a and 2b activites. We have recruited some workers to Phase 2a as part of a screening survey and an intensive two-week exposure analysis of a high exposure rubber products factory. Additional factory identification and recruitment activities are underway. Factory participation for Phase 2b has proven to be more difficult than initially envisioned. We originally anticipated recruiting subjects from adjacent or even distant provinces. Accordingly we are now simultaneously exploring suitable facilities,

both in Shanghai and outside Shanghai. In addition, although originally we planned to rely heavily on IPHS staff to introduce the project to Factory Management we are currently evaluating several sites outside Shanghai, based on initial contact by Professor Ni, President of the Chinese Academy of Occupational Medicine.

Benzene and Metabolite Analyses

At UCHSC we set out to develop a minimal step method for the extraction and quantitation of the benzene metabolites from blood and bone marrow: phenol (PH), catechol (CAT), hydroquinone (HQ) and trans,trans-muconic acid (MA). The rationale for this approach was to design the simplest procedures possible for extraction and analysis of these compounds that facilitate implementation in Shanghai, utilizing existing instrumentation (GC/MS). Our reasoning proved to be prescient. The final method was based on liquid phase extraction with ascorbic acid, acetonitrile and toluene. Deuterated derivatives for each metabolite were purchased or synthesized and employed as internal standards. The limit of detection for PH, CAT and HQ in blood samples was in the nanogram range. There is no commercial source for deuterated trans,trans-muconic acid-[1,2,5,6-^{13}C]. Therefore synthesis was achieved by refluxing a solution of triphenylphosphine and ethyl[1,2-$^{13}C_2$]bromoacetate in a mixture of acetonitrile and dichloromethane (ml) that was washed, dehydrated over magnesium sulfate and dissolved in dimethylformamide (DMF) to which glyoxal trimeric dehydrate was added to give the muconic acid diethyl ester. This was then dissolved in THF and lithium hydroxide in water. The solvents were evaporated under reduced pressure and the residue was suspended in a mixture of toluene and hydrochloric acid (pH 2.0). Publication of the method and analysis of background levels of benzene metabolites in the blood and bone marrow of unexposed volunteers awaits measurements that are still forthcoming from SMCDCP and that have been frustrated by events at SMCDCP discussed below.

Following on the recommendation of the SRP at the annual meeting in August, we investigated the possibility of measuring sPMA in urine of ME Phase 2b subjects. A direct, linear relationship exists between the internal dose of benzene and the rate of excretion of sPMA. Urinary sPMA is therefore an useful biomarker of exposure to benzene. The measurement can be easily performed using a small volume of urine, collected at an appropriate time relative to potential exposure. The strategy we have adopted for analysis of urinary sPMA is an enzyme-linked immunoabsorbant assay (ELISA). Therefore, we have negotiated a site license to employ ELISA assay owned by AB Biomonitoring, Ltd, Cardiff, UK. This methodology will be implemented following approval of the addition of urinary collection to our Phase 2b clinical protocol.

*****Recent Development as/of February 19, 2004*****

Despite two years time, the SMCDC has failed to successfully adapt these analytical methods for analysis of blood and bone marrow, and, for that matter, has not provided entirely satisfactory results for the measurement of benzene in air or any results for

exhaled breath analysis. The stated reasons are many, including serious illness in senior technical personnel resulting in a chronic lack of leadership, instrumentation problems that remain unresolved, and the inability or lack of resolve to commit adequate personnel and time to the project. We recently completed a detailed analysis of CDC analytical capability that included inspection and review of methods and procedures by Professor Zheng Xixing of Fudan University and David Chang of ExxonMobil. We have concluded that CDC has provided less than desired performance for analytical support for benzene or benzene metabolites. Among the issues that remain unresolved are: 1) in all cases, standard curves remain inadequate and are extrapolated from high values; 2) split sample analysis reveals an unacceptable and consistently low bias in contrast to paired samples analyzed by an AIHA certified laboratory; 3) despite several deadlines and adequate guidance they have not developed a satisfactory method of analysis for benzene in exhaled breath or benzene metabolites in blood and bone marrow. These issues have been the subject of frequent discussion with SMCDC staff and management as they have unfolded over the last year. Therefore, after protracted negotiations and little sign of success, we have terminated our agreement with SMCDC to conduct analyses as part of the project. We are collaborating with Fudan University to transfer all analytical activities to JCML with a minimum of delay. Because this is a very recent development we have not had an opportunity to evaluate every impact that this contingency will have on the project.

Quality Assessment Issues

Elements of QA/QC have been referred to throughout this report as they specifically refer to other areas, such as Exposure Assessment, Control Selection, JCML clinical laboratory operations and benzene metabolism. In order to improve overall coordination, professor Liang Youxin has been assigned the task of supervising QA/QC for exposure assessment, and the data entry process has been streamlined. Gail Joregenson from Exxonmobil visited JCML in early 2004 to consult with and evaluate the overall process and to coordinate late phase database changes which will be reported in the next progress report.

Conclusions

During the last 6 months we have successfully begun JCML clinical and research operations and are routinely diagnosing blood and lymphoid diseases for the 23 participating hospitals in Shanghai. Operating a combined clinical and research laboratory of this magnitude in the local Shanghai environment creates a number of unique challenges. In most areas of clinical operation, based on objective criteria, we meet or exceed the standards of many of our US counterparts. We have successfully undergone our first external pathology diagnostic review, which was a milestone. We are preparing for the second review which will be conducted in April 2004. In the research arena all processes are up to speed except immortalization of cell lines. For EBV immortalization, the problems have been identified and largely resolved as of February, 2004. In some clinical areas we need to provide additional support, training for and coordination with our clinicians and laboratory colleagues in Shanghai in order to

improve local standards of practice to meet CAP operational criteria. These relatively small details will continue to be addressed, but for the moment we are on track and functional. I believe we are on the way to becoming both a US and Chinese referral laboratory. The caseload number and variety of diseases encountered is remarkable. We are accruing an acceptable or greater than predicted number of study cases based on our initial projections, and the rate continues to grow.

Control selection for CC/DP has required additional training and orientation of clinical coordinators and the recognition that the 23 participating hospitals have markedly different capabilities and standards when it comes to tracking patient admissions. We are developing individualized approaches to deal with these issues and standardize as much as possible the control selection process. Exposure assessment under the leadership of the Fudan team got off to a predictably slower start than JCML. Progress on EA improved after we reorganized the exposure assessment operation and conducted an intensive training initiative to streamline the process. This effort has been a collaborative one, requiring the expertise and attention of all the major US collaborators on this project as well as our colleagues at Fudan University. The EA operation is improving with a renewed understanding of the need for ongoing training and continued guidance by US personnel. Although we have had very good experiences interacting with and recruiting factory workers, we have experienced some challenges in encouraging factory management participation in the ME study. We have taken a multifaceted approach to dealing with this process including: attempting to fine tune the approach of IPHS to initiating factory involvement, exploring political solutions, actively pursuing leads outside of town.

It has come as somewhat of a surprise that our biggest challenge has turned out to be obtaining high quality analytical support. However, in the absence of any demonstrable improvement in SMCDCP analytical capabilities over the past year, the need for a change in tactics has become imminent. Our immediate focus is on achieving a rapid and efficient transition to bring the analytical capabilities necessary for the project on line at JCML. To this end we are experiencing active cooperation and support from Fudan University. The goal is to have this implemented before the next 6 month reporting period. Analytical support for benzene air monitoring should be implemented faster than that.

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JCML began routine full service clinical operations around the beginning of August 2003. These activities include: routine hematology, bone marrow aspirate analysis, bone marrow and lymph node histopathology, clinical chemistry-liver enzymes, serology, flow cytometric analysis of blood, bone marrow and lymph node preparations, cytogenetics, FISH and molecular genetics. As of December 31st, after the first 5 months of operations, approximately 190 cases had been referred to JCML for diagnosis as part of the clinical operation. (The case contact rate has continued to increase with over 270 cases having been seen by the end of 6 months.) An external pathology review was conducted in October 2003. As of October 31, 106 cases have successfully undergone secondary pathology review. Preliminary estimates suggest approximately a 50% concordance between initial hospital case diagnosis (if any) and the JCML diagnosis, and in excess of a 90% concordance between the JCML diagnosis and secondary pathology review. The latter is an acceptable standard of concordance between US laboratories.

Clinical Service Statistics

An important measure of clinical laboratory service performance is the time from sample collection to the availability of test results (i.e. turn-around times (TAT)). The TAT for routine hematology in support of the 23 referring hospitals is normally under 2 hours with no excursions in routine clinical cases beyond 4 hours as of 12/31/03. Routine analyses of bone marrow aspirates average 3-7 days. For acute cases, preliminary aspirate analyses are available within 1-3 days. Flow cytometric analysis of bone marrow or lymph node preparations are completed within 1-4 days. The TAT for cytogenetics analyses averages 6-10 days with 70-80% being completed within 7 days. FISH analyses are 90% completed within less than 7 days. For acute cases, cytogenetics are completed within 5 days and FISH analyses within 2-3 days. The AML abnormal frequency rate is 57.14% for cytogenetics and 60% combined for cytogenetics and FISH. For the lymphoma cases, the abnormal cytogenetics rate is close to 80%. These statistics exceed the performance of most US clinical laboratories. The TAT for bone marrow or lymph node histology is 5-7 days. At present, the TAT for bone marrow or lymph node histology is 5-7 days and case-specific availability of control slides is spotty. This is not an acceptable standard of performance, and efforts are underway to correct the situation.

DNA and RNA are routinely isolated from samples received the same day, with all samples processed within 24 hours. Random RNA samples are subjected to electrophoresis, with visual confirmation of the 18S and 28S fractions demonstrating quality RNA. Additional quality control for DNA samples is provided by the amplification of the FLT-3 gene, performed on all samples from a given week on the first day of the following week. At present, routine molecular analyses performed in the laboratory include analysis of FLT-3 internal tandem duplication by PCR and FLT-3

amino acid substitution at D835 using RFLP, BCR/ABL confirmation by rtPCR, and TCR beta or gamma chain gene rearrangements by PCR and hetero/homoduplex analysis. Additional study mandated molecular analyses (e.g. HHV8, NQ01) are not time sensitive and will be processed as time allows.

Initially, MNC from all blood and bone marrow samples coming into JCML were preserved in two aliquots each using 3-4 ml of starting material. This volume of starting material is sub optimal. However, randomly selected samples demonstrate greater than 90% viability upon thawing. Initial EBV viral lots harvested from cells obtained in Shanghai have failed to transform both patient samples and test samples. Therefore, EBV supernatant was prepared in Colorado and shipped to Shanghai. This material has been used to successfully immortalize test cultures. Efforts are underway to increase the transformation efficiency of the culturing process and increase the starting blood sample volume. Patient cultures are now in the process of being immortalized using MNC that have been cryopreserved for subsequent immortalization. Sample thawing viability for December was determined to be 99.4%.

Case Control (CC) /Disease Progression (DP) Case Accrual

By December 31st approximately 190 cases were referred to JCML for diagnosis as part of the clinical operation, and, as of October 31, 106 cases have successfully undergone secondary pathology review. The case contact rate has not reached a steady state but continues to increase with over 270 cases accrued by the end of 6 months. Diagnoses qualifying for inclusion in the CC or DP studies represented 83% of the total case contacts with 17% of cases being diagnosed as non-qualifying or excluded conditions. Individual disease diagnoses included: AML 42, NHL 66, AA 17, and MDS 26. Again, these numbers should be interpreted with caution because JCML has not yet reached a steady state with respect to rate of clinical case accrual. Whether this reflects a progressively increasing case accrual or fluctuating admissions is not understood at this time.

Exposure Assessment

A variety of problems associated with procedure de-bugging, coordination and implementation led to an initial lag in exposure assessment operations relative to JCML case accrual. Early on, these issues were magnified by the high case accrual rate. However, we have re-organized the exposure assessment teams, which have undergone additional training and are now operational. By mid-January over 460 subjects had been entered into the exposure assessment program. The first stage assignment (exposed, unexposed, uncertain) produced a rapid initial sorting. From that stage, 12 subjects were classified as "exposed" for benzene, and are undergoing a more thorough quantitative assessment. The "uncertain" category included 67 subjects for which further information is needed (e.g. review of IPHS database work site inspections). Many of these uncertain ratings are expected to be transferred into the "benzene exposed" category. An additional 35 "other exposures" (i.e. other workplace hazards of concern besides benzene) have

been identified and classified and another 270 subjects were classified as having no exposure or "unexposed".

Independently, the Exposure Assessment (EA) team has compiled the Chinese literature for information on benzene exposure. District IPHS written records (not in the central database) and specific factory records are also being located. A Fudan team member has also initiated quality assurance overviews for the IPHS database searching process.

Control Selection for CC/DP

We have periodically reviewed the demographic characteristics and diagnoses for controls in order to ensure that procedures outlined in the protocol are being implemented correctly in the field. These periodic audits have shown that the matching criteria are being followed (i.e. controls are being successfully matched according to age and gender). However, we have determined that, on occasion, the protocol is not uniformly applied in all hospitals. Specifically, the selection of a control subject with the closest time of admission to that of a study subject is not always adhered to. Therefore, we met with clinical coordinators and reemphasized the control selection procedure with regard to time of admission/diagnosis. Some hospitals do not have a central admissions log, making it impossible to ensure that the control with the closest time of diagnosis is identified. In lieu of this, we are currently reviewing the procedures in every hospital separately, to assess whether there is any systematic bias in selecting controls.

Molecular Epidemiology (ME) Study

Phase 1 activities. The longitudinal exposure analysis of workers is progressing well. We have been successful in identifying several factories from which benzene workers were examined by hospitals over the past several years, and have correlated these with benzene monitoring data in the IPHS database. Professor Ni has identified a subset of factories that historically may have experienced high concentrations of benzene exposure. These are being investigated on a factory-by-factory basis. To date, we have identified a small number of facilities that have both good monitoring data and good medical records. A review and abstraction of medical records for workers in these facilities is underway. In addition, we also have located the medical records for 194 individual cases of benzene poisoning that presented at local hospitals over approximately the past 5 years. Analysis of this data set has begun, and medical record retrieval and abstraction is underway for all of these activities.

Phase 2a and 2b activites. We have recruited some workers to Phase 2a as part of a screening survey and an intensive two-week exposure analysis of a high exposure rubber products factory. Additional factory identification and recruitment activities are underway. Factory participation for Phase 2b has proven to be more difficult than initially envisioned. We originally anticipated recruiting subjects from adjacent or even distant provinces. Accordingly we are now simultaneously exploring suitable facilities,

both in Shanghai and outside Shanghai. In addition, although originally we planned to rely heavily on IPHS staff to introduce the project to Factory Management we are currently evaluating several sites outside Shanghai, based on initial contact by Professor Ni, President of the Chinese Academy of Occupational Medicine.

Benzene and Metabolite Analyses

At UCHSC we set out to develop a minimal step method for the extraction and quantitation of the benzene metabolites from blood and bone marrow: phenol (PH), catechol (CAT), hydroquinone (HQ) and trans,trans-muconic acid (MA). The rationale for this approach was to design the simplest procedures possible for extraction and analysis of these compounds that facilitate implementation in Shanghai, utilizing existing instrumentation (GC/MS). Our reasoning proved to be prescient. The final method was based on liquid phase extraction with ascorbic acid, acetonitrile and toluene. Deuterated derivatives for each metabolite were purchased or synthesized and employed as internal standards. The limit of detection for PH, CAT and HQ in blood samples was in the nanogram range. There is no commercial source for deuterated trans,trans-muconic acid-[1,2,5,6-^{13}C]. Therefore synthesis was achieved by refluxing a solution of triphenylphosphine and ethyl[1,2-$^{13}C_2$]bromoacetate in a mixture of acetonitrile and dichloromethane (ml) that was washed, dehydrated over magnesium sulfate and dissolved in dimethylformamide (DMF) to which glyoxal trimeric dehydrate was added to give the muconic acid diethyl ester. This was then dissolved in THF and lithium hydroxide in water. The solvents were evaporated under reduced pressure and the residue was suspended in a mixture of toluene and hydrochloric acid (pH 2.0). Publication of the method and analysis of background levels of benzene metabolites in the blood and bone marrow of unexposed volunteers awaits measurements that are still forthcoming from SMCDCP and that have been frustrated by events at SMCDCP discussed below.

Following on the recommendation of the SRP at the annual meeting in August, we investigated the possibility of measuring sPMA in urine of ME Phase 2b subjects. A direct, linear relationship exists between the internal dose of benzene and the rate of excretion of sPMA. Urinary sPMA is therefore an useful biomarker of exposure to benzene. The measurement can be easily performed using a small volume of urine, collected at an appropriate time relative to potential exposure. The strategy we have adopted for analysis of urinary sPMA is an enzyme-linked immunoabsorbant assay (ELISA). Therefore, we have negotiated a site license to employ ELISA assay owned by AB Biomonitoring, Ltd, Cardiff, UK. This methodology will be implemented following approval of the addition of urinary collection to our Phase 2b clinical protocol.

*****Recent Development as/of February 19, 2004*****

Despite two years time, the SMCDC has failed to successfully adapt these analytical methods for analysis of blood and bone marrow, and, for that matter, has not provided entirely satisfactory results for the measurement of benzene in air or any results for

exhaled breath analysis. The stated reasons are many, including serious illness in senior technical personnel resulting in a chronic lack of leadership, instrumentation problems that remain unresolved, and the inability or lack of resolve to commit adequate personnel and time to the project. We recently completed a detailed analysis of CDC analytical capability that included inspection and review of methods and procedures by Professor Zheng Xixing of Fudan University and David Chang of ExxonMobil. We have concluded that CDC has provided less than desired performance for analytical support for benzene or benzene metabolites. Among the issues that remain unresolved are: 1) in all cases, standard curves remain inadequate and are extrapolated from high values; 2) split sample analysis reveals an unacceptable and consistently low bias in contrast to paired samples analyzed by an AIHA certified laboratory; 3) despite several deadlines and adequate guidance they have not developed a satisfactory method of analysis for benzene in exhaled breath or benzene metabolites in blood and bone marrow. These issues have been the subject of frequent discussion with SMCDC staff and management as they have unfolded over the last year. Therefore, after protracted negotiations and little sign of success, we have terminated our agreement with SMCDC to conduct analyses as part of the project. We are collaborating with Fudan University to transfer all analytical activities to JCML with a minimum of delay. Because this is a very recent development we have not had an opportunity to evaluate every impact that this contingency will have on the project.

Quality Assessment Issues

Elements of QA/QC have been referred to throughout this report as they specifically refer to other areas, such as Exposure Assessment, Control Selection, JCML clinical laboratory operations and benzene metabolism. In order to improve overall coordination, professor Liang Youxin has been assigned the task of supervising QA/QC for exposure assessment, and the data entry process has been streamlined. Gail Joregenson from Exxonmobil visited JCML in early 2004 to consult with and evaluate the overall process and to coordinate late phase database changes which will be reported in the next progress report.

Conclusions

During the last 6 months we have successfully begun JCML clinical and research operations and are routinely diagnosing blood and lymphoid diseases for the 23 participating hospitals in Shanghai. Operating a combined clinical and research laboratory of this magnitude in the local Shanghai environment creates a number of unique challenges. In most areas of clinical operation, based on objective criteria, we meet or exceed the standards of many of our US counterparts. We have successfully undergone our first external pathology diagnostic review, which was a milestone. We are preparing for the second review which will be conducted in April 2004. In the research arena all processes are up to speed except immortalization of cell lines. For EBV immortalization, the problems have been identified and largely resolved as of February, 2004. In some clinical areas we need to provide additional support, training for and coordination with our clinicians and laboratory colleagues in Shanghai in order to

improve local standards of practice to meet CAP operational criteria. These relatively small details will continue to be addressed, but for the moment we are on track and functional. I believe we are on the way to becoming both a US and Chinese referral laboratory. The caseload number and variety of diseases encountered is remarkable. We are accruing an acceptable or greater than predicted number of study cases based on our initial projections, and the rate continues to grow.

Control selection for CC/DP has required additional training and orientation of clinical coordinators and the recognition that the 23 participating hospitals have markedly different capabilities and standards when it comes to tracking patient admissions. We are developing individualized approaches to deal with these issues and standardize as much as possible the control selection process. Exposure assessment under the leadership of the Fudan team got off to a predictably slower start than JCML. Progress on EA improved after we reorganized the exposure assessment operation and conducted an intensive training initiative to streamline the process. This effort has been a collaborative one, requiring the expertise and attention of all the major US collaborators on this project as well as our colleagues at Fudan University. The EA operation is improving with a renewed understanding of the need for ongoing training and continued guidance by US personnel. Although we have had very good experiences interacting with and recruiting factory workers, we have experienced some challenges in encouraging factory management participation in the ME study. We have taken a multifaceted approach to dealing with this process including: attempting to fine tune the approach of IPHS to initiating factory involvement, exploring political solutions, actively pursuing leads outside of town.

It has come as somewhat of a surprise that our biggest challenge has turned out to be obtaining high quality analytical support. However, in the absence of any demonstrable improvement in SMCDCP analytical capabilities over the past year, the need for a change in tactics has become imminent. Our immediate focus is on achieving a rapid and efficient transition to bring the analytical capabilities necessary for the project on line at JCML. To this end we are experiencing active cooperation and support from Fudan University. The goal is to have this implemented before the next 6 month reporting period. Analytical support for benzene air monitoring should be implemented faster than that.