

ADDITIONAL GUIDELINES FOR THE TRANSFER OF RECOMBINANT DNA MOLECULES INTO HUMAN SUBJECTS

In addition to submitting a registration form to the IBC, those researchers proposing human gene therapy protocols must submit information addressing each of the following points. The submission may be in narrative form, but must address each point in the following order:

1. What is the structure of the cloned DNA that will be used?

a. Describe the gene (genomic or cDNA), the bacterial plasmid or phage vector, and the delivery vector (if any). Provide complete nucleotide sequence analysis or a detailed restriction enzyme map of the total construct.

b. Describe the regulatory elements the construct contains (e.g., promoters, enhancers, polyadenylation sites, replication origins, etc.). Name the source from which these elements are derived. Summarize what is currently known about the regulatory character of each element.

c. Describe the steps used to derive the DNA construct.

2. What is the structure of the material that will be administered to the patient?

a. Describe the preparation, structure, and composition of the materials that will be given to the patient or used to treat the patient's cells.

i. If DNA, describe the purity (both in terms of being a single DNA species and in terms of other contaminants) and the sensitivity of the assays that will be used to determine this.

ii. If a virus, describe any special features of the cell lines, media, or sera used to propagate it. Describe the purification methods and assays with their sensitivity that will be used to detect and eliminate any contaminating materials (including helper virus or other organisms) that may have biological effects.

iii. If co-cultivation is employed, describe the cells used for co-cultivation. Describe the purification methods and assays with their sensitivities that will be used to detect and eliminate any contaminating materials. Specifically, describe the tests used to assess the material to be returned to the patient for the presence of live or killed donor cells or other non-vector materials originating from those cells.

iv. If other methods are to be used, describe the purification methods and assays with their sensitivities that will be used to detect and eliminate any contaminating materials. Name the possible sources of contamination.

b. Describe any other material to be used in the preparation of the material to be administered to the patient.

i. If a viral vector is proposed, describe the nature of the helper virus or cell line.

ii. Describe the nature of any carrier particles that are to be used.

3. What cells are the intended targets of the recombinant DNA?

a. Describe how ex vivo targeted cells will be characterized before and after treatment.

b. Describe the theoretical and practical basis for assuming that only the target cells will incorporate the DNA.

c. Provide the percentage of target cells that contain the added DNA.

d. Describe whether the added DNA is extrachromosomal or integrated and whether it is unrearranged.

e. Describe the assays with their sensitivities to monitor this.

f. Provide the number of copies of added DNA present per cell and describe the stability of the added DNA both in terms of its continued presence and its structural stability.

4. How efficient and specific is gene transfer and expression?

a. Describe the animal and cultured cell models used to assess the in vivo and in vitro efficacy of the gene transfer system, comparing and contrasting these to the proposed human treatment.

b. Provide 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 determined?

c. 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.

d. Describe to what extent expression is only from the desired gene (and not from the surrounding DNA).

e. Describe to what extent the insertion modifies the expression of other genes.

f. Provide the percentage of cells that express the desired gene, whether the product is biologically active, and if so, the percentage of normal activity that results from the inserted gene.

g. Describe the extent to which the gene is expressed in cells other than the target cells.

5. Is a retrovirus delivery system being used?

a. Describe the cell types that have been infected with the retroviral vector preparation and describe which cells, if any, produce infectious particles.

b. Describe the stability of the retroviral vector and resulting provirus in terms of loss, rearrangement, recombination, or mutation. Describe steps taken in designing the vector to minimize instability or variation and any assays, with their sensitivities, used to measure stability. Provide information on how much rearrangement or recombination with endogenous or other viral sequences is likely to occur in the patient's cells.

c. Describe laboratory evidence that is available concerning potential harmful effects of the transfer (eg, development of neoplasia, harmful mutations, regeneration of infectious particles, or immune responses).

d. Describe steps taken in the design of the vector to minimize its pathogenicity and describe assays with their sensitivities to determine this.

e. Provide any evidence from animal studies that vector DNA has entered untreated cells, particularly germ-line cells.

f. Provide whether a similar protocol has been conducted in non-human primates and/or other animals. If so, describe the results. Specifically, provide any evidence that the retroviral vector recombined with any endogenous or other viral sequences in the animals.

6. Is a non-retrovirus delivery/expression system being used?

a. Describe animal studies that 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).

b. Provide how long the animals have been studied after treatment.

c. Describe any safety studies that have been conducted, including data about the level of sensitivity of such assays.