preparation of the material to be administered to the patient. For example, if a viral vector is proposed, what is the nature of the helper virus or cell line? If carrier particles are to be used, what is the nature of these?

Appendix M–II–B–2. Preclinical Studies, Including Risk-Assessment Studies

Provide results that demonstrate the safety, efficacy, and feasibility of the proposed procedures using animal and/or cell culture model systems, and explain why the model(s) chosen is/are most appropriate.

Appendix M–II–B–2–a. Delivery System

Appendix M–II–B–2–a–(1). What cells are the intended target cells of recombinant DNA? What target cells are to be treated *ex vivo* and returned to the patient, how will the cells be characterized before and after treatment? What is the theoretical and practical basis for assuming that only the target cells will incorporate the DNA?

Appendix M–II–B–2–a–(2). Is the delivery system efficient? What percentage of the target cells contain the added DNA?

Appendix M–II–B–2–a–(3). How is the structure of the added DNA sequences monitored and what is the sensitivity of the analysis? Is the added DNA extrachromosomal or integrated? Is the added DNA unrearranged?

Appendix M–II–B–2–a–(4). How many copies are present per cell? How stable is the added DNA both in terms of its continued presence and its structural stability?

Appendix M–II–B–2–b. Gene Transfer and Expression

Appendix M–II–B–2–b–(1). What animal and cultured cell models were used in laboratory studies to assess the *in vivo* and *in vitro* efficacy of the gene transfer system? In what ways are these models similar to and different from the proposed human treatment?

Appendix M–II–B–2–b–(2). What is the minimal level of gene transfer and/or expression that is estimated to be necessary for the gene transfer protocol to be successful in humans? How was this level determined?

Appendix M–II–B–2–b–(3). Explain in detail all results from animal and cultured cell model experiments which assess the effectiveness of the delivery system in achieving the minimally required level of gene transfer and expression.

Appendix M–II–B–2–b–(4). To what extent is expression only from the desired gene (and not from the surrounding DNA)? To what extent does the insertion modify the expression of other genes?

Appendix M–II–B–2–b–(5). In what percentage of cells does expression from the added DNA occur? Is the product biologically active? What percentage of normal activity results from the inserted gene?

Appendix M–II–B–2–b–(6). Is the gene expressed in cells other than the target cells? If so, to what extent?

Appendix M–II–B–2–c. Retrovirus Delivery Systems

Appendix M–II–B–2–c–(1). What cell types have been infected with the retroviral vector preparation? Which cells, if any, produce infectious particles?

Appendix M–II–B–2–c–(2). How stable are the retroviral vector and the resulting provirus against loss, rearrangement, recombination, or mutation? What information is available on how much rearrangement or recombination with endogenous or other viral sequences is likely to occur in the patient's cells? What steps have been taken in designing the vector to minimize instability or variation? What laboratory studies have been performed to check for stability, and what is the sensitivity of the analyses?

Appendix M–II–B–2–c–(3). What laboratory evidence is available concerning potential harmful effects of the transfer (e.g., development of neoplasia, harmful mutations, regeneration of infectious particles, or immune responses)? What steps will be taken in designing the vector to minimize pathogenicity? What laboratory studies have been performed to check for pathogenicity, and what is the sensitivity of the analyses?

Appendix M–II–B–2–c–(4). Is there evidence from animal studies that vector DNA has entered untreated cells, particularly germ-line cells? What is the sensitivity of these analyses?

Appendix M–II–B–2–c–(5). Has a protocol similar to the one proposed for a clinical trial been conducted in non-human primates and/or other animals? What were the results? Specifically, is there any evidence that the retroviral vector has recombined with any endogenous or other viral sequences in the animals?

Appendix M–II–B–2–d. Non-Retrovirus Delivery/Expression Systems

If a non-retroviral delivery system is used, what animal studies have been conducted to determine if there are pathological or other undesirable consequences of the protocol (including insertion of DNA into cells other than those treated, particularly germ-line cells)? How long have the animals been studied after treatment? What safety studies have been conducted? (Include data about the level of sensitivity of such assays.)

Appendix M–II–B–3. Clinical Procedures, Including Patient Monitoring

Describe the treatment that will be administered to patients and the diagnostic methods that will be used to monitor the success or failure of the treatment. If previous clinical studies using similar methods have been performed by yourself or others, indicate their relevance to the proposed study. Specifically:

Appendix M–II–B–3–a. Will cells (e.g., bone marrow cells) be removed from patients and treated *ex vivo*? If so, describe the type, number, and intervals at which these cells will be removed.

Appendix M–II–B–3–b. Will patients be treated to eliminate or reduce the number of cells containing malfunctioning genes (e.g., through radiation or chemotherapy)?

Appendix M–II–B–3–c. What treated cells (or vector/DNA combination) will be given to patients? How will the treated cells be administered? What volume of cells will be used? Will there be single or multiple treatments? If so, over what period of time?

Appendix M–II–B–3–d. How will it be determined that new gene sequences have been inserted into the patient's cells and if these sequences are being expressed? Are these cells limited to the intended target cell populations? How sensitive are these analyses?

Appendix M–II–B–3–e. What studies will be conducted to assess the presence and effects of the contaminants?

Appendix M–II–B–3–f. What are the clinical endpoints of the study? Are there objectives and quantitative measurements to assess the natural history of the disease? Will such measurements be used in patient follow-up? How will patients be monitored to assess specific effects of the treatment on the disease? What is the sensitivity of the analyses? How frequently will follow-up studies be conducted? How long will patient follow-up continue?

Appendix M–II–B–3–g. What are the major beneficial and adverse effects of treatment that you anticipate? What measures will be taken in an attempt to control or reverse these adverse effects if they occur? Compare the probability and magnitude of deleterious consequences from the disease if recombinant DNA transfer is not used.