**BRMAC Meeting #27**
**Human Stem Cells as Cellular Replacement Therapies for Neurological Disorders**
July 13-14, 2000
Hilton Hotel, Gaithersburg, MD

**PRODUCT: Discussion Questions**

Stem cells are distinct from other cellular products in that they are intended to differentiate in order to assume their final therapeutic phenotype. Frequently, the process of terminal phenotypic differentiation will be initiated *ex vivo* prior to patient administration. The capacity for stem cells to generate phenotypically distinct cell lineages through differentiation is fundamental to their utility but also imposes significant challenges in developing meaningful product specifications, predicting outcomes and assessing safety. Neural stem cells represent an example typifying the phenotypic potentiality of stem cells. The proposed use of neural stem cells generally is to produce functional neuronal circuitry through differentiation of developmentally immature precursors. In contrast to their potential therapeutic benefits, neural stem cells may also possess the ability to develop aberrant neural connections that result in adverse clinical effects, including seizures and inhibition or hyperactivation of existing circuitry. These events may occur as a consequence of unregulated neurotransmitter release, release of unintended neurotransmitters, or differentiation into an undesired cellular phenotype that contributes to the formation of gliotic scars. Careful scrutiny must be given these unintended events. Current knowledge of the molecular and cellular biology of most cellular products is unlikely to allow analytical specifications that provide information sufficient for precise product characterization. Accordingly, it will be necessary to develop guiding principles that make best use of available information in order to identify cellular characteristics that can be defined analytically, to establish well-defined manufacturing process controls, and to design appropriate biological assays. Collectively, these principles will facilitate development of stem cells as safe and effective therapies.

## 1. Human Stem Cell Sources

Human stem cells may be obtained from a variety of sources. These include autologous or allogeneic adult cells, allogeneic fetal cells or tissues, as well as established allogeneic stem cell lines of embryonic or adult origin. Stem cells may also be derived from mutation expressing transformed cells or immortalized cell lines that are the product of genetic engineering. The extent of information that might be reasonably obtained through safety testing and characterization of stem cells will vary substantially depending upon their source.

a. What source controls are appropriate for donor tissue? Are existing standards for blood banking and/or organ transplantation appropriate? In cases where cell lines might be established or where culture times might be prolonged, would it be appropriate to retest donors for HIV to eliminate donors in the "window" period?

b. Does the BRMAC concur with the position that records linking cell preparations with donors and patients should be maintained? If so, what records should be kept, who would have access to them, and for how long should they be maintained?

c. If there were circumstances when the cell or tissue source of origin is not traceable or is poorly characterized, would there be conditions acceptable for considering use of such stem cells in humans?

d. Does the BRMAC recommend that molecular genetic analysis be a component of stem cell source qualification? If so, which genetic markers should be evaluated (mutational analysis of parkin gene and α-synuclein, Parkinson's disease; SOD-1, ALS; HD gene, Huntington's disease)? Are existing genetic tests sufficiently reliable as to be useful for stem cell source qualification? In situations when testing of stem cells derived from embryonic or fetal sources reveals mutations that are positively associated with neurodegenerative disease onset, what would be the obligations for informing donors?

e. Are there different intrinsic safety concerns for stem cells based on their source of derivation (e.g., autologous adult donation, allogeneic adult donation, embryonic germ cells, or embryonic stem cells)?

f. Does the BRMAC recommend different characterization studies for stem cells depending upon the procedures and sources of their derivation or are these issues generic to this entire product category?

## 2. Manufacturing of Stem Cells

a. To what extent do stem cell isolation and maintenance procedures determine desirable cell fates and preclude undesired cell fates?

b. What are likely to be critical manufacturing process controls? Examples include qualification of the source(s) from which stem cells are obtained, standardization of procedures used to procure, expand or maintain stem cells, and the development of validated tests that allow monitoring of stem cell product identity and heterogeneity.

What evidence supports these conclusions and what further studies are needed to resolve these questions?

## 3. Characterization of Stem Cell Preparations and Selection of Specifications

The ability to reliably identify stem cells derived from various cellular types is essential for assessing purity of cellular preparations and evaluating consistency achieved in the isolation or manufacture of stem cells. In addition, a correlation must be established between the assessment of stem cell identity and the outcomes obtained following their administration. Finally, unambiguous identification of the therapeutic cell type is essential for accurate dosing. Therefore, an explicit, detailed description of stem cell preparations with respect to identity and purity is required.

a. Stem cell preparations may consist of heterogeneous cell populations. Some may be essential to the intended effect, some deleterious, and some inert. Both to minimize the possibility of untoward reactions and to ensure consistent dosing, both purity and the profile

of impurities should be controlled. In a complex product of this type, what principles should guide selection of purity specifications?

b. Have specific markers been identified that permit unambiguous identification of cell phenotype, determination, or fate?

c. Have specific markers been identified that are *necessary* to ensure that product cells will assume the correct, functional, therapeutic phenotype?

d. Have specific markers been identified that are *sufficient* to ensure that product cells will assume the correct, functional, therapeutic phenotype?

e. Have specific markers been identified that indicate that isolated stem cells will lead to adverse events such as ectopic tissue differentiation, deleterious cell fate, tumorigenesis, or some other undesired outcome?

f. What approaches should be used to evaluate proposed specifications?

## 4. Potency Assays for Stem Cell Products

An ideal potency assay would guarantee that each lot of the product that performs acceptably will have the desired clinical effect. When this cannot be achieved, it is useful nevertheless to devise an assay for which an acceptable result is associated reliably with desirable product performance characteristics.

a. What recommendations does the committee have for development of stem cell potency assays?

b. At present, it is unlikely the molecular determinants that would ensure desirable product characteristics are known completely. Given the difficulty of assessing intended cellular function, are there surrogate measures that might be predictive for assuring intended bioactivity of stem cell implants? If not, what studies would provide this information?

c. What degree of inter-assay variability would be considered acceptable?

d. In what manner might a dosage unit of potency be expressed? Would such units be based on the number of total cells in a preparation or defined by a subset of stem cells expressing a specific marker predictive of differentiative phenotype (e.g. percentage of cells expressing neural-specific markers such as nestin, type III β-tubulin, neuron-specific nuclear protein or tyrosine hydroxylase) and presumed clinical outcome?