Polycythemia Vera Expert Panel Meeting
Project Recommendations
December 1, 2008

INTRODUCTION

Investigations into the excess of polycythemia vera (PV) in Carbon, Luzerne, and Schuylkill Counties of Northeast (NE) Pennsylvania began in late 2006, and the initial studies were completed in mid-2008. The federal Agency for Toxic Substances and Disease Registry (ATSDR) and the Pennsylvania Department of Health (PADOH) collaborated to conduct the investigations. On August 25, 2008, ATSDR and PADOH held a public meeting to present the results of these initial investigations to the community, documenting an increased incidence of PV with a clustering effect in parts of the tri-county area. These high-incidence areas include a variety of environmental hazards, both past and present, including National Priority List (NPL or Superfund) sites and waste-coal power plants.

PV is a blood disease that occurs in about 1 out of every 100,000 people each year. Although PV is a cancer, it can be controlled with proper medical care. The cause of PV is not known. In 2004, a genetic marker called JAK2 was found in nearly all persons with PV. A test for JAK2 helps to confirm the diagnosis of PV and was used in the investigation to verify the PV diagnosis of all participants.

To suggest further activities for assessing the PV cluster in the affected area, an expert panel of PV researchers, environmental scientists, and public health officials was

convened by ATSDR and PADOH. This panel met on the day of the public meeting to propose, discuss, and prioritize additional research projects that would shed light on the PV cluster in NE Pennsylvania or would improve the general knowledge base on this and similar diseases. The panel identified potential projects in four different areas: epidemiologic/clinical, genetic/biomolecular, toxicological, and environmental analysis. Altogether, the panel discussed 12 separate projects. Following this meeting, panel members provided additional feedback on the proposed projects based on their areas of expertise. ATSDR and PADOH, with the primary goal of understanding the cause of the PV cluster, took this feedback into consideration and then developed a prioritized list of research activities. This report summarizes and prioritizes the projects, and discusses how these projects might best be accomplished.

METHODS

ATSDR and PADOH evaluated the projects by using the expert panel's feedback, along with 5 other factors: the scope of work, complexity, resource requirements, cost, and time frame for completion. The projects then were ranked by assigning a recommendation of high, intermediate, or lesser priority.

RESULTS

Epidemiologic/Clinical Studies

The expert panel identified five possible epidemiological or clinical projects as potential follow-up activities.

Case-control study in NE Pennsylvania. Expert panel members felt strongly that a case-control study is necessary to assess risk factors for the PV cluster. Such a study would evaluate potential risk factors associated with the disease by comparing persons with the disease (cases) to community members without the disease (controls). The study would examine the relationship of the potential risk factors with disease by comparing the prevalence of the risk factors in those persons with and without disease and looking for significant differences.

Such a study would pose many challenges. First, because the cause of PV is unknown, it would require examination of a large number of potential risk factors, and the number of potential hypotheses to explore is almost unlimited. Overlooking possible explanations is easy if the right questions are not asked. Second, potentially spurious associations can result from a number of biases, including patient recall of events long in the past and improper choice of controls. The likely long induction period between exposure and onset of disease means that researchers must consider factors from decades earlier. These challenges are among the reasons why identifying causes for health concerns such as PV

are often difficult and unsuccessful. Nonetheless, a case-control study of the type proposed here is the most generally accepted approach to assessing disease risk factors. The results of this study could also affect the relevance and design of other projects. This project was thus given a high-priority rating.

Comparative epidemiologic study. Another epidemiologic study would compare the tri-county cluster to other populations in locations with similar environmental conditions. These locations might include West Virginia or other parts of Pennsylvania. This project would focus on identifying other populations with similar environmental conditions and evaluating the occurrence of PV in those populations.

For example, 16 of the 20 waste-coal power plants in the United States are located in Pennsylvania, and 3 others lie across the border in West Virginia. Examining PV incidence in these other areas, using protocols similar to those already used in NE Pennsylvania, would eliminate the problem of misdiagnosis and assist in appropriately characterizing cases. If PV incidence in these areas is elevated, the study would enhance the researcher's ability to associate potential risk factors with disease. If PV incidence is not elevated, comparing characteristics of the affected and unaffected areas may identify factors present in the high-incidence but not the low-incidence locations. The latter scenario would represent an analytic multiple-group study, with the locations of high and low or normal incidence defining the case groups.

This activity would require aggressive case-finding efforts, medical record review, and testing for the JAK2 genetic marker, which was recently included in the World Health Organization's revised diagnostic criteria for PV. The study would use the protocol and methods from the current investigation, and thus could be completed in a relatively short period of time. Because it would have direct implications for the affected communities and may help elucidate the cause(s) of PV, this project was given a high-priority rating.

Prospective assessment of PV trends. This project would continue to monitor and document the incidence of PV in the tri-county area and would assess whether rates of PV remain elevated, have stabilized, are decreasing, or have returned to baseline rates seen throughout Pennsylvania. It would also identify a prospective cohort of patients for enrollment in the various studies and document the accuracy of reporting and diagnosis to the state cancer registry now that more precise diagnostic tests are available. PADOH has access to state cancer registry data and has assessed additional data in the cancer registry since ATSDR's initial investigation. Because the information gathered would directly benefit the local community and patients with PV, and the study would have relatively low resource requirements, this project was given a high-priority rating.

Descriptive study of other myeloproliferative diseases (MPDs) in the study area. MPDs are closely related, and current research indicates that they are possibly elements of a single disease process. In addition to PV, MPDs include essential thrombocythemia (ET), primary myelofibrosis (PMF), and chronic myeloid leukemia (CML). If the incidence of any or all of the MPDs is elevated, the study could provide useful

information regarding the current PV cluster, elucidate the interrelationship between the various MPDs and their progression, and provide supportive information regarding possible causation. As was done with the initial PV investigation, the various MPD diagnoses would require confirmation by using genetic markers. This activity would also assess the validity of state cancer registry data regarding other MPDs.

The resources necessary to perform such a descriptive study are minimal, but such a project would not directly address the cause of the current PV cluster. Since diagnostic inaccuracies likely exist with all the MPDs, this project would benefit the local community by identifying misdiagnosed and undiagnosed cases. This project was given an intermediate-priority rating.

Clinical outcomes study. The expert panel suggested a prospective study to determine the clinical outcomes of PV patients. This study would compare the clinical course of PV patients in the tri-county area with that of patients from other locations. If a unique causative factor is responsible for PV in the tri-county area, those patients may experience an atypical clinical course and outcome. In other general studies of PV, controlling for cause is not possible when clinical course, response to therapy, and other aspects of disease are studied. Therefore, the PV cohort in the tri-county area is a potentially unique population. The possible factors for evaluation include the frequency and occurrence of disease symptoms and complications, progression to PMF or CML, survival time, response to therapy, and cause of death.

Such a study has great potential but is more likely to produce general knowledge rather than information specifically beneficial to the tri-county area patients. In addition, it would require a prolonged period of time (years to decades) to generate useful information. This project was given a lesser priority rating.

Genetic/Biomolecular Studies

The expert panel identified four genetic or biomolecular studies as potential follow-up activities.

Gene-profiling analysis of PV patients in the tri-county area. This project involves comparing gene-profiling analyses of the archived PV specimens of patients from the study area to specimens from control PV patient populations from other geographic sites. This work would help determine if a genetic or hereditary component for PV exists and if unique attributes exist in the cluster area population. Although PV is not an inherited disease, evidence suggests that an inheritable predisposition to acquire the disease exists.

This study would require comparatively little time and resources and is potentially high yield if a genetic link is established for PV. Specific needs would include a sophisticated research laboratory with demonstrated expertise in this highly technical area, access to archived specimens, and additional specimens from the control populations. Because of its prospective nature but potentially high yield, this project was given an intermediate-priority rating.

JAK2 mutation survey. This prevalence survey of the JAK2 mutation and related genetic abnormalities in the local population would collect population-based samples and relevant demographic information from the local community to determine the background prevalence of the JAK2 mutation. One option would be to test anonymous leftover samples collected from blood donors or from persons undergoing routine clinical laboratory testing. Current research suggests that persons with the JAK2 mutation who are asymptomatic for PV represent preclinical cases that will ultimately progress to a clinically diagnosed disease state. Studies from other locations have suggested the prevalence of this mutation in asymptomatic persons is very low. If additional JAK2-positive persons are identified in the affected area, the study may require similar surveys to see if the prevalence differs from other locations. A higher prevalence would indicate the need for follow-up studies of targeted clinical screening and would warrant surveillance of JAK2-positive persons for early detection of disease.

This project would not directly help identify the cause of the current PV cluster but would have a relatively low resource requirement and short time-frame to complete. It also would provide useful data on the background prevalence of the JAK2 mutation and the need for additional screening in the study area, and could be used as a model for PV screening in other areas. This project was thus given an intermediate-priority rating.

Cytogenetic studies of PV patients in the tri-county area. This project involves testing archived specimens from the NE Pennsylvania PV cohort for specific alterations (either

genetic or morphologic) that are potentially indicative of both a toxic exposure and the type(s) of toxin(s) involved. Many toxic agents produce genetic and/or morphological signatures in cells that can persist well after the exposure. Fluorescence *in situ* hybridization (FISH) analysis is a molecular cytogenetic technique in which fluorescently labeled DNA probes are hybridized to metaphase spreads or interphase nuclei. Applications of this technique include identifying structurally abnormal chromosomes, including several associated with cancer such as BCR/ABL and TEL/AML1 translocations; identifying marker chromosomes; detecting very small deletions (microdeletions); and rapidly detecting numerical chromosome abnormalities. The identification of specific patterns of abnormalities would support the hypothesis that an environmental insult is responsible for PV in general and/or the current cluster specifically. A negative result would not necessarily preclude the presence of such a factor because this technique is largely experimental. Additional considerations are the study's requirement of a sophisticated laboratory infrastructure and access to the archived specimens. This project was given a lesser priority rating because of its theoretical nature and technical requirements.

Tissue banking. Another genetic/biomolecular project would establish a tissue bank for specimens from PV patients in the involved geographic area. A tissue bank provides a repository for biological specimens that, as the knowledge base related to a disease or condition expands, are potentially valuable in a variety of future studies. This project may or may not directly benefit persons with PV in the cluster area, and it would likely involve longitudinal collection of specimens to monitor changes over time. Acquisition

and storage of biological specimens is costly, but the inclusion of specimen collection as part of another proposed project might help alleviate these costs. Additional issues relating to specimen access, distribution, and oversight would present logistical challenges. This project was given a lesser priority rating given its limited direct impact in addressing the cause and source of the cluster and the relatively long time period required.

Toxicological Studies

The expert panel identified two toxicological studies as potential follow-up activities.

Polycyclic aromatic hydrocarbon (PAH) exposure survey. This project would evaluate the extent of PAH exposure in PV patients compared with both healthy persons in the local population and persons in other locations. Specifically, PAH effects on the aryl hydrocarbon receptor (AhR) are assessed in PV and normal CD34+ cells by monitoring the up-regulation of a battery of AhR target genes including CYPIA1 and CYPIB1. PAHs, which are linked to bone marrow toxicity in mice, are ubiquitous and associated with numerous sources (both environmental and lifestyle), including waste coal plant emissions and Superfund sites in the study area. Such a correlation might suggest a potential cause—although not a specific source—for the elevated incidence of PV in the local community; however, establishing a cause-and-effect relationship would not be feasible through this survey alone. Since the AhR up-regulation is a transient process, this method would only identify recent PAH exposure; however, many of the potential

PAH exposure sources are not fully remediated or are still active (including all of the waste-coal power plants), thus the data are still potentially useful.

This project would require a sophisticated research laboratory with demonstrated expertise in this area and access to specimens from the current PV cases and comparison populations. Given the relatively short time required, and the potential for direct benefit to PV patients and the general population of the area, this project was given an intermediate-priority rating.

Toxicological assay. This project would develop a bone marrow stem cell culture and subject the culture to a specific chemical or mixture of chemicals. This would determine the chemical or chemical mixtures' ability to cause the genetic instability and damage that could lead to PV. Specifically, this would develop a physiological *in vitro* assay for stem cell-derived progenitor cells for evaluating toxic compounds in the study area. The Comet assay and the micronucleus test would be used to screen for DNA damage caused by environmental mutagens. An *in vitro* cell culture system that captures essential features of the *in vivo* erythroid micronucleus genotoxicity assay would be used to identify environmental agents that might increase DNA damage in hematologic cells. This research could provide a valuable tool to evaluate the potential for suspected toxic chemicals to cause PV and other MPDs. A successful application of this technology is described in the literature, and this project could serve as a model for other diseases with suspected environmental causes.

The project is highly specialized, requiring a sophisticated research laboratory with demonstrated expertise in this area and a development phase during which the method is modified and tested. Thus, the overall project time and costs are difficult to evaluate. These factors, along with the exploratory nature of this project resulted in a lesser priority rating.

Environmental Analysis Study

The expert panel identified a single environmental project as a potential follow-up activity. This investigation would assess PV patients' historical residential locations with respect to environmental hazards during various time periods. Such an investigation would not prove a cause-and-effect relationship but could identify potential risk factors for further investigation. The study could identify possible toxic agents, potential exposure levels, and exposure pathways for a given population over a specified time period. Environmental studies dealing with historical exposures are often challenging due to a lack of adequate environmental testing data and other important information. To increase the statistical power of such an environmental study, researchers could also consider evaluating persons who previously lived in the study area and were diagnosed with PV after they had moved away. Such patients were identified but were not included in investigations done to date as these focused on persons residing in the area at the time of diagnosis. Because the latency period for PV is probably lengthy, and the diagnosis is often delayed for a number of years due to the gradual onset of symptoms, any role of the environment likely relates to exposures that occurred well in the past.

The success of this project would depend largely upon the availability of historical records and testing data for the cluster area. This work would require expertise in geospatial and statistical methods and access to the current investigation data files. Because most of this data and the required resources are readily available, this project was given a high-priority rating.

DISCUSSION

The projects listed here represent follow-up activities that the panel members specified collectively; they do not represent the universe of relevant future research. Each of the projects has the potential to provide new information about PV; however, some are more limited than others in providing information about the current cluster. The validity and usefulness of some projects are potentially affected by the results of others; thus, in many cases the projects are viewed as interrelated rather than as independent of one another. Accordingly, the overall research portfolio requires coordination and collaboration among the researchers involved.

ATSDR and PADOH believe four of the 12 evaluated projects would provide a direct benefit to the PV cluster area, are feasible given existing resources, and deserve the highest priority. These projects include the case-control study, comparison of the current cluster with locations having similar environmental conditions, continued PV incidence

monitoring, and further evaluation of the historical residential locations and potential environmental risk factors of the cluster cases.

ATSDR is addressing the proposed environmental study by evaluating the historical residential locations of the PV cluster patients and their relationship to potential environmental pathways and exposure sites. This work will continue the analyses from the initial investigation by using statistical and geospatial analytical tools and will involve a comprehensive review of existing historical documents relating to environmental exposures. The project will attempt to determine if a significant number of PV patients lived in a particular area during a period when a known or suspected environmental exposure occurred. Such relationships would provide a basis for further investigation of specific chemicals and sites. ATSDR is currently evaluating possible approaches for comparing the cluster population to populations with similar environmental challenges. Issues of concern when undertaking this comparison include study location(s), data quality and availability, and technically appropriate procedures for implementing this unusual study design.

PADOH will continue to monitor PV incidence in the tri-county area and will examine the feasibility of medical record review and JAK2 testing for all newly diagnosed PV patients. PADOH reviews cancer registry data each year and uses these data to calculate PV incidence rates in the tri-county region. These rates are compared with previous years and the overall state rate to identify trends. If JAK2 testing and medical record review are used to validate the diagnosis of all new PV patients, this project will also help

monitor improvements in diagnostic accuracy and reporting to the state cancer registry. The project is ongoing and will continue until the tri-county rates are commensurate with the rest of Pennsylvania.

ATSDR and PADOH determined that the remaining projects, because of their scope and technical requirements, are best addressed by academic and/or private research groups. The agencies will provide technical support to all external partners involved in this work.

The PV Expert Panel consisted of the following participants: Emmanuel Besa, MD (Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA); Patricia Colsher, PhD (West Virginia Dept. of Public Health); Ron Hoffman, MD, and Mingjiang Xu, MD (Mt. Sinai Medical Center, NY); Arthur Frank, MD (Drexel University, Philadelphia, PA); Samuel Lesko, MD (Northeast Regional Cancer Institute, Scranton, PA); Paul I. Roda MD, FACP (Geisinger/Hazleton Cancer Center, Hazleton, PA); Evelyn Talbott, DrPH (University of Pittsburgh, PA); Steve Ostroff, MD, Robin Otto, Gene Weinberg, Dr.PH, Jim Logue, Dr.PH, Mark White, MD, MPH, and Greg Bogdan, Dr.PH (PADOH); Bob Lewis, Mark Iannuzzo, and John Mellow (Pennsylvania Dept. of Environmental Protection); Brad White (U.S. Environmental Protection Agency); Bob German, Dr.PH and Frances Michaud (Centers for Disease Control and Prevention, Atlanta, GA); Steve Dearwent, PhD, Vince Seaman, PhD, and Lora Werner, MPH (ATSDR Region 3, Philadelphia, PA).

ATSDR has made this information available to all stakeholders and posted it on the PV Web site:

http://www.atsdr.cdc.gov/sites/polycythemia_vera/index.html.