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March 25, 2004

MULTICENTER STUDY OF HYDROXYUREA IN SICKLE CELL ANEMIA (MSH) PATIENTS' FOLLOW UP - EXTENSION I

PROTOCOL

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CHAPTER 1

BACKGROUND AND STUDY RATIONALE

1.1 INTRODUCTION

Sickle cell disease is a complex syndrome with multiple organ system disturbances brought about through the interplay of genetic, humoral, vascular, and environmental factors. The clinical course can be one of abrupt and insidious exacerbations and remissions, subsequently resulting in impairment of function, permanently damaged organs, and ultimately death.

1.2 MULTICENTER STUDY OF HYDROXYUREA IN SICKLE CELL ANEMIA (MSH) AND OTHER STUDIES OF HYDROXYUREA

The MSH randomized, double-blind clinical trial followed 299 patients with sickle cell anemia from January 1992 to February 1995. The study ended before the originally planned termination date because of strong evidence for the efficacy of hydroxyurea in the reduction of the frequency of acute vaso-occlusive (painful) crises among adult patients with moderate to severe sickle cell anemia (defined by at least three reported crises in the year prior to study entry).[1] At the conclusion of the MSH clinical trial, patients who had been assigned to placebo were offered the opportunity to start hydroxyurea therapy, and patients assigned to hydroxyurea were offered the opportunity to continue the therapy. Expected bone marrow suppression was observed among 79% patients assigned to hydroxyurea. Bone marrow suppression according to the operational definition used in the MSH occurred also in approximately 35% of the patients assigned to placebo. No deaths were attributed to therapy with hydroxyurea, and no severe or unexpected adverse outcomes were attributed to treatment with hydroxyurea

An increased number of patients assigned to hydroxyurea compared to MSH patients assigned to placebo (13 versus 4) were reported at routine six-month visits to have complaints of

bruising and bleeding. The increase was not associated with an increase in medical contacts; repeat reports of these complaints (i.e., complaints at more than one six-month visit) were made by only three patients assigned to hydroxyurea and two patients assigned to placebo. The only well documented causes of bleeding were reported for patients assigned to hydroxyurea -- menometrorrhagia in two patients and an anal fissure in one patient. Platelet counts were not suppressed to thrombocytopenic levels in any of the 17 patients with complaints. The increased frequency of bruising or bleeding reported may have been due to chance (a "false positive" or Type I error), errors in reporting, or to a true effect of hydroxyurea.

Other than bone marrow suppression, the MSH patients reported the effects of hydroxyurea listed in the product package insert (i.e., hair loss, skin rash, etc.) with similar frequencies among patients assigned to placebo and patients assigned to hydroxyurea. Over the course of one to three years of therapy, hydroxyurea did not appear to increase the frequency of important adverse clinical outcomes. Since the beneficial effects of hydroxyurea are thought to depend on continuing administration, sickle cell anemia patients may be taking hydroxyurea for more than three years. Observation of an adequate number of sickle cell anemia patients with more than three years of exposure to hydroxyurea will be necessary to obtain information on the occurrence of infrequent adverse outcomes.

There has been controversy concerning the potential of hydroxyurea for mutagenesis, teratogenesis and oncogenesis in humans. Alterations in sperm have been reported in laboratory rodents,[2] and teratogenesis in a variety of laboratory animals.[3-6] Although there are only sporadic case reports of pregnancies among women treated with hydroxyurea for malignancies, and a small number of men and women in the MSH who conceived (against study advice), none have had reproductive outcomes which would establish mutagenesis or teratogenesis in humans.[1,7]

The Polycythemia Vera Research Group has reported a higher frequency of acute leukemia among patients treated with hydroxyurea (3/59, 5.1%) than among historical, control patients who had phlebotomy alone (2/134, 1.2%), but the level of evidence is consistent with the interpretation that the difference is due to chance ($p > 0.10$).[8-11] Acute leukemia is known to occur spontaneously in patients with polycythemia vera.[11] Chromosome abnormalities have been reported to occur in polycythemia vera patients whose only treatment has been phlebotomy, but also more frequently among those who have been treated with alkalating agents, hydroxyurea or radioactive phosphorous (as single agents or in combination).[12] Although it would be logical for the occurrence of chromosomal abnormalities to precede the occurrence of acute leukemia, this relationship has not been observed by the Polycythemia Vera Study Group.[12] In a population of patients with polycythemia secondary to congenital heart disease, no occurrences of acute leukemia have been observed among those treated with hydroxyurea for as long as 15 years.[13]

From 1997 through 2002 an effort was made to contact all patients in an observational, follow-up study entitled the MSH Patients' Follow-Up. In the MSH Patients' Follow-Up all except 27 patients' clinical status was established on follow-up visits. Many of these 27 patients were reported informally to be alive and in stable condition by their treating physicians, but were unable to complete clinic visits for personal reasons. Continuing search of mortality data bases is made, also, to identify deaths that occur among these patients. The data collected in the MSH Patients' Follow-Up establish the following. First, over nine years of observation, there is a high level of mortality in the MSH patients population (86/299, 29%), a severely affected group. Second, the most frequent causes of death were pulmonary and sudden death associated with vaso-occlusive crises.[14] Third, there was an observational associations among survival and use of hydroxyurea, level of fetal hemoglobin, occurrence of acute chest syndrome and vaso-occlusive crises, and reticulocyte levels.[14] Neutrophil levels were not associated with mortality.[14] Fourth, no severe

chromosomal abnormalities were observed in the limited number of patients who had complete karyotype studies. Fifth, no cases of leukemia were observed. Sixth, three patients were diagnosed with cancer.[14] Carcinoma in situ was diagnosed as a screening finding in one patient and an incidental finding in another patient. Both carcinomas in situ were completely removed in the course of diagnostic procedures, and both patients died of causes unrelated to carcinoma in situ. One patient died of an invasive, endometrial carcinoma at 64 years of age after taking hydroxyurea for over nine years. Seventh, to date no evidence of association of hydroxyurea with perinatal mortality, developmental delay or impaired school performance has been found, but many of the children are not yet school age. Data on offspring of patients in the MSH are still being collected. Eighth, increased hemoglobin levels and decreased bilirubin levels continue in the observational follow-up to be associated with use of hydroxyurea.

1.3 CONCLUSIONS

Hydroxyurea may be an acceptable therapy for sickle cell anemia, but only a small number of patients with sickle cell anemia have been treated with hydroxyurea for long periods of time. Long-term follow-up of the MSH patients for clinical outcomes and laboratory findings could confirm the absence of consistent adverse effects in the MSH or provide evidence of mutagenesis, teratogenesis and oncogenesis, or specific organ/system damage.

1.4 REFERENCES

1. Charache S, Terrin ML, Moore RD, Dover GJ, Barton FB, Eckert SV, McMahon RP, Bonds DR and the Investigators of the Multicenter Study of Hydroxyurea in Sickle Cell Anemia. Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. N Eng J Med 1995; 332:317-22.

2. Evenson DP, Jost LK. Hydroxyurea exposure alters mouse testicular kinetics and sperm chromatin structure. Cell Prolif 1993; 26:147-59.

3. DePass LR, Weaver EV. Comparison of teratogenic effects of aspirin and hydroxyurea in the Fischer 344 and Wistar strains. J Toxicol Environ Health 1982; 10:297-305.

4. Ferm VH. Severe developmental malformations: malformations induced by urethane and hydroxyurea in the hamster. Arch Pathol 1966; 81:174-7.

5. DeSesso JM, Goeringer GC. The nature of the embryo-protective interaction of propyl gallate with hydroxyurea. Reprod Toxicol 1990; 4:145-52.

6. Adlard BPF, Dobbing J. Maze learning by adult rats after inhibition of neuronal multiplication in utero. Pediatr Res 1975; 9:139-42.

7. Jackson N, Shukri A, Ali K. Hydroxyurea treatment for chronic myeloid leukaemia during pregnancy. Br J Haematol 1993; 85:203-4.

8. Berk PD, Wasserman LR, Fruchtman SM, Goldberg JD. Treatment of polycythemia vera: a summary of clinical trials conducted by the Polycythemia Vera Study Group. In: Wasserman LR, Berk PD, Berlin NI, eds. Polycythemia vera and the myeloproliferative disorders. Philadelphia: W.B. Saunders, 1995; 166-94.

9. Fruchtman SM, Kaplan ME, Peterson P, Mack K, Berk PD, Wasserman LR. Acute leukemia (AL), hydroxyurea (HU), and polycythemia vera (PV): an analysis of risk from the Polycythemia Vera Study Group. Blood 1994; 84:Suppl 1:518a. abstract.

10. Boivin P. Indications, procedure and results for the treatment of polycythemia vera by bleeding, pipobroman and hydroxyurea. Nouv Rev Fr Hematol 1993; 35:491-8.

11. Weinfeld A. Swolin B, Westin J. Acute leukaemia after hydroxyurea therapy in polycythemia vera and allied disorders: prospective study of efficacy and leukaemogenicity with therapeutic implications Eur J Haematol 1994; 52:134-9.

12. Swolin B, Weinfeld A, Westin J. A prospective long-term cytogenetic study in polycythemia vera in relation to treatment and clinical course. Blood 1988; 72:386-95.

13. Triadou P, Maier-Redelsperger M, Krishnamooty R, et al. Fetal haemoglobin variations following hydroxyurea treatment in patients with cyanotic congenital heart disease. Nouv Rev Fr Hematol 1994; 36:367-72.

14. Steinberg MH, et al. Effect of hydroxyurea on mortality and morbidity in adult sickle cell anemia: risks and benefits up to 9 years of treatment. JAMA, in press.

CHAPTER 2

OVERVIEW OF STUDY OBJECTIVES AND DESIGN

2.1 INTRODUCTION

The MSH Patients' Follow Up – Extension I will be an observational, prospective study of the cohort of patients enrolled in the MSH and available for further follow-up. The MSH Patients' Follow Up – Extension I cohort will be defined as all those patients alive at the end of their participation in the MSH or MSH Patients' Follow-Up who give consent and are able to return for follow-up evaluations in the participating MSH Clinical Centers. Efforts will be made to continue surveillance of vital status for the patients who are not able to return for follow-up evaluations.

2.2 OBJECTIVES

The objectives of the Multicenter Study of Hydroxyurea in Sickle Cell Anemia (MSH) Patients' Follow Up – Extension I (MSH Patients' Follow Up – Extension I) will be to determine whether there are any long-term adverse effects of treatment of sickle cell anemia with hydroxyurea. Untoward effects of hydroxyurea could be reflected in mortality, adverse reproductive outcomes, the development of additional serious medical conditions, impairment or failure of specific organs or systems (e.g., kidney, liver, central nervous system).

The specific objectives in the long-term follow-up of the MSH patient population will be: to identify newly arising serious medical conditions and progressive impairment or failure of specific organs or systems; to assess all cause mortality and classify causes of death; to classify birth defects occurring in the offspring of patients since enrollment in the MSH; to assess quality of life for the patients; and to compare the function of specific organs or systems, all cause and cause specific mortality, frequency of birth defects and quality of life to those observed in relevant defined patient

populations such as the Clinical Study of Sickle Cell Disease (CSSCD) adult cohort and the general African-American population of the United States.

2.3 DESIGN FEATURES

2.3.1 Eligibility

Patients must meet only one inclusion criteria, a history of enrollment in the MSH. Patients will be excluded from the study only if there has been failure to obtain informed consent.

2.3.2 Patient Management

Clinical care for patients enrolled in the study will be the responsibility of their personal physicians. Findings of concern to the patients will be reported promptly to patients and their physicians, but all management decisions will be made by the patients and their physicians. Information on recommended therapy for the routine management of sickle cell anemia will be available in the form of NHLBI booklets and other publications.

2.3.3 Ascertainment of End Points

At annual medical visits a complete evaluation of each patient including history, physical examination, review of laboratory findings (from the MSH Patients' Follow Up – Extension I Clinical Center and other Medical Centers), and blood specimen collection will be performed. This data collection will be used to identify all hospitalizations and serious medical conditions as well as obtain blood specimens for cytogenetic studies. A list of data collection forms and schedule is given in Appendix I and laboratory determinations in Appendix II.

2.3.4 Size of Study and Duration of Follow-Up

The enrollment is anticipated to include all surviving MSH patients willing to participate (210 patients). These patients will complete clinic visits annually for at least five years (five visits). A study time line is given in Appendix III.

2.3.5 Data Monitoring and Patient Safety

Annually the MSH Patients' Follow-Up Data and Safety Monitoring Board (DSMB) will review summaries of data provided by the Medical Coordinating Center. If any conclusions bearing on patient safety are made (e.g., hazards associated with hydroxyurea therapy), they will be communicated to the MSH Patients' Follow-Up Clinical Center Directors and patients promptly.

Individual serious events such as deaths, birth defects or occurrences of cancer will be reported to the NHLBI and DSMB chairman as they occur.

2.4 OUTCOMES TO BE MONITORED

Outcomes to be monitored include: death; stroke; renal failure; hepatic failure; coma; sepsis and other serious infections; birth of a child or termination of pregnancy; cytogenetic and DNA analysis results (chromosome breakage, translocation, abnormal chromosomes and micro- satellite instability); and health outcomes of offspring.

CHAPTER 3

PATIENT ELIGIBILITY AND PATIENT ORIENTATION

3.1 INTRODUCTION

The MSH demonstrated important reductions in the frequency of acute vaso-occlusive (painful) crises among sickle cell anemia patients who had more than two crises per year and were given hydroxyurea therapy as compared to placebo. Hydroxyurea therapy requires close medical supervision and frequent phlebotomy to monitor for bone marrow toxicity. Other than expected myelosuppression, the MSH patients assigned to hydroxyurea did not experience acute adverse effects of treatment with hydroxyurea during two to three years of treatment.

There is a genuine uncertainty in the medical community as to whether or not hydroxyurea therapy for sickle cell anemia entails substantial long term risks. Since the MSH was conducted with adult patients, the MSH Patients' Follow Up - Extension I is designed for adult patients. The uncertainty concerning long-term risk of cancer, mutagenicity and teratogenicity (observed in animals but not in humans) is problematic for the continued use of hydroxyurea therapy among adults with sickle cell anemia. Uncertainty as to the risks of hydroxyurea therapy in sickle cell anemia will be remedied for adult patients in large part by information to be obtained from the MSH Patients' Follow Up - Extension I.

During orientation, the nature of the study, the procedures to be followed, and the level of commitment to the MSH Patients' Follow Up - Extension I required for study participation will be explained to the patient. Orientation will also provide an opportunity to address each potential participant's concerns and questions regarding the MSH Patients' Follow Up - Extension I.

3.2 ELIGIBILITY CRITERIA

Patients must meet only one inclusion criteria, a history of enrollment in the MSH. Patients will be excluded from the study only if there has been failure to obtain informed consent.

3.3 PATIENT ORIENTATION

Patients will be informed that this is an observational follow-up study; that the purpose is to learn the health outcomes of patients who were in the MSH; that the long-term risks of hydroxyurea therapy are still unknown. The procedures for eligibility, enrollment, follow-up and close-out will be explained to each individual being recruited for the MSH Patients' Follow Up - Extension I.

3.4 INFORMED CONSENT

Written informed consent must be obtained from each patient, and no Clinical Center will begin enrolling patients before its consent form is on file at the Medical Coordinating Center. The exact language used on a Clinical Center's consent form may vary from institution to institution, but the text must be comprehensible to persons with a 7th grade reading level, and no form will be considered as having been given final approval until it has been reviewed by the Medical Coordinating Center, NHLBI, and Data and Safety Monitoring Board (DSMB) Chair. A draft, model consent form is included in Appendix VI. Items relevant to obtaining informed consent for participation in the MSH Patients' Follow Up - Extension I are listed below:

1. The purpose of the study is to determine the health outcomes of patients who were in the MSH; whether hydroxyurea has any effects on the occurrence of cancer or reproductive outcomes over a five year period of time is of particular interest.
2. The extent of patient involvement is one out-patient visit every year. About 3 tablespoons of blood (45 ml) will be taken on each visit; over the course of the entire study, about 15

tablespoons (450 ml maximum) of blood will be drawn. Other annual health evaluations paid for by the study will include urinalysis, electrocardiography, biochemistry and special blood analyses (ferritin, catecholamine and arterial blood gas analyses), and a complete blood count. Three times in the course of the study, each patient will have special studies of the lungs (pulmonary function tests and high resolution computed tomography of the chest). Each clinic visit will take about two to three hours. A member of the clinic staff will administer a questionnaire concerning each patient's medical and reproductive history, major medical events and the quality of the patient's life in the interval since the previous visit.

3. The main risk of hydroxyurea therapy is bone marrow depression, with attendant risks of bleeding, infection, and increased symptoms of anemia. Bleeding and infection due to treatment were not encountered during the MSH. Other risks which have been described, but not encountered in the MSH, include GI disturbances, dermatological abnormalities (including skin rash and hair loss), and liver or kidney dysfunction. There may be other risks. Although they are unlikely to occur, we want to know if they do occur. If hydroxyurea increases the risk of developing cancer, the risk is small. The patient should be aware that non-invasive cancer occurred and was cured in two MSH patients, who died of other causes and caused one death among the 299 MSH patients, of whom 89 are know to have died.

4. There is also a risk of teratogenesis and mutagenesis. If pregnancy should occur in a female patient, she should be counseled, and she should not continue taking hydroxyurea while pregnant. Men who have taken hydroxyurea and father babies must understand the possibility of fetal abnormality, and they and their partners should receive the same

counseling given women in the study who become pregnant. We want complete information concerning any baby born during or after the MSH clinical trial to MSH patients or their partners. We also want to complete telephone interviews with the parents (or guardian(s)) of any children of MSH patients as these children grow up, and to keep track of those children's progress in school.

5. Patients will be reimbursed $30 per annual visit for travel and clinic visits costs.

6. If patients become ill, because of sickle cell anemia or some other illness, the study has made no provision to pay for medical care. It also has made no provision to pay for analgesics.

7. By signing the consent, the patient agrees to give the MSH Patients' Follow Up - Extension I investigators permission to get records from any medical facility attended during the study. The study records will be kept confidential; patients will not be identified by name; but, data may be shared with the National Heart, Lung, and Blood Institute, the manufacturer of hydroxyurea, or the U.S. Food and Drug Administration (FDA). The FDA can audit medical records at the Clinical Center.

8. The alternative to participating in the study is for the patient's medical care to continue as before. Patients may withdraw from the study at any time, without detriment to them or disadvantage in treatment from any person or institution affiliated with the MSH Patients' Follow Up - Extension I.

9. By signing the consent, the patient acknowledges that he/she understands what he/she has been told, and that all of his/her questions regarding the study have been answered.

10. In accord with local institutional requirements, means for seeking more information about patient protection and redress from injury due to the study, must be spelled out.

11. This Protocol is conducted with the oversight of independent individuals responsible for patients well being (e.g., the local Institutional Review Board) and in compliance with privacy protection requirements such as those included in the Health Insurance Portability and Accountability Act (HIPAA).

CHAPTER 4

FOLLOW-UP PROCEDURES

4.1 INTRODUCTION

Patients will be scheduled in the MSH Patients' Follow Up - Extension I for five annual visits. Every attempt should be made to register every living MSH patient in the MSH Patients' Follow Up - Extension I. Patients can be enrolled and followed at any MSH Patients' Follow Up - Extension I Clinical Center. At the time of each annual visit, a medical review is conducted including ascertainment of death, reproductive events, adverse events, or offspring outcomes, and socioeconomic and quality of life measures. Clinical studies are performed and specimens for laboratory determination are collected.

All specified events ascertained in a patient or an offspring from the annual medical examination and history or diagnostic procedures will be reported on the appropriate event form (patient or offspring) accompanied by appropriate documentation from the medical record.

Appendix I shows the schedule of data collection and forms.

4.2 FOLLOW-UP VISITS

At the annual visits, medical reviews will be conducted including measurement of weight, ascertainment of possible adverse events, major procedures, current therapies (including hydroxyurea) and pregnancies of patients or partners.

According to study schedule special studies may be performed (high resolution computed tomography, pulmonary function tests, arterial blood gases analyzed, electrocardiography, urinalysis). Blood specimens will be collected and prepared for local laboratories or shipment to one

of the Core Laboratories. Each patient's current address and telephone number will be updated and maintained in the Clinical Center files.

4.2.1 Ascertainment of Specified Events and Possible Adverse Effects in Patients

At the annual visit, the patient will be questioned about any major medical procedures or diagnoses. Specified major events will be documented to ascertain the nature of the event, including indication(s) and treatment(s). Reportable events and diagnoses (see Section 5.5) will be followed up by the clinic coordinator for collection of documentation such as emergency room reports, hospitalization reports and office visit records. These will be forwarded to the Medical Coordinating Center where they will be compiled for classification.

The event report forms and documentation will identify the occurrence of death, stroke, cancer, hospitalizations unrelated to acute vaso-occlusive (painful) crisis, pregnancy in patient or partner, or birth of a child. Outcomes of all pregnancies in patients or partners will be documented. If any study patient dies, efforts will be made to obtain complete post-mortem information. Discharge summaries, and narratives of the fatal events will be reported on study forms and sent to the Medical Coordinating Center.

All reportable events, whether treated on an out-patient or in-patient basis, will be reviewed by the Medical Coordinating Center Principal Investigator. Adverse treatment effects will be reported to the U.S. Food and Drug Administration (FDA).

4.2.2 Ascertainment of Specified Events in Offspring

At annual visits, patients will be queried regarding specified medical events among any offspring conceived since the patient's enrollment in the MSH clinical trial (1992 forward). These will include perinatal and newborn difficulties, birth defects and hospitalizations for conditions other than those for acute vaso-occlusive (painful) crises. In addition, Clinical Center staff will ascertain

the offspring's routine care physician, and obtain from that source summaries of growth and histories of developmental milestones, to be used for completion of an annual review of the offspring's health. A centrally located neuropsychologist will use staff under his direction to contact parents (or guardians) of these offspring for a standard telephone interview to assess developmental progress.

4.2.3 Laboratory Specimen and Data Collection

Blood specimens may be collected at each annual visit. Annually, Clinical Center staff will use the specimens to collect local laboratory data for routine hematology and biochemistry, also urinalysis and electrocardiography will be performed. These results will be reported on study forms.

Specimens for DNA and cytogenetic studies will be collected once in the course of the study for each patient and shipped by overnight courier to the appropriate Core Laboratories. Serum specimens will be collected annually, frozen at -80°C and shipped in batches to the NHLBI Biological Specimen Repository.

4.3 PATIENT FOLLOW-UP AND MANAGEMENT

The major difficulty to be overcome in successfully completing follow-up procedures in the MSH Patients' Follow Up - Extension I is maintaining contact with all living patients. The major responsibility for this task rests with the Clinical Center staff. Medical Coordinating Center staff will assist the Clinical Center staff with schedules for follow-up and recommendations for techniques (e.g., use of criss-cross telephone directories, contact with friends and relatives) to recontact patients whose follow-up is interrupted.

Strategies to increase or optimize contact with MSH patients include:

1. providing comprehensive care for management of sickle cell anemia, including hydroxyurea therapy;

2. maintaining (at least monthly) telephone contact with the patient, (keeping it on the friendly, caring side), or a member of the family or close friend especially for those patients who are difficult to reach;
3. regular telephone contact with a primary care giver;
4. maintaining up-to-date, accessible records of MSH Patients' Follow Up - Extension I enrollees' whereabouts including address, telephone numbers, work, family, friends, doctor(s), social workers, religious or care organizations, etc.

Medical management of patients during the MSH Patients' Follow Up - Extension I remains entirely with the primary caregiver(s). MSH Patients' Follow Up - Extension I study leadership, the NHLBI, and Clinical Center Directors endorse current standards of good medical practice for routine care of patients enrolled in the MSH Patients' Follow Up - Extension I. The MSH Patients' Follow Up - Extension I will provide neither direct routine care nor reimbursement for care of patients enrolled in the study.

4.4 CLOSE-OUT VISIT

The close-out visits (annual visit 5) should be completed for all patients within a relatively short period of time. Every effort will be made to schedule patients for the close-out visit. Vital status will be ascertained on all patients lost to follow-up including at least one search via the National Death Index.

4.5 DEBRIEFING CONTACT

After final data have been collected and final reports prepared and submitted for publication, patients will be scheduled for a debriefing contact. They will be informed of the primary results of the study and the recommendations of the MSH Patients' Follow Up - Extension I investigators.

CHAPTER 5

DATA AND SPECIMEN COLLECTION

5.1 INTRODUCTION

Data collection in the MSH Patients' Follow Up - Extension I comprises data assessed or measured at annual visits and information documenting events which typically occur between scheduled annual visits. The annual visit forms are administered during the patients' regular visits. The information documenting reportable events in patients and their offspring will be abstracted briefly onto study forms and supplied with supporting documents copied from the patient's or offspring's medical record (hospital, doctor's office, etc.)

5.2 REGISTRATION

Every attempt must be made to register every living MSH patient.

Every attempt must be made to register all offspring conceived and born since the patient's enrollment in the MSH (January 1992-April 1993) including all offspring born during MSH Patients' Follow-Up and MSH Patients' Follow Up - Extension I.. The offspring registration will include a neonatal and hemoglobin assessment. Lists of MSH patient offspring reported during the MSH clinical trial will be provided to Clinical Center staff to begin the offspring registration process.

5.3 HISTORY AND PHYSICAL EXAMINATION

The purpose of the annual history and examination is to review the patient's and offsprings' medical condition so as to ascertain any reportable condition or event that has occurred to a patient or an offspring since the patient's MSH Close-Out Visit (January 1995) or the last completed MSH Patients' Follow Up - Extension I annual visit.

5.4 LOCAL LABORATORY DATA

Clinical Centers will collect blood, serum and urine specimens at each annual visit. Routine hematology, biochemistry and urinalysis studies will be performed locally; results will be reported on study forms.

Appendix II shows the analyses to be performed locally for routine hematology, biochemistry and urinalysis.

5.5 CLINICAL EVENTS

The main outcomes of the MSH Patients' Follow Up - Extension I are any possible adverse event or condition of therapy with hydroxyurea, either during the conduct of the MSH trial or during hydroxyurea therapy initiated after the end of blinded treatment in the MSH. The annual visit provides an opportunity to perform a physical examination, query the patient about any major medical events and assess results of laboratory determinations. Conditions reported on study forms from the physical examination, medical history and laboratory results will be reviewed at the Medical Coordinating Center. Clinical Center staff may subsequently be asked to submit reports of specific events, including date(s) of occurrence, and attach documentation from the medical record (hospital, doctor's office, etc.) Possible events occurring in patients' offspring will similarly be ascertained, reported and documented. In addition to live births, the results of terminated pregnancies is of particular importance. All pregnancies in patients and their partners, as well as their outcomes, are reportable. Reportable patient events include:

Death

Stroke

Renal failure

Hepatic failure

Cancer

Sepsis and other serious infections

Birth of a child or termination of pregnancy

Reportable offspring events and data include:

Neonatal abnormalities

Developmental milestones in the first three years of life

Cancer

Clinic Center Directors and Coordinators must keep these conditions in mind at all times, not only at annual visits (including the first annual visit). Any of these events may be reported as soon as they occur and documentation is available. Regular contact with patients, their care givers, and offsprings' pediatricians will increase the likelihood of prompt reporting of events.

5.6 OUTCOMES

The reports of events in patients and their offspring will be reviewed by the Medical Coordinating Center Principal Investigator. His assessment will be codified onto study forms, using ICD-9 codes. This assessment and classification will form the basis of reporting study outcomes to the Data and Safety Monitoring Board, to the U.S. FDA, and as deemed necessary and for publication of final study results.

5.7 BLOOD SPECIMENS FOR CENTRAL PROCESSING

Clinical center staff will prepare frozen serum specimens for the NHLBI serum bank, and specimens for fetal hemoglobin, DNA and cytogenetic studies for the Core Laboratories, ship them, and file copies of corresponding specimen transmittal lists to the Medical Coordinating Center. Clinical Centers will label specimens for the Core Laboratories and NHLBI Biological Specimen

Repository according to procedures provided by the Medical Coordinating Center, such that patient confidentiality and quality control can be maintained through anonymity of specimens. The Core Laboratories will perform the requisite studies and transmit electronic files of results to the Medical Coordinating Center on an agreed schedule.

CHAPTER 6

PARTICIPATING UNITS

6.1 INTRODUCTION

The MSH Patients' Follow Up - Extension I will be organized around 21 Clinical Centers, a Medical Coordinating Center, a Core Laboratory, and the National Heart, Lung, and Blood Institute (NHLBI) Sickle Cell Disease Scientific Research Group. The Clinical Center staff will be trained in accordance in the procedures set out in the study Manual of Operations. The objective is to standardize all study procedures carried out in the Clinical Centers and at the operational central units.

Study monitoring will be carried out by the Data and Safety Monitoring Board (DSMB), Steering Committee and Executive Committee. Monitoring will include adherence to Protocol, achievement of recruitment goals, patient safety and quality of data. Subcommittees of the Steering Committee (e.g., writing, publications, ancillary studies) will review proposals for secondary analysis and ancillary studies.

An organizational chart of the MSH Patients' Follow Up - Extension I is presented in Exhibit 6-1.

6.2 PARTICIPATING UNITS

6.2.1 Medical Coordinating Center

The Medical Coordinating Center staff will comprise the Principal Investigator/Medical Coordinating Center Director, Study Manager/Deputy Director, Statistician and Coordinator. Medical Coordinating Center staff for the MSH Patients' Follow Up - Extension I will provide expertise in the areas of study design, quality control, data processing and data analysis. The Medical Coordinating Center will provide biostatistical and epidemiological advice for the overall

conduct of the MSH Patients' Follow Up - Extension I; collaborate with the MSH Patients' Follow Up - Extension I investigators in all phases of the study including planning, participant recruitment and follow-up, development and maintenance of a data management system for MSH Patients' Follow Up - Extension I, preparing required statistical analyses; generate Core Laboratory work lists, report forms, blood specimen transmittal lists, and progress reports; implement a quality assurance plan; and, assist in the preparation of manuscripts for publication. Medical Coordinating Center staff will undertake the primary responsibility for the collection, processing, storage, and analysis of the study data as well as to ascertaining that the provisions of the Protocol are carried out by each participating Clinical Center.

The Medical Coordinating Center has fiscal and administrative responsibility for the contracts which govern the Clinical Centers and the Core Laboratory.

Medical Coordinating Center staff will provide substantial technical and scientific guidance in developing the Protocol and Manual of Operations, study forms, Clinical Center procedures, quality control systems, and laboratory specimen preparation, processing and reporting. The Medical Coordinating Center will also collaborate in formulating agendas, producing reports and providing minutes for Data and Safety Monitoring Board and Steering Committee meetings.

6.2.2 Clinical Centers

Each site for follow-up of patients in the MSH Patients' Follow Up - Extension I will be known as a Clinical Center. During this study, Clinical Center Directors and Coordinators will participate in Steering Committee and subcommittee meetings. The Clinical Center will collect the data in accordance with the provisions of the study Protocol and Manual of Operations at the local level, and will coordinate patient care so that each patient receives optimal medical management. The 21 collaborating centers, which are funded by subcontracts through the Medical Coordinating

Center, will each have staff to serve as a Clinical Center Director and a Coordinator. Exhibit 6-2 lists the Clinical Centers participating in the MSH Patients' Follow Up - Extension I.

6.2.3 Core Laboratories

The Core Laboratory has responsibility for receiving blood samples from the Clinical Centers and performing fetal hemoglobin analyses, DNA analyses or cytogenetic analyses.

6.2.4 National Heart, Lung, and Blood Institute

The MSH Patients' Follow Up - Extension I is an initiative of the National Heart, Lung, and Blood Institute (NHLBI) staff -- (Division of Blood Disease and Resources) Sickle Cell Disease Scientific Research Group (SRG). The Biostatistics Research Branch (Division of Epidemiology and Clinical Applications) will assist study investigators and key study personnel through all phases of the study. A member of the SRG will serve as a voting member on the Steering Committee, and other study committees. The NHLBI membership on the Steering Committee is for the purpose of providing direction from the NHLBI throughout the phases of Protocol development, recruitment, follow-up, data analysis and interpretation.

The NHLBI staff will provide technical assistance in the monitoring of issues concerning recruitment, treatment, follow-up, quality control, and adherence to Protocol to assist the study investigators in assessing potential problems affecting the study and potential changes in the Protocol. They have contractual responsibility for the management of the study funds. A Data and Safety Monitoring Board will be appointed by the NHLBI to provide overall monitoring of the study.

6.3 STUDY ADMINISTRATION

6.3.1 Study Chairman

The Chairman and Vice Chairman of the Steering Committee will be elected by the Steering Committee.

6.3.2 Steering Committee

The Steering Committee will be composed of all MSH Patients' Follow Up - Extension I investigators and Coordinators from each Clinical Center, investigators and co-investigators of the Medical Coordinating Center, and NHLBI staff representatives. The Steering Committee will be responsible for organizing and planning the study. Steering Committee members are expected to participate in writing committees and other subcommittees as needed. The Steering Committee will meet annually in Bethesda, Maryland. There will be only one designated voting member per Clinical Center; a designated voting member (e.g., Clinical Center Director or representative) must be present to vote.

Writing committees will prepare for publication data pertinent to that special area, and submit manuscripts for approval by the Publications Committee. Members of these writing subcommittees will be able to review data for manuscript preparation directly with the Medical Coordinating Center staff.

The Publications Committee will review and evaluate all proposed oral/poster presentations and manuscripts that will utilize study data. Recommendations of the Publications Committee will be forwarded to the NHLBI for review. Approval of the NHLBI will be required before any study data can be submitted for peer review.

Clinical Center Directors may bring any issue concerning the MSH Patients' Follow Up - Extension I in their own clinics to the attention of the Steering Committee for reporting in a peer-

reviewed journal. If the Steering Committee recognizes the issue as one of the study-wide importance, the initiating Clinical Center Director will have available the study-wide data for publication. If there is no study-wide report produced in a timely fashion (i.e., within one year), the proposing Clinical Center Director may report data from his or her own Clinical Center in compliance with the routine requirements of the NHLBI for report preparation and submission. If the Steering Committee does not agree that the matter is of study-wide importance, the Clinical Center Director will be permitted to prepare a report immediately based on his or her Clinical Center's data in compliance with NHLBI policies.

6.3.3 Executive Committee

The members of the Executive Committee will be Chair of the Steering Committee, Chairs of all special subcommittees, Principal Investigator of the Medical Coordinating Center, and NHLBI staff. In addition, five (5) members of the Steering Committee will be elected on a rotating basis to serve for a period of one year, with possibility of re-election. One of the representatives to the Executive Committee will be elected from the Coordinators.

The Executive Committee will provide final recommendations and plans developed by the Steering Committee to the Sickle Cell Disease SRG and the Data and Safety Monitoring Board (DSMB). In addition, this committee will provide scientific advice at the operational level subsequent to the planning phase. This committee will review any proposed changes in the Protocol, forms, and Manual of Operations. The Executive Committee will plan to convene monthly (12 times but at least twice each year) by conference call, if necessary, with additional meetings to be called as needed by the Chair.

6.3.4 Data and Safety Monitoring Board

The Data and Safety Monitoring Board (DSMB) will be composed of five members who would be appointed for the duration of the study by the NHLBI. The DSMB will act in a senior advisory capacity on policy matters and will review study progress to observe that study goals are being met. DSMB members may not participate in the study as investigators. The DSMB will rely upon the Executive Committee, the Steering Committee, the Medical Coordinating Center and the Sickle Cell Disease SRG to provide information to aid the DSMB in its duties.

The DSMB will review the initial study Protocol and approve all changes made to it during the course of the study, review Data and Safety Monitoring Reports, and make recommendations on major Protocol changes. The Executive Committee will report any unexpected or unusual findings to the DSMB which may be convened ad hoc for a special review of the MSH Patients' Follow Up - Extension I any time circumstances so warrant. The Board will meet at least yearly, to review the annual MSH Patients' Follow Up - Extension I report.

EXHIBIT 6-1

MSH PATIENTS' FOLLOW UP - EXTENSION I

ORGANIZATIONAL CHART

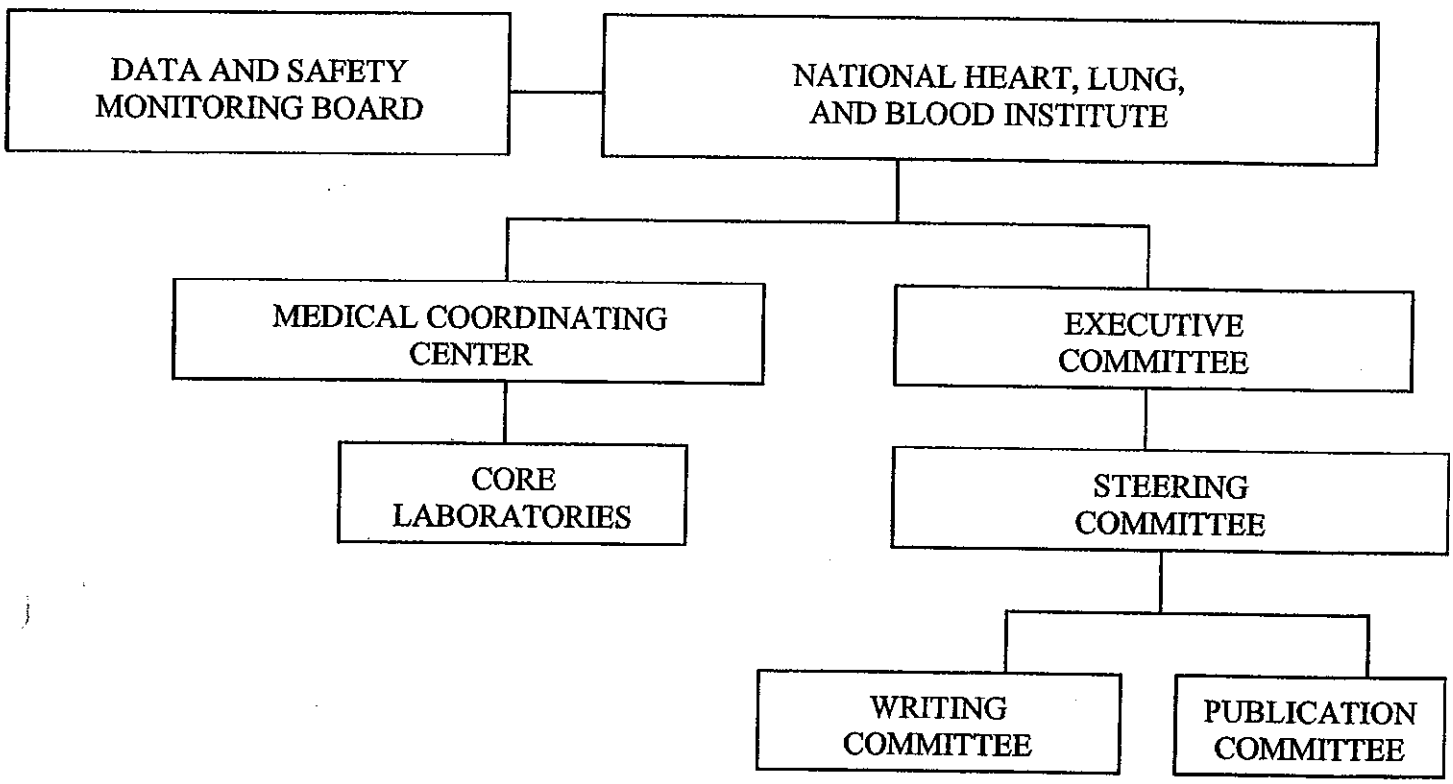

EXHIBIT 6-2

Clinic No.	Clinic	Maximum Projected No. of Patients
01	University of North Carolina (Chapel Hill, North Carolina)	16
02	Duke University (Durham, North Carolina)	10
03	Medical College of Georgia (Atlanta, Georgia)	10
04	Jefferson Medical College (Philadelphia, Pennsylvania)	14
05	University of Mississippi (Jackson, Mississippi)	16
06	University of Miami (Miami, Florida)	7
07	University of California (San Francisco, California)	6
08	University of Illinois (Chicago, Illinois)	42
09	Howard University (Washington, D.C.)	11
10	University of Medicine and Dentistry of New Jersey (Newark, New Jersey)	7
11	Emory University (Atlanta, Georgia)	11
13	St. Luke's - Roosevelt Medical Center (New York, New York)	15
14	Children's Hospital of Oakland (Oakland, California)	4
15	Medical College of Virginia (Richmond, Virginia)	15
16	Case-Western Reserve University (Cleveland, Ohio)	4
17	The Hospital for Sick Children (Toronto, Canada)	4
18	Brigham and Women's Hospital (Boston, Massachusetts)	5
19	New York Methodist Hospital (Brooklyn, New York)	4
21	University of Alabama (Birmingham, Alabama)	5
22	University of Pittsburgh (Pittsburgh, Pennsylvania)	4
28	Michael Reese Medical Center (Chicago, Illinois)	6
Totals		217

CHAPTER 7

CONDUCT OF THE STUDY

7.1 INTRODUCTION

The study time-line is presented in Appendix III. During the start-up period of approximately six months, the Protocol and Manual of Operations are developed, the Core Laboratory is identified, the Medical Coordinating Center and NHLBI Biological Specimen Repository put their procedures into place, and Clinical Centers continue maintaining contact with all living MSH patients. Towards the end of the start-up period, the Data and Safety Monitoring Board meets to approve the Protocol and a series of conference calls will be conducted for the Steering Committee, clinic staff members and Medical Coordinating Center personnel as training sessions. Clinical Center personnel are certified. Patient registration (Year 1 visits) begin as soon as Clinical Centers are certified to enroll patients, and continues through the 12th month from the beginning of the planning phase.

Follow-up continues five years after the beginning of enrollment, at which time the last of the five-year visits are completed. Clinical Center, Medical Coordinating Center and National Heart, Lung, and Blood Institute staff will collaborate throughout all phases of the study.

7.2 TRAINING AND CERTIFICATION

The goal of training and certification is to standardize all procedures relating to the conduct of the study.

Clinical Center directors and coordinators must participate in at least one training session and successfully complete the certification process, which will require submission of a final consent form approved by the Medical Coordinating Center and local IRB, documentation of Health Insurance Portability and Accountability Act (HIPAA) compliance, documentation of appropriate

Human Subjects Protection training, and satisfactory completion of practice procedures and data collection with prior MSH Patients' Follow-Up or sample patients. Study procedures include: scheduling and preparing for patient visits; phlebotomy, specimen preparation, packaging and shipment; conduct of orientation, eligibility, enrollment and follow-up visits; completion of all study forms and procedures directed therein, including medical reviews and examinations and interviewing patients; referral of patients for counseling and/or other follow-up; requesting and abstracting documentation from primary care facilities for possible events; and responding to edit messages from the Medical Coordinating Center.

Training is conducted and certification is issued by the Medical Coordinating Center staff.

7.3 DATA EDITING AND MANAGEMENT

7.3.1 Introduction

The Medical Coordinating Center will serve MSH Patients' Follow-Up as the repository of all forms, documents and minutes. Thus, Clinical Centers will send to the Medical Coordinating Center the original of each MSH form completed and retain a copy for Clinical Center files. All MSH Patients' Follow-Up data collection forms and copies of transmittal lists for blood specimens shipped in the course of data collection will be sent to the Medical Coordinating Center. Medical Coordinating Center staff will monitor the arrival of forms and transmittal lists to identify form delinquencies based on appointment schedules and anticipated study forms. Medical Coordinating Center staff will monitor Core Laboratory and NHLBI Biological Specimen Repository specimen receipt dates for specimen delinquencies based on appointment schedules, anticipated specimens, and reports of specimens received in the laboratory and repository.

7.3.2 Receipt and Inventory

Medical Coordinating Center staff will receive, log in and prepare all forms for data entry. The Medical Coordinating Center will receive MSH Patients' Follow-Up forms submitted with transmittal lists. Clinical Centers should send specimens directly to the Core Laboratory and NHLBI Biological Specimen Repository with copies of transmittal lists to the Medical Coordinating Center. Only forms accompany transmittal lists sent to the Medical Coordinating Center. Mail from the Clinical Centers will be opened immediately and forms will be stamped with the date of receipt. Transmittal lists are compared with forms received and data transmitted from the Core Laboratory. Any discrepancies between crucial patient identifiers (e.g., name code, ID number or date of study entry/follow-up visit) that Medical Coordinating Center staff find on forms received will be brought to Clinical Center attention immediately by a telephone call.

7.3.3 Expected Receipt of Forms

The expected dates for receipt at the Medical Coordinating Center of patients' forms will be: two days after clinic visit for Core Laboratory Specimen Transmittal Lists, the end of calendar quarters for the NHLBI Serum Repository, and two weeks for all forms required at each annual visit (see Appendix I). Forms or specimens not sent to the Medical Coordinating Center or Core Laboratory within two weeks of the expected date will be denoted as delinquent.

7.4 QUALITY CONTROL PROCEDURES

7.4.1 Monitoring the Clinical Centers

Medical Coordinating Center staff will produce monthly reports from the data entered from forms submitted for each entered patient. A sufficiently low data collection performance will be responded to by a site visit from Medical Coordinating Center and NHLBI staff. Failure to improve performance after such a site visit may result in an end to support for a Clinical Center.

On a regular basis, the Executive Committee (composed of staff from the Medical Coordinating Center, NHLBI, and rotating members from the Steering Committee) will confer by conference telephone call to review recruitment goals and protocol violations reported for each Clinical Center. Clinics will be notified of violations with suggestions for remedial action. Repeated violations will result in a site visit by the Principal Investigator of the Medical Coordinating Center and an NHLBI staff member.

At each scheduled Steering Committee meeting (see Appendix III), a report of progress toward accomplishment of study goals will be presented, both for the study as a whole and for individual Clinical Centers. These reports may include certification status, number of patients registered, completeness of scheduled visit data collection, completeness of specimen collection, completeness of events reporting, adherence to study protocol and results of actions taken to improve data collection.

7.4.2 Site Visits

Prior to suspension of payments or separation of a Clinical Center from the study, the Principal Investigator of the Medical Coordinating Center will visit the Clinical Center and provide a site-visit report to the Data Safety and Monitoring Board (DSMB) for recommendation on final action. The Clinical Centers with the greatest difficulty in meeting proposed goals for patient enrollment may also be site visited, and recommendations made for improved performance together with a report to the DSMB. Clinical Centers which are not having problems with performance may also be visited once during the study, to assure quality of data produced. For regularly scheduled site visits as well as site visits for special causes, Medical Coordinating Center staff will generate computer printouts of form data for comparison to Clinical Center form copies and to actual patient charts.

7.4.3 Monitoring the Core Laboratory

The Core Laboratory will be monitored for timely submission of data to the Medical Coordinating Center based on receipt of copies of transmittal lists from the Clinical Centers. Medical Coordinating Center staff will set aside selected study identification numbers and create name codes for use with these reserved identification numbers on labels for the submission of replicate specimens for a program of external quality control monitoring. Summaries of Core Laboratory activities and data will be provided in performance reports including counts of specimens received, data delivered to the Medical Coordinating Center and reproducibility on blind replicates. The Medical Coordinating Center will include in its reports data on Core Laboratory performance and internal quality assurance monitoring for DSMB review.

7.4.4 Medical Coordinating Center

Medical Coordinating Center activities in MSH Patients' Follow-Up will be checked internally to help enhance the quality of data and analyses. Persons (such as the Principal Investigator or Deputy Director) not involved in the preparation of the data editing programs will fill out test study data forms, making deliberate errors. These forms will be keyed and processed through the data editing system to see if all of the errors were caught by the data management system. An audit of a sample of original data forms against the data on the Medical Coordinating Center computer will be used to detect problems with the data entry and with editing software prepared and provided by the Medical Coordinating Center.

New analysis programs (including runs using statistical packages such as SAS) will be tested by running against a small subfile of 10 or 20 participants and independently producing the tabulations and statistical calculations manually from the original data. This will help to assure the correct variables have been selected from the analysis file, the variables and cut-points have been

defined properly, and transformations of the original variables on the analysis file have been formulated correctly.

7.5 PAYMENTS FOR CLINICAL CENTERS

Payments from the Medical Coordinating Center to the Clinical Centers will be based on the numbers of patients enrolled and data collection completed. Payments will be made on a quarterly schedule.

CHAPTER 8

POLICY MATTERS

8.1 INTRODUCTION

Procedural guidelines are established to ensure that all Clinical Centers adhere to the Protocol, to facilitate optimum use of data generated by the study, and to ensure optimal use of the resources of the Core Laboratory and Medical Coordinating Center (for quality control in the study see Section 7.4).

8.2 QUALITY ASSURANCE

Members of the Steering Committee will establish criteria defining protocol violations. Violations may involve repetitive failure to obtain Follow-Up information or failure to file reports in timely fashion (form delinquencies).

Any Clinical Center Director experiencing problems with violations will be asked to submit a proposal outlining how recurrence will be prevented. The Data and Safety Monitoring Board will be made aware of the occurrence of protocol violations.

The Medical Coordinating Center will document violations in performance reports, as well as notifying the Clinical Centers of them. Repeated minor violations which are not corrected will result in suspension of payments to the clinic, which will resume when minor violations are corrected.

Prior to suspension of payments, the Principal Investigator of the Medical Coordinating Center will visit the Clinical Center and provide a site-visit report to the Data and Safety Monitoring Board (DSMB) for recommendation on final action. The clinics with the greatest difficulty in meeting their proposed goals for recruitment will also be site visited, and recommendations for improvement made to them, with a report to the DSMB Chair. Centers which are not having

problems with performance will also be visited once during the study, to assure quality of data produced.

8.3 TYPES OF MSH PATIENTS' FOLLOW-UP RESEARCH

The Steering Committee will exercise responsibility for all end point, data bank, and ancillary studies, and for all publications and presentations evolving from the MSH Patients' Follow Up - Extension I research.

Investigators at all MSH Patients' Follow Up - Extension I sites, including the Medical Coordinating Center and the NHLBI Program Office, have equal status with regard to developing protocols, participating in such studies as are approved and collaborating in the development and publication of research papers based on MSH Patients' Follow Up - Extension I material.

The procedures in this section for end point, data bank, and ancillary studies, and for publication of MSH Patients' Follow Up - Extension I research results are similar to those used in the MSH. These procedures are intended to assure that study data conform to the requirements of study design, are accurately presented, authorship is appropriately acknowledged, and the text of all publications is well written.

MSH Patients' Follow Up - Extension I research and the resulting presentations and publications may be grouped into the following study categories:

1. End point studies;
2. Data bank studies;
3. Ancillary studies; and
4. Independent studies.

8.3.1 End Point Studies

An end point study is a study pertaining to the fundamental goals of the project (namely, the evaluation of the safety and efficacy of hydroxyurea in the treatment of sickle cell anemia) or which involves data, such as mortality rates, which cannot be released prior to the end of the study. These studies will summarize the findings of the MSH Patients' Follow Up - Extension I, based on the entire study population, and will be written at the conclusion of follow-up or data collection.

8.3.2 Data Bank Studies

A data bank study is a study which uses data routinely collected on patients when they are enrolled in the MSH Patients' Follow Up - Extension I and analyzes these to answer some scientific question. Data used in this research are not directly related to the fundamental goals of the study. In general, such studies are conducted with the idea of producing a scientific paper for publication based on the results of analysis.

8.3.3 Ancillary Studies

An ancillary study is a study which uses supplementary data collected on patients who are enrolled in the MSH Patients' Follow Up - Extension I, over and above the data collection required by the Protocol. Such studies are usually restricted to consideration of a specific test technique or involve only supplemental data collected in MSH Patients' Follow Up - Extension I.

8.3.4 Independent Studies

Independent studies of concern to the MSH Patients' Follow Up - Extension I are studies conducted in patients with sickle cell anemia in an MSH Patients' Follow Up - Extension I Clinical Center but involve patients who are not enrolled in the MSH Patients' Follow Up - Extension I. It is understood that each Clinical Center has the right to conduct studies which are independent of the MSH Patients' Follow Up - Extension I in patients with sickle cell anemia who are not enrolled in

the MSH Patients' Follow Up - Extension I. Independent studies of patients who were enrolled in the MSH must be reviewed by the Executive Committee. MSH Patients' Follow Up - Extension I investigators agree not to conduct independent studies which would compete with the MSH Patients' Follow Up - Extension I during the period of follow-up of MSH patients.

8.4 CLINICAL CENTER DIRECTOR ACCESS TO MSH PATIENTS' FOLLOW-UP DATA FILES AT THE END OF THE STUDY

At the end of the study, Medical Coordinating Center staff will produce a well documented data tape containing a refined (and reduced) set of the MSH Patients' Follow Up - Extension I data for the purpose of analysis by the MSH Patients' Follow Up - Extension I investigators and eventual release to the public domain in accordance with NHLBI policy. Clinical Center Directors may analyze these data in their own centers, but prior to submission of articles for publication must submit the analyses proposed for publication to the Medical Coordinating Center where they will be reviewed and computations replicated, and to the NHLBI for approval. Clinical Center Directors who perform their own analyses are responsible for obtaining all support necessary for the data bank or ancillary study outside of regular study resources. The Medical Coordinating Center will be the center of study analysis activities as long as the MSH Patients' Follow Up - Extension I investigators continue in their collaborative efforts.

8.5 PUBLICATION

The authors of any publications stemming from the study will be those who actually write the document, plus the group as a whole ("Doe J, Roe K, and the Multicenter Study of Hydroxyurea in Sickle Cell Anemia Patients' Follow-Up"), with all investigators and coordinators listed in the appendix at the end of the paper or reference made to a publication listing all investigators. Study manuscripts may only be submitted for publication for main end point studies and approved data

bank and ancillary studies (see also Section 6.3.2). All manuscripts related to study patients must be reviewed and approved by the Publications Committee. All manuscripts to be submitted for publication must first be approved by the NHLBI in accordance with regulations governing publication of research supported by NHLBI contracts, and must include acknowledgment of NHLBI contract support.

8.6 CONFLICT-OF-INTEREST

MSH Patients' Follow Up - Extension I investigators and their immediate family will not buy, sell, or hold stock options in any of the companies (currently, Bristol-Myers Squibb Company, Par Pharmaceuticals Inc., Duramed Pharmaceuticals) manufacturing medication under study from the time the recruitment of patients begins until funding for the study in the investigator's unit ends and the results are made public; or from the time the recruitment of patients begins until the investigator's active and personal involvement in the study or the involvement of the institution conducting the study (or both) ends.

Each investigator will agree not to serve as a paid consultant to the companies during these same periods. The guidelines will also apply to the investigator's spouse and dependents. The Medical Coordinating Center will hold and update annually conflict-of-interest statements from each investigator.

Certain other activities are not viewed as constituting conflicts-of-interest but must be reported annually to the Medical Coordinating Center: the participation of investigators in education activities supported by the companies (permitted only if no honorarium is paid to the investigator); the participation of investigators in other research projects supported by the companies; and, occasional scientific consulting to the companies on issues not related to hydroxyurea and for which there is no financial payment or other compensation.

APPENDIX I

MSH Patients' Follow-Up - Extension I
Data Collection Forms and Schedule

A. Scheduled data collection	Year 1 2003	Year 2 2004	Year 3 2005	Year 4 2006	Year 5 2007
Registration (Form 40)	✓	As needed	As needed	As needed	As needed
Demographics/Socioeconomic (Form 41)	✓	✓	✓	✓	✓
Health Status Questionnaire (Form 42)	✓	✓	✓	✓	✓
Medical History & Examination (Form 43)	✓	✓	✓	✓	✓
Hydroxyurea History (Form 44)	✓	✓	✓	✓	✓
Protocol Review and Specimen Collection (Form 45)	✓	✓	✓	✓	✓
Offspring Developmental Review (Form 46)	✓	✓	✓	✓	✓
Chest X-ray (Form 47)	✓	✓	✓	✓	✓
ECG Summary (Form 48)	✓	✓	✓	✓	✓
Local Laboratory Results (Form 49)	✓	✓	✓	✓	✓
NHLBI Biological Specimen Repository (Form 61 - Transmittal List)	✓	✓	✓	✓	✓
DNA/Cytogenetic Studies (Form 62 - Transmittal List)	Once during AV06 – AV10				
Fetal Hemoglobin (Form 65 - Transmittal List)	✓	✓	✓	✓	✓
67 – Arterial Blood Losses	✓	✓	✓	✓	✓
68 - Spirometry	Every other year in AV06 – AV10				
69 – High Resolution Computed Tomography	Every other year in AV06 – AV10				

B. Event Reports
Patient Event (Specified events) (Form 50)
Offspring Registration and Newborn Assessment (Form 51)
Offspring Event (Specified events) (Form 52)

APPENDIX II

MSH Patients' Follow Up - Extension I
Laboratory Determinations

Routine Hematology

Hemoglobin
Packed cell volume
Mean corpuscular volume
White blood cell count
Mean corpuscular hemoglobin
Mean corpuscular hemoglobin concentration
Red blood cell count
Red cell distribution width
Platelet count
Reticulocyte count
Differential
% Lymphocytes
% Bands
% Polymorphonucleocytes
% Monocytes
% Basophils
% Eosinophils

Biochemistry

Urea nitrogen
Alanine aminotransferase (ALT)
Aspartate aminotransferase (AST)
Uric acid
Creatinine
Glucose
Calcium
Phosphate
Bilirubin (total and direct)
Albumin
Total protein
Alkaline phosphatase
Ferritin
Catecholamines

Urinalysis
Specific gravity
Findings of microscopic examination

DNA/Cytogenetic studies
Chromosome break counts
Translocation counts
Counts of abnormal chromosomes
Analysis of microsatellite instability

Serum Bank
Specimens to be stored for future availability

Special Laboratory/Radiology

Electrocardiography
High resolution computed tomography of the chest
Pulmonary function tests
Arterial blood gas analysis
Hemoglobin F

APPENDIX III

MSH Patients' Follow Up - Extension I
Study Time Line

YEAR Month	Phase	Medical Coordinating Center	Clinical Centers	DSMB	Steering Committee
2003 January February March	I. PLANNING	Draft Protocol, consent form and study forms			Plan Data Collection
April May June	I. REVIEW TRAINING CERTIFICATION	Develop database; draft Manual of Operations	IRB Reviews Training	Meeting 6/03	
July August September October November December	II. DATA COLLECTION ANNUAL VISIT 1	Final protocol, consent form and study forms	Collect data		Meeting 9/03
2004 January February March April May June July August September October November December	II. DATA COLLECTION ANNUAL VISIT 2		Collect data	Meeting 6/04	Meeting 9/04

2005 January February March April May June July August September October November December	II. DATA COLLECTION ANNUAL VISIT 3		Collect data	Meeting 6/05	Meeting 9/05
2006 January February March April May June July August September October November December	II. DATA COLLECTION ANNUAL VISIT 4		Collect data	Meeting 6/06	Meeting 9/06
2007 January February March April May June July August September October November December	II. DATA COLLECTION ANNUAL VISIT 5 (CLOSE-OUT) III. FINAL DATA AND ANALYSIS		Collect data Clean data	Meeting 6/07	Meeting 9/07

APPENDIX IV

PRECISION OF ESTIMATES

The MSH Patients' Follow Up - Extension I is intended to estimate the frequency of occurrence of potentially toxic effects of treatment with hydroxyurea occurring between the date patients were enrolled in the MSH Clinical Trial and end of the patient follow-up in the MSH Patients' Follow Up - Extension I (September 30, 2007). Frequency of occurrence will be reported both for patients assigned to hydroxyurea during the MSH clinical trial, and for the entire cohort enrolled in the MSH clinical trial since a number of patients assigned to placebo during the MSH clinical trial began treatment with hydroxyurea at the conclusion of the trial.

Consent for follow-up is anticipated from 210 patients, including 109 patients assigned to hydroxyurea. If these patients are followed to the conclusion of the MSH Patients' Follow Up - Extension I, there will be 3,315 patient-years of follow-up (2,265 during the MSH clinical trial and MSH Patients' Follow-Up and 1,050 during the MSH Patients' Follow Up - Extension I) available for observation of events, with varying amounts of follow-up time per patient. Among patients assigned to hydroxyurea during the MSH, there would be 1,719 patient-years of follow-up (1,174 during the MSH and MSH Patients' Follow-Up and 545 during the MSH Patients' Follow Up - Extension I).

Let d= the number of events observed, y= the total number of person-years, and r be the estimated event rate, calculated as r=d/y. If no events are observed, one-sided α-level confidence intervals will be estimated as followed for the event rate in the MSH cohort. Let r_u = the value of r such that the probability of observing 0 events from a Poisson distribution with mean given by the product of r and y equals α. If no events of a particular type are observed, the upper limit of a one-sided 95% confidence interval for the event rate from a total of y person-years of follow-up would be

given by $r_u = -\ln(0.05)/y$. From the estimates of follow-up above, the respective 95% confidence limits would be r_u=1.44 events/1000 person-years for the entire cohort and r_u = 2.65 events/1000 person-years for patients assigned to hydroxyurea during the trial. Thus, with relatively complete follow-up, the MSH Patients' Follow Up - Extension I can detect potential toxic effects of hydroxyurea which occur at a rate of 1 per 1,000 person-years, but events with a rate of occurrence of 1 per 10,000 patient-years may not be detected.

When one or more events occur, two-sided confidence intervals will be calculated, using the methods described in our previous response. In Table 1, the upper and lower limits 95% confidence intervals are given for follow-up of the entire MSH cohort, and for follow-up only of the patients randomly assigned to hydroxyurea, using the follow-up available from those patients who have given consent.

In addition to estimating event rates, comparisons will be performed of the frequency of events in the MSH Patients' Follow-up versus other cohorts, such as adult CSSCD participants. Most such adverse events will have low frequency of occurrence, and power to detect differences in rates of occurrence may be limited. Total mortality may be among the most frequent adverse outcomes. In the CSSCD, the mortality (rate $\pm$ s.e.) among participants who would have met the MSH entry criterion of $\geq$ 3 crises per year was 3.74 $\pm$ 1.036 per 100-person-years based on 348 patient years of follow-up. Using the normal approximation to the Poisson distribution as described in Section 3.8.3, it can be estimated that with complete follow-up of MSH patients, there would be power=0.70 to detect a 69% reduction in mortality compared to the CSSCD cohort with $\geq$ 3 crises/person-year, power=0.80 to detect a 78% reduction in mortality compared to the CSSCD cohort with $\geq$ 3 crises/person-year, and power=0.90 to detect a 90% reduction in mortality compared to the CSSCD cohort. The standard error of rate estimates from CSSCD adults with $\geq$ 3 crises per year will be

considerably larger than that from the MSH cohort, so that modest changes in the number of MSH patients followed have little effect on the power of the comparison with the CSSCD. (Comparisons using age- and gender-adjusted rates, as described in Appendix V, may have slightly more power than the above estimates.)

TABLE 1

RATE ESTIMATES AND 95% CONFIDENCE INTERVALS FOR EVENTS PER 1,000 PERSON-YEARS BY NUMBER OF EVENTS OBSERVED FOR FOLLOW-UP OF FULL COHORT AND FOLLOW-UP OF PATIENTS RANDOMLY ASSIGNED TO HYDROXYUREA

Follow-up of 210 MSH Patients (Both Treatment Groups)				Follow-up of 109 MSH Patients (Hydroxyurea Group Only)		
Number of events	Rate Estimate	95% C.I.		Rate Estimate	95% C.I.	
		Lower	Upper		Lower	Upper
1	0.30	0.01	1.68	0.58	0.01	3.24
2	0.60	0.07	2.18	1.16	0.14	4.20
3	0.90	0.19	2.65	1.75	0.36	5.10
4	1.21	0.33	3.09	2.33	0.63	5.96
5	1.51	0.49	3.52	2.91	0.94	6.79
6	1.81	0.66	3.94	3.49	1.28	7.60
7	2.11	0.85	4.35	4.07	1.63	8.39
8	2.41	1.04	4.75	4.65	2.01	9.17
9	2.71	1.24	5.15	5.24	2.40	9.94
10	3.02	1.45	5.55	5.82	2.79	10.70
15	4.52	2.53	7.46	8.73	4.89	14.39
20	6.03	3.69	9.32	11.63	7.11	17.97
25	7.54	4.88	11.13	14.54	9.41	21.47
30	9.05	6.11	12.92	17.45	11.77	24.92

C.I. = confidence intervals.

Confidence intervals calculated from the Poisson distribution using exact methods.

Estimated confidence intervals are based on 3,315 patient-years of follow-up for the full cohort and on 1,719 patient-years of follow-up for the patients assigned to hydroxyurea.

APPENDIX V

ANALYSIS PLAN

V.1 INTRODUCTION

The objective of the Multicenter Study of Hydroxyurea in Sickle Cell Anemia (MSH) Patients' Follow Up - Extension I (MSH Patients' Follow Up - Extension I) will be to determine whether there are any long-term adverse outcomes of treatment of sickle cell anemia with hydroxyurea. These outcomes may be the occurrence of specified events (e.g., death, development of leukemia) or of abnormal values on one or more laboratory tests (e.g., renal function) consistent with end organ damage or other toxicities. Data from the MSH clinical trial (including patients who do not enroll in the MSH Patients' Follow Up - Extension I) will be pooled with data collected during the MSH Patients' Follow Up - Extension I. If data are available on the occurrence of these outcomes in reference populations, such as the CSSCD adult cohort or all African-Americans, the occurrence of these outcomes among patients in the MSH Patients' Follow-up will be compared with these reference populations. Methods for summarizing the occurrence of outcome events are described below in Sections V.2 and V.3.

A large number of potentially toxic outcomes will be examined. For each outcome, 95% confidence intervals will be calculated, or tests will be conducted at an $\alpha = 0.05$ level. To preserve power to detect potentially toxic effects of hydroxyurea therapy, these tests and confidence intervals will not be adjusted to take account of the multiplicity of outcomes examined. This observational study will not provide the same strength of evidence for harm or benefit as would be obtained from a randomized trial with a single well-defined outcome measure, but can screen for findings which warrant further investigation.

Up to the time of close-out in the MSH clinical trial, only one patient assigned to placebo was deliberately treated with hydroxyurea by his study physicians. Compliance with therapy varied among patients assigned to hydroxyurea; 31 patients discontinued therapy for clinical or personal reasons. MSH

patients assigned to placebo were offered the opportunity to start hydroxyurea therapy at the time of close-out. Some MSH patients originally assigned to hydroxyurea have discontinued their medication, and MSH patients assigned to placebo were offered the opportunity to take hydroxyurea. For analyses of frequency of adverse outcomes, three groups will be of special interest: 1) patients assigned to hydroxyurea in the MSH; 2) patients assigned to placebo who began treatment with hydroxyurea after close-out in the MSH; and, 3) patients assigned to placebo who have never taken hydroxyurea. Analyses of the frequency of an outcome, such as acute non-lymphocytic leukemia, will be performed among patients ever treated with hydroxyurea and among patients assigned to hydroxyurea in the MSH, who would have had a longer duration of exposure.

In addition to the occurrence of events, the MSH Patients' Follow Up - Extension I will collect repeated assessment of binary or continuous measures (e.g., findings on physical examination, laboratory measurements or quality of life measurements). Methods for analysis of these data in addition to calculation of means or proportions at each visit are discussed in Section V.7.

V.2 ESTIMATION OF OCCURRENCE OF OUTCOME EVENTS

Patients were enrolled in the MSH clinical trial at an approximately uniform rate over the course of 15 months, and will be followed in the MSH Patients' Follow Up - Extension I to a common closing date. Thus, even if there were no patients lost to observation (e.g., through death) for some outcomes, follow-up times would be unequal. For this reason, the crude proportion of patients with events is not an ideal summary measure. Average rates of occurrence for such events will be estimated by $r = d/y$, where d is the number of observed events, and y is the total number of patient years of follow-up.

The occurrence of most events of interest (e.g., death, development of leukemia) is anticipated to be low. For rare events, the mathematical assumptions that lead to a Poisson distribution for events are often reasonable. These assumptions include:

1. The numbers of events occurring in disjoint intervals are independent random variables;
2. For any time t, the number of events occurring between t and t + h depends only on the length of the interval, h, and not on t; and
3. The probability of two or more events occurring in an interval approaches zero as the length of the interval approaches zero.

We anticipate that a Poisson model will offer an adequate approximation to the distribution of the number of events of each type observed. Two-sided 95% confidence limits will be calculated when one or more occurrences of a given type of event are observed. If no occurrences are observed, a one-sided 95% confidence limit will be calculated. A two-sided α-level confidence interval for these event rates will be given by finding r_l, the value of r such that the probability of observing $\geq$d events from a Poisson distribution with mean equal to the product of r and y equals $\alpha/2$, and r_u, the value of r such that the probability of observing $\leq$d events from a Poisson distribution with mean equal to the product of r and y equals $\alpha/2$.

For example, if one event is observed, the probability of observing ≥ 1 event from a Poisson distribution with mean ry is $p(\geq 1) = 1\text{-}p(0) = 1\text{-}\exp(-r_l y)$. To find the upper confidence limit when one event is observed, one must solve $p(\leq 1) = \alpha/2$ for r_u, when $p(\leq 1) = p(0)+p(1) = \exp(-r_u y)(1 + r_u y)$; for α=0.05 and y=2217, this yields r_u=2.5 events per 1000 patient-years. Setting $p(\geq 1) = \alpha/2$ implies $r_l = -\log(1-\alpha/2)/y$ when 1 event is observed. With 3315 person-years of follow-up (100% follow-up of the MSH cohort), this gives a lower bound, r_l= 0.01 per 1000 patient-years. If no events of a particular type are observed, then a one-sided 95% confidence limit for the rate of occurrence of that event will be obtained by finding $r_u = \ln(0.05)/y$, the maximum value of r such that the probability of observing 0 events from a Poisson distribution with mean equal to the product of r and y is ≤ 0.05.

The Poisson process model is useful for comparison of age- and gender-adjusted rates of occurrence in the MSH cohort with rates in reference populations for whom only aggregate data (total events and total person-time) have been published. More complicated models might be considered to analyze variations among MSH Clinical Centers beyond that accounted for by age and gender differences among clinic populations.

Adverse outcomes of interest in the MSH Patients' Follow Up - Extension I, such as acute leukemia, occur at rates of a few per 100,000 person-years among adult African-Americans. Unless these rates of occurrence are greatly increased among MSH patients exposed to hydroxyurea, it is likely that there will be no occurrences of some adverse outcomes and only a few occurrences for others. For many of the potential adverse outcomes being considered, there may be too few events to even fit a model more complicated than the simple Poisson model. To decide which of two alternative models fit the data better would require even more events. For example, if an outcome is observed in fewer than ten patients, it would be difficult to compare suitability of a Poisson process model to more complicated models which assume that occurrence rate increases with duration of exposure. If there are no events in most MSH Clinical Centers, it will be difficult to fit a model describing among-clinic variation. For more frequent outcomes, it may be desirable to consider models allowing for random variation among clinics in age- and gender-specific event rates. For example, Tsutakawa, Shoop and Marienfeld describe an empirical Bayes procedure for modeling variability in cancer mortality rates among geographic units, in which age- and gender-specific rates within each unit each follow a Poisson distribution, but the rates for the various units have a normal distribution with mean μ and variance σ^2.

A secondary method of describing the occurrence of events will be the percentage of patients with an event by the end of follow-up (i.e., at the largest observed value of follow-up time,

combining follow-up in the MSH clinical trial and MSH Patients' Follow-up), estimated using the Kaplan-Meier method. Confidence limits (95%) for the proportion with the outcome will be calculated using the log[-log] transformation of the estimate of the survival function at the end of follow-up.

V.3 COMPARISONS WITH REFERENCE POPULATIONS OR WITHIN THE MSH COHORT

The frequency of acute non-lymphocytic leukemia in all patients in the MSH, in patients assigned to hydroxyurea in the MSH and in patients assigned to placebo who began treatment with hydroxyurea after close-out in the MSH would be compared to the following reference populations:

1. published data (if any) on the occurrence of acute non-lymphocytic leukemia among the African-American adult population;
2. the occurrence rate of acute non-lymphocytic leukemia in the Cooperative Study of Sickle Cell Disease (CSSCD) adult cohort;
3. the occurrence rate of acute non-lymphocytic leukemia in patients within the CSSCD adult cohort with ≥ 3 crises per year; and,
4. the occurrence rate of acute non-lymphocytic leukemia in MSH patients assigned to placebo who have never taken hydroxyurea.

The follow-up experience included from MSH patients assigned to placebo would include: 1) follow-up of placebo patients during the MSH, and 2) follow-up after the MSH for those patients who did not begin treatment with hydroxyurea. Patients who elect not to take hydroxyurea at the end of the MSH may be disproportionately placebo patients who have been doing well without hydroxyurea treatment or placebo patients who could not tolerate hydroxyurea. Comparisons of these patients with patients randomly assigned to treatment with hydroxyurea are potentially biased.

V.3.1 Comparisons With Reference Populations

Several methods of analysis will be considered for comparing patients treated with hydroxyurea to selected reference populations. Age-adjusted occurrence rates will be calculated between groups of patients in the MSH and external reference populations (e.g., adult African-Americans) and compared using a z-statistic. This method could also be used for comparison of occurrence rates between MSH patients assigned to hydroxyurea and MSH patients never exposed to hydroxyurea.

Effects of exposure to hydroxyurea may be analyzed with time-dependent covariates in a Cox model. A time dependent indicator variable for hydroxyurea exposure would initially equal 1 for all patients assigned to hydroxyurea and 0 for all patients assigned to placebo. This indicator would become equal to 1 for patients in the placebo group if they initiate treatment with hydroxyurea. A second time dependent indicator variable could be used to indicate when patients exposed to hydroxyurea stopped treatment. Interactions between these indicator variables and functions of follow-up time will be used to study whether or not there is a non-zero hazard associated with hydroxyurea exposure, and whether or not this hazard changes with time (e.g., if a latency period exists).

In comparisons of the MSH cohort with CSSCD adult participants, it must be recognized that MSH patients were selected at entry to have had at least three acute vaso-occlusive crises in the year preceding enrollment and that patients with known sickle ß-thalassemia were excluded. Fewer than 10% of the CSSCD adult cohort had $\geq$ 3 crises/year, and mortality among such patients in the CSSCD was about twice that of patients with fewer crises/year[1]. Therefore, it would be desirable to perform comparisons of the occurrence of mortality, end organ failure or other complications of

sickle cell disease to those CSSCD adult patients with ≥ 3 crises in a specified year as well as with the entire CSSCD adult cohort.

For comparisons with the CSSCD cohort or the adult African-American population from published data for the occurrence of specified events (e.g., death, development of leukemia), age- and gender-adjusted rates will be calculated for the entire MSH patient cohort (or subgroups of this cohort) using the direct method of adjustment, with the age and gender distribution in the MSH cohort as the standard population. Strata will be defined to avoid strata with zero events in the MSH cohort. Assuming occurrence of events has a Poisson distribution within each age-gender stratum, the adjusted rates will be given by $r_{ref} = \Sigma_i w_i d_i/y_i$, with estimated variance $v_{ref} = \Sigma_i w_i^2 d_i/y_i^2$, for $w_i =$ the sample size in the i-th age/gender stratum of the MSH cohort, d_i the number of events and y_i the number of patient-years of follow-up in the corresponding stratum of the reference population. For the MSH cohort, the estimated rate of occurrence will be calculated simply as $r_{msh} = d/y$, with estimated variance given by d/y^2, where d is the number of events in the MSH cohort and y the total person-years for the MSH cohort. Differences in the occurrence of events between the two populations may be tested using the asymptotically normal statistic $Z = (r_{msh}-r_{ref}) /[V_{msh}+V_{ref}]^{1/2}$.

V.3.2 Comparisons by Assigned MSH Clinical Trial Treatment or By Exposure to Hydroxyurea

Comparison of occurrences of study outcomes between patient groups defined by assigned study treatment during the MSH clinical trial will be performed with the log-rank test. Survival curves by treatment group will be presented using the Kaplan-Meier method. It may also be of interest to compare occurrence of more frequent outcomes according to exposure to hydroxyurea, among three groups: patients originally assigned to hydroxyurea, patients in the placebo group who have began hydroxyurea treatment after the close of the MSH, and placebo patients who have never taken hydroxyurea. Exposure to hydroxyurea would be treated as a time-dependent covariate in a

Cox proportional hazards model. At the time each event occurs, cumulative exposure to hydroxyurea for each patient would be classified according to information collected through the time of the most recent follow-up visit. The statistical power of comparisons according to events occurring during the MSH Patients' Follow-Up may be limited by the balance in numbers between those taking hydroxyurea and those not taking hydroxyurea in analyses according to current exposure to hydroxyurea.

For some reproductive outcomes it may be of interest to compare patients according to exposure to hydroxyurea during pregnancy using a chi-square test or logistic regression. It should be recognized that these comparisons using exposure after the clinical trial are potentially biased, as use of hydroxyurea after the end of the MSH clinical trial depends on self-selection rather than random assignment.

V.5 OUTCOMES OF PREGNANCY

Frequency of various outcomes of pregnancy and findings during the first year of life for children born to MSH Patients' Follow-Up cohort will be reported. These data may be compared to published reports on occurrence of complications of pregnancy, still-birth, prematurity, etc., in patients with sickle cell disease. These reproductive outcomes will also be considered according to randomized treatment assignment in the MSH clinical trial. Outcomes considered to be possible teratogenic effects of hydroxyurea exposure may also be reported according to whether the mother was being treated with hydroxyurea during pregnancy.

V.6 CYTOGENETIC FINDINGS

Frequency of occurrence of genetic abnormalities (chromosome breakage, sister chromatic exchange, breakage near known oncogenes or mutations found in selected DNA sites) will be

reported according to study visit (years 1 and exit) and according to MSH clinical trial treatment assignment and subsequent treatment with hydroxyurea.

V.7 LABORATORY MEASURES

Mean values will be presented for laboratory measures (e.g. renal function tests). The percentage of patients with values outside normal ranges and the percentage of patients with changes beyond specified limits from MSH baseline values will be presented for each laboratory measure at each MSH Patients' Follow-Up visit, as well as the cumulative percentage of patients with these findings on each laboratory test. The means and percentages abnormal will be compared with data from the CSSCD cohort (if available), using both descriptive displays and two-sample t-tests and chi-square tests or adjusted analyses taking account of age and gender (e.g. by analysis of covariance or Mantel-Haenszel tests). In secondary analyses, these laboratory outcomes will be compared according to randomized treatment assignment in the MSH clinical trial. If comparable laboratory measures were collected during the MSH clinical trial, these data will be combined with data from the MSH Patients' Follow-Up in reporting mean values and percent of patients with abnormal findings according to time since MSH clinical trial entry and cumulative abnormal findings per patient-year of follow-up.

V.8 OTHER OUTCOMES

Frequency of occurrence of other clinical findings (e.g., ECG abnormalities) will be defined and reported overall, according to MSH clinical trial treatment assignment and according to cumulative hydroxyurea therapy.

Mean values of measures of quality of life will be reported for each study visit. If the same instruments for assessing quality of life are used in the MSH Patients' Follow-Up as were used in the

MSH clinical trial, these data will be combined to report mean values on quality of life measures according to time since entry into the MSH clinical trial. These data will be reported for all patients in the cohort and according to MSH clinical trial treatment assignment. The generalized estimating equation (GEE) method for longitudinal data analysis may be used to test for changes in differences of quality of life measures over time between MSH treatment groups.

V.9 REPORTS

V.9.1 Performance Reports

Monthly study status reports will include information on recruitment. Monthly study status reports will provide information on the number of MSH patients who have outcomes of concern. In annual reports, the number of enrolled patients as well as the percent of goal (living MSH patients) will be provided by Clinical Center and for the study.

The annual study status reports will also include information on the number and percent of forms and specimens delinquent by Clinical Center, the edit status of individual forms by Clinical Center and summary of the status of Core Laboratory evaluations of clinical chemistries or DNA. In addition, the percentage of follow-up procedures specified by the Protocol (telephone contacts, quality of life data collection or blood specimen collection) which are actually performed by Clinical Center staff will be reported in each performance report.

V.9.2 Data Monitoring Reports

The Medical Coordinating Center will distribute to the NHLBI Project Office and Chairman of the Data and Safety Monitoring Board (DSMB) the monthly study status report. More extensive reports will be prepared for review by the Data and Safety Monitoring Board at six- to twelve-month intervals. The Data and Safety Monitoring Reports will include complete enrollment, performance,

and quality assurance information, and will present observed events in terms of rates as discussed above. Because this is a study of rare events, it may not be possible to pre-determine all the conditions that may be reported. Some conditions may be reported by Clinical Center Directors which will then be relayed to the Chairman of the Data and Safety Monitoring Board immediately; others will be reported in the DSMB Reports.

V.9.3 Annual Reports for the NHLBI Program Office

At annual intervals, detailed reports on Medical Coordinating Center activities will be prepared for submission to the NHLBI Program Office. These reports will provide information on the status of the study, the status of Medical Coordinating Center activities with respect to coordination, data management and data analysis. These reports will summarize the accomplishments in relationship to the study goals and will indicate the participation of Medical Coordinating Center staff in study meetings and conference calls, and provide a summary of the communications with Clinical Centers which are documented by numbered memoranda. It will also outline any changes in personnel in the Medical Coordinating Center and certified personnel in Clinical Centers which have occurred during the course of the year. It will provide a current list of accepted abstracts and publications.

References

1. Platt OS, Thornington BD, Brambilla DJ, Milner PF, Rosse WF, Vichinsky E and Kinney TR. Pain in sickle cell disease. Rates and risk factors. N Engl J Med, 1991; 325:11-6.

2. Tsutakawa RK, Shoop GL and Marienfeld CJ. Empirical Bayes estimation of cancer mortality rates. Statistics in Medicine, 1985; 4:201-12.

3. Kaplan EL and Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc, 1958; 53:47-481.

4. Kalbfleisch JD and Prentice RL. The Statistical Analysis of Failure Time Data, John Wiley & Sons, New York, 1980, pp. 10-15.

5. Prentice RL. Linear rank tests with right censored data. Biometrika,1978; 65:167-179.

6. Cox DR. Regression models and life tables (with discussion). J R Stat Soc B, 1972; 34:187-220.

7. Liang KY and Zeger S. Longitudinal data analysis using generalized linear models. Biometrika 1986; 73:13-22.

APPENDIX VI

MULTICENTER STUDY OF HYDROXYUREA (MSH)
PATIENTS' FOLLOW UP - EXTENSION I
(Model Consent Form)

1. Nature and Purpose of the Study

Sickle Cell anemia is a disease that passes from parents to children through genes. When you joined the Multicenter Study of Hydroxyurea in Sickle Cell Anemia (MSH), you helped find out that the medicine called hydroxyurea can reduce the number of painful attacks that adults with sickle cell anemia have. The purpose of the MSH Patients' Follow Up - Extension I is to learn whether this medicine has any other effects that occur after a longer period of time. Hydroxyurea might increase the risk of some cancers. Although this risk is not certain to exist and at most is small, we want to learn whether or not this risk really exists. Also, we do not know if it will be safe for patients who take hydroxyurea to have a pregnancy. We want to find out about all babies that MSH patients and their partners have. We can learn about these risks by keeping in touch with both patients who take hydroxyurea and those who do not take hydroxyurea.

As with other new treatments, there may be other risks to using hydroxyurea that are not now known. So far we have not seen any new risks, but we are still looking. If any new bad effects are observed, patients in the study will be told as soon as possible and action taken to protect their safety. Over 200 patients with sickle cell anemia are expected to join this study.

2. What to Expect

If you agree to join this study, you will take medicine according to the direction of your own doctors. You will know the medicines you are taking. We will ask you to help us keep a record

of what you are taking. We would like you to join the study and see the doctors here for one, special research visit each year for a total of five visits. The visit will take an afternoon or morning. At each visit a blood sample from a vein will be drawn with a needle and a test tube. Each sample will use only about three tablespoons of blood. We will use the blood to find out about your general health and to study the cells in your blood. Part of this sample will be used at the present time, part will be frozen or made into slides and stored for studies to be planned in the future. The portion of the blood that is frozen will be stored at a central laboratory for the National Institutes of Health. Blood samples will be handled in a manner that protects your privacy. A smaller blood sample from an artery will be used to check how well your lungs get oxygen into your blood at every visit. We will perform a high resolution computed tomography of your chest (a chest X-ray with extra detail), electrocardiogram and lung function tests at the time of all but two of the visits.

We will use some of the blood sample to look for changes in the genes in blood cells. Part of the frozen sample will be used in the future for more studies of genes. If you agree now, doctors in other approved studies may use the frozen samples at a later date for studies other than sickle cell anemia. Your agreement or refusal to use the sample in other studies will not affect its use for current or future studies of sickle cell anemia. The study will not be giving patients any individual results of gene tests. Please mark the consent you give for study of your blood and the genes in your blood (check one):

☐ for the MSH Patients' Follow Up - Extension I only.

☐ for the MSH Patients' Follow Up - Extension I and for other medical research projects.

_____
Initials

We will ask if you have had to visit a hospital or clinic because of any serious illness such as heart disease, lung disease or cancer. We will fill out a form about how much you can do and how well you feel. You will be asked to sign forms so that we can get records from your private doctors or hospitals where you have been treated.

In addition, a research assistant will contact you via telephone to interview you about your child's development, communication, daily living, and socialization skills. This telephone interview will take approximately 1 1/2 hours. If results of this developmental interview reveal that your child is having developmental difficulties, we will refer you for appropriate clinical interventions.

3. Risks and Discomforts

The blood samples for this study involve needle sticks. You may be able to avoid an extra needle stick if a blood sample is taken at the same time as a blood sample for your usual care.

4. Hydroxyurea Therapy Decisions

You and your doctors decide if you should take hydroxyurea therapy. This therapy must be closely watched, if you take it, to avoid excess effects on your blood that can lead to bleeding, infections, or weakness due to low blood counts. Some MSH patients have had bleeding, infections or weakness due to low blood counts, but none due to the therapy as far as we can tell. Also, we want to know if there are any other discomforts such as upset stomachs and skin rashes. So far, these have not occurred more than could be foreseen in MSH patients.

5. Benefits

Any important medical information about you or about the results of this study will be available to the doctors who take care of you.

_____
Initials

6. Privacy

In this study only your own clinic will know your name. We will make note of your initials, age, sex, weight, and height. Only that identification will be stored in the study computer. You will not be identified personally in any report from this study. Your personal medical records will be kept private. At the end of the study a computer tape of the study results will be made for future use. It will not include any information that could identify you directly. Information may be given to the National Institutes of Health or the Food and Drug Administration, but your name will not be used in such files.

7. Other Choices

If you join this study, it is your own choice. You may refuse to take part in it or you may leave it at any time. If you do not join, or leave, doing so will not harm your present or future care at the hospital or clinic. Instead of taking part in this study you may go to your doctor for your usual treatment of sickle cell anemia.

8. Costs Paid For by the Study

You will not be charged for any of the study visits or procedures.

9. Costs Not Paid For by the Study

If you need medicine, the study will not pay for it. If you must visit your private doctor or emergency room, or must stay in the hospital, those costs will not be covered by this study.

10. Payments to You

You will be paid $30.00 for travel and other clinic visit costs. If you do not attend study clinic visits or become unable to take part in the study then you will no longer be paid that money.

_____
Initials

11. Rights

You will be given a copy of this consent form to keep. If at any time you have questions or concerns about the study you may call either _____, the study staff, or _____, a person whose job is to watch over the well being of patients in medical research projects. Should you have any bad effect of treatment during the study, care will be provided to you. The cost of treating such bad effects is not covered in the study, and no money has been set aside to pay for these bad effects.

12. Questions

This study has been explained to you by Dr. _____ and your questions were answered. If you have any other questions about this study you may call Dr. _____ at _____.

I have talked about the MSH Patients' Follow Up - Extension I with the study doctor and read this form. I have had time with the study doctor to ask questions and talk over concerns about the study. I willingly give my consent to join this study.

_____
Initials

I agree to release of medical information by study doctors to my private doctor.

Signature of Patient	Date
Signature of Witness	Date
Signature of Physician	Date

Initials