The Design of Clinical Trials for Cell Transplantation into the Central Nervous System

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INTRODUCTION

A recent systematic review, including a tentative meta-analysis of the published results, pointed out the need for controlled trials to evaluate the allotransplantation of the cells as a treatment for Parkinson's disease.[1] Cell therapy within the CNS raises many critical questions that need to be answered before a clinical trial should be launched. A critical point of concern is that the major requirements have not always been fulfilled in some of the known trials, and many of them will be systematically examined in this work, from the animal experiments to the analysis of the results. Currently, cell therapy in the CNS has to be confined in the field of experimental research, and one must keep in mind that cell therapy remains in most cases a palliative, and not a real curative, treatment for various types of lesions of the CNS, including degenerative, vascular, or traumatic. Even in the cases of orphan diseases, the potential benefit to risk ratio has to be evaluated a priori, and then after any trial.

THE EXAMPLE OF PARKINSON'S DISEASE

Preclinical data

Animal and *in vivo* experiments are mandatory for formulating the action mechanism, the efficacy that can be predicted, and the procedures of cell therapy in Parkinson's disease (PD). It is now a well accepted fact that autotransplantation using adrenal tissue must be abandoned.[1] The same is true for the xenograft of the porcine mesencephalic embryonic neurones.[2] To the contrary, animal results were available at the time of the first allograft human trials, which indicated that the heterotopic implantation of the embryonic mesencephalic neurons into the dopamine (DA)-depleted striatum of several animal species could reinnervate the striatum and ameliorate some functional deficits. The formation of the synaptic contacts with the intrinsic host striatal neurons, the long-term survival of the grafted neurons, and the symptoms related to the amelioration of DA deficit were documented in various species, including monkeys.[3,4] Xenografts, using human cells injected in animal brains, were found useful for validating the best period for fetal tissue procurement (typically, 5-9 weeks after conception).

The determination of these results enables us to define the procedures of a planned subsequent trial. At this stage, the clinical team must validate the methods for selection of the patients, the transplantation methodology, brain imaging, and evaluation (Table 1). Here, it can be opined that the preclinical experiments may be found helpful to define the protocols for surgery and brain imaging in the best manner.

Pilot trials

In the field of cell therapy, contrary to drug phase I trials, pilot trials cannot be performed on normal, volunteering subjects. This means that the questions to be answered by these "phase I-like" trials may differ from therapeutic results. While the team members improve their skills for cell therapy, they should take serious measures to collect data regarding several kinds of side effects. The team must take into account the following:

the surgical and the anesthetic risks involved (e.g., bleeding, infection, mental confusion, etc.),

iatrogenic side effects of the immunosuppressants,

side effects related to dopamine release, or regional modifications by transplanted cells (e.g., dystonia, dyskinesias, hallucinations, etc.),

side effects related to the unwanted development of cells in some part of the CNS and/or ventricles (e.g., hydrocephalus),[5]

and psychic disturbances related to the particular procedure (e.g., one of our patients remarked "the brains of foreign people grow in my own head").

In addition, data for general, unexpected adverse events have to be properly acquired in an effective manner, using, e.g., the World Health Organization (WHO) severity scale, or equivalent scales.[6]

The examination of graft protocols by ethical commit-

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TABLE 1. *The Transplantation Team for PD*

Medical Personnel	Requirement Level
Transplant biologists	Mandatory
Neurosurgeons (stereotaxy)	Mandatory
Imaging experts (MRI, PET)	Mandatory
Neurologists	Mandatory
Psychiatrists	Desirable
Neurophysiologists	Desirable
Immunologists	Desirable
Ethician	Desirable
Pathologist	Mandatory
Methodologist	Mandatory

A similar composition is needed, whichever the disease treated. In HD trials, the psychiatrists and the neurophysiologists are mandatory.

tees is of particular importance in the field of cell therapy. It is of utmost importance to examine the benefit-to-risk ratio and to address the ethical concerns regarding the procurement of fetal tissue from abortion.

Another important aspect of this work is to demonstrate the survival and maintenance of grafted neurons (Table 2). Generally positron emission tomography (PET), using labeled levodopa, is considered the premium standard for PD.[7] However, the PET imaging is not a direct quantitative measurement of the surviving and/or grafted dopaminergic neurons; functional alterations of the DA receptors and the transporters may alter the results. Long-term improvements of motor functions or reductions of the dopaminergic drugs may validate the survival of the graft, if positive correlations exist with the PET imaging.[8,9] When the postmortem examination of the grafted brains is available, it validates the clinical diagnosis of PD. It also directly shows the presence of the heterotopic grafted tyrosine hydroxylase (TH)+ neurons in the striatum and allows a numerical estimate of the number of surviving neurons (viability) of the striatal volume, reinnervated by DA fibers, with reciprocal synaptic contacts with the host's brain. It also shows the evidence of immune events around the transplanted neurons.[10,11] The results of postmortem examination of the brain validate preclinical animal experiments, which were performed to build the clinical trial. Both of these procedures demonstrate similar results.

The main objective remains the demonstration of clinical efficacy, as a palliative, or in some putative examples, as a curative treatment. This is not in accordance with phase I drug trials. In the example of PD, patients are their own controls, and the improvement of the severity of the disease/symptoms has to be in direct relationship to the development of the graft. This necessitated the original development of the core assessment program for intracerebral transplantations (CAPIT)[12] and, several years later, the development of the core assessment program for surgical interventional therapies in Parkinson's disease (CAPSIT-PD) protocols.[13] The first attempts were unilateral and the main objective was to obtain motor effects that were supposed to be mainly contralateral to the surgery, with one side of the body serving as "control."

The following guidelines were generally adhered to: quantitative measurements of the speed (timed tests) and the quality (video recordings) of the standardized movements, repeated auto-scoring of the daily time spent in "on" and "off" state, measurements of the unified Parkinson's disease rating scale (UPDRS) III scores in standardized conditions (defined OFF after a 12 h fasting and repeated measures after a single standard dose of levodopa), repeated sessions of the CAPIT procedures several months before, and then for years after the graft, in parallel with brain imaging by PET.[14–19]

Several conclusions could be drawn following the long-term follow-up of unilaterally grafted PD patients: the procedure shows improvements in brain functioning for some but not all patients, with bilateral improvements that remained asymmetric; the effects were maintained for several years, and the increase of the capability of levodopa intake on the grafted side, the demonstration of pharmacologically induced liberation,[20] paralleled the progressive loss of DA activity in the nongrafted striatum. In addition, occurrence of dyskinesias in the "off" state was reported.[17]

Further pilot trials addressed several other questions: 1) the improvement of the viability of grafted neurons by drugs, e.g., lazaroids,[21] 2) the "best" amount of donors needed to optimize the clinical effects,[22] and 3) the comparison of bilateral and unilateral grafts.[23]

One major problem is the lack of uniformity in reported results. Variable results are usually reported with

TABLE 2. *Collection of Data Indicating the Survival of Grafted Neurons in the Host's Brain*

Indicator	Type of Evidence
Long-lasting improvement of motor state in "off" condition	Indirect evidence
Long-lasting reduction of the requirement of the DA drugs	Indirect evidence
PET imaging with labeled Levodopa	Indirect evidence
Correlation, PET/motor measurements	Indirect evidence
Functional improvement of movement-related brain activation (PET/MRI)	Indirect evidence
Demonstration of DA release by transplant	Indirect evidence
Postmortem examination of grafted brain	Direct evidence

several different explanations, such as uneven selection of patients, within centers or between different centers, and modifications of the graft procedures (harvesting of tissues, solid grafts *vs* cell suspensions, number of donors, number and location of grafts within the putamen and/or the caudate nuclei, immunosuppressant etc.).

The major lesson from these experiments is the report of individual patients with a long-lasting motor improvement, a reduced need for dopaminergic drugs, a long-term survival of more than 100,000 neurons per side, and a correlation between the clinical improvement and the increase in the capability of striatal levodopa intake, demonstrated by the PET imaging. Graft remains in the field of palliative treatments, but the "positive" cases are promising for CNS repair. However, the major variations in the results between centers, and between patients, indicate that additional steps forward are needed to leave transplantation out of the domain of research.

Controlled trials

Two controlled trials have been designed and performed for severe PD.[24,25] Their designs incorporated a "sham-operated" *versus* a transplant group. The first trial included 40 subjects, with a factorial design to test the effect of age. The second trial reported 34 subjects in three cohorts, which allowed a comparison between the sham-operated patients and subjects receiving one or four donors per side. There were also several other differences between those two trials. The follow-up was one year for the former group and two years for the latter. The Denver group (trial 1)[24] used three main endpoints: primary outcome (subjective rating of the change of severity of the disease), UPDRS scale, and Schwab-England scale. The New York City group (trial 2)[25] selected UPDRS III "off" scale as a major endpoint. The immunosuppressant was used only in the second trial.

Both trials were negative regarding the main endpoints for the whole cohorts, despite an increase in the striatal fluorodopa intake as compared to placebo cohorts. Interestingly, some patients appeared to respond better to transplantation: patients under 60 years of age in the former trial and less severe patients (but not younger) in the second trial. The therapeutic impact appeared to be a "stabilization" of the UPDRS score in the grafted patients, as compared with a deterioration in the control group. As far as the "more severe" group is concerned, grafted as control patients appeared to maintain the same motor score with time, i.e., the sham-operated patients did not deteriorate. It could be interesting to make a tentative meta-analysis of both trials. After 1 year, control patients did not change. Treated patients improved by approximately 18% (in trial 1), and ~20% for the four-donor group in trial 2. In view of small cohorts being followed, it remains doubtful whether this would be clinically useful. It must be pointed out that in trial 2, the grafted cohorts appeared to deteriorate at the end of the immunosuppression and the reported negative evolution did not result from low viability of grafts (<100,000 TH+ neurons in a postmortem case). Moreover, disabling "off" dyskinesias were reported in approximately 50% of the grafted patients in both series.

TABLE 3. *A Few Typical Modes of Action of Cell Therapy in the CNS*

Mode of Action	Disease
Liberation of neuromediators	PD (DA), Alzheimer (Ach)
Reconnection with host's brain	HD, stroke
Neurotrophic effects	All diseases, including ALS
Active or passive bridges	Spinal transection
Remyelination	MS
Induction of host's stem cells	All diseases
Expression of enzymatic activity	PD (DA)

ALS = amyotrophic lateral sclerosis.

The conclusions drawn by both teams are that fetal mesencephalic allograft cannot, at present, be recommended as a treatment for severe PD. However, several lessons can be learned and the efficacy can be improved employing more neurons and better targets, and/or neurotrophic factors. A detailed discussion is presented later.

THE DESIGN OF CLINICAL TRIALS FOR CELL TRANSPLANTATION

Basic requirements

Preclinical experiments must define the potential mode of action of cell therapy (Table 3). To obtain significant information, it is of critical importance to select a good model for the disease under consideration. For some genetic diseases [e.g., Huntington's disease (HD), etc.], phenotypic models, such as intoxication by the drug 3-nitropropionic acid, give a very different ground for cell therapy than genotypic models (transgenic animals).[26] The models must produce data concerning the viability of the grafted cells, the motor and/or nonmotor expected benefits, and the evolution of the graft into the host's brain.[27] If we consider the example of stroke,[28,29] the models differ according to the size and the reversibility of ischemia, which is induced by sudden occlusion of an artery, and do not address the vascular diseases which may be a therapeutic goal (e.g., experimental atherosclerosis, arterial hypertension, etc.). In the case of Alzheimer disease (AD), one can use phenotypic models like hippocampal lesions or destruction of cholinergic forebrain nuclei, or genotypic models with overexpression of amyloid protein or precursors. The choice of the model critically depends on the mode of action of cell therapy, and/or the use of the model (e.g., xenograft of

human cells into large animal brains to improve the surgical methods). Repeated animal experiments are mandatory for validating a treatment before launching any trial, even in lethal diseases (e.g., HD). They also indicate the amount of tissue to be grafted to expect clinically significant results.

The source of tissue is another major objective under consideration for preclinical experiments. It is now generally accepted that the fetal tissue obtained from voluntary abortion is not a suitable source of cells.[30] The viability depends on many factors, and a quite complex dissection is mandatory to avoid grafting the non-neural cells with unacceptable side effects. Moreover, the age at the time of abortion may be a critical factor and it differs according to pathology, e.g., HD and PD. There are major concerns about the viability, purity, and standardization of prepared tissue. Moreover, there is a major ethical concern regarding the need for the production of a large amount of tissues for the therapy.[30] Autografts are abandoned in the case of adrenal graft for PD, and remain a possible option concerning Schwann cells in the example of multiple sclerosis (MS). Intrinsic sources of tissue are, nevertheless, available (e.g., stem cells from several tissues, such as bone marrow, or from olfactory mucosa).[31] Autograft would appear as a very expensive option, in view of the necessary amplification of cell lines, but has major advantages from the immunological point of view. Most genetic and degenerative CNS diseases may also preclude autografts, with the consideration of the possibility of expanding cells affected by genetic or "degenerative" intrinsic lesions.[30]

Human-derived precursor cells may be obtained from many different tissues, and the analysis of research in this domain is far beyond the scope of this report.[30,31] Human cell lines have shown major advantages, mainly from the immunological or viability point of view, whereas the xenografts yielded poor results for PD. Cell lines may arise from CNS, blood, skin, olfactory mucosa, or other tissues. It is required to differentiate therapeutic cells according to their origin, their degree of differentiation from embryonic progenitors, uncommitted or regionally specified progenitors, committed precursors, or postmitotic neuroblasts. Harvesting and biochemical inductors may vary and they must be standardized before clinical use. The security of cell therapy is a major concern for nonlethal diseases and must be validated before the pilot trials.

Selection of patients

As brain transplantation surges ahead as a new and promising treatment aimed at repairing disabled neural networks, patients in the very advanced stage of disease may desire to avail the opportunity of a "last chance" treatment. Whereas, from an ethical point of view, pilot trials were performed in severely disabled PD patients (stage IV and V according to the Hoehn and Yahr scale), the selection of the patients may define different groups. To ensure the validity of the results, the group of selected patients needs to have a minimal variability. The severity of the disease has to be defined according to the preclinical data and the assumed mechanism of action of the graft. Published series of PD patients indicates that the selection of patients younger than 60 years of age, and having a less severe disease (e.g., stage III), may allow better results.[24,25] This can be drawn from pilot trials, and is of critical importance in controlled series. As far as HD is concerned, the assumed action of striatal graft is to rebuild part of the striatal neural networks under severe conditions and to protect the cerebral cortex from atrophy.[33,34] This postulate may not be true, and some data indicate that severe cortical lesions already exist in the initial stages of the clinical disease.[35] Nevertheless, as graft was performed with striatal neurons, it appears necessary to graft patients early in the disease, before the appearance of the cortical symptoms.

In other CNS lesions, such as spinal cord trauma or ischemic stroke, it is mandatory to choose homogeneous cohorts according to the following: 1) the severity of the lesion (complete or incomplete transection of the cord, the extent of the brain infarction), 2) the location of the lesion (e.g., cortical brain infarction in a given area irrigated by middle cerebral artery), and 3) the time between lesion and the treatment. Many cellular and trophic events take place after a lesion, and animal experiments are critical for defining the optimal stage of the disease. If we consider the example of the MS patients, different objectives can be achieved: to improve remyelination and/or to protect axons and cell bodies from death.[31] The selected cohorts of MS patients have to minimize variations on many grounds, including clinical disease (remittent or progressive), duration of evolution, major systems being symptomatic (visual, motor, sensory, cerebellar etc.), and the extent and the severity of the disease evaluated with standardized MRI. Ethical concerns may be raised by all pilot trials, and have to be carefully addressed.

Graft procedures

A discussion on the sources of the cells is presented above, and a choice has to be made among many different sources, from postmitotic fetal neurons to stem cells or adult cell lineages, genetically engineered. This point is beyond the scope of the present report (Table 4)

Irrespective of the source, the various methods of harvesting the cells, to ensure the purity, the number of cells available for one patient, the mean number of cells by volume injected, the biochemical characteristics (e.g., enzymatic activity or production of neurotrophic factors) have to be standardized and have to ensure a clinical grade for security. There are no major differences with

TABLE 4. *Some Examples of Potential Sources of Tissue for Brain Transplantation*

Postmitotic Cells	Precursor Cells
Human fetal neural	Olfactory mucosa precursor cells
Xenogenic fetal neurons	Stem cells from bone marrow
Autograft (adrenal, Schwann cells)	Immortalized cell lines
Autologous cells genetically engineered	Embryonic progenitors, committed precursors, or postmitotic neuroblasts
Cografts	Uncommitted or regionally specified progenitors
Olfactory mucosa neurons or glial cells	Committed precursors Postmitotic neuroblasts

the production of a clinical drug, and the security remains a major concern for gene therapy or immortalized cell lines. In some instances, the pregraft exposure of the cells to drugs and neurotrophic factors (e.g., reelin) have to be checked by preclinical research.

The administration of cells is not always carried out by stereotactic surgery. In the examples of stroke and MS, it may appear that intravenous injection of cells, if assumed to cross the blood-brain barrier, may permit to give a cell treatment quite soon after the lesion.[32] This may also apply to spinal cord transection. The demonstration of possible fusion between injected cells and resident cells is a possible cause of misinterpretation of experimental results.[32] For PD patients, the precise location of injected neurons within the striatum may be critical.[36] The use of the semipermeable capsules, implanted in the CNS or cerebral ventricles, allow xenografts and indirect gene therapy with an acceptable level of security.[37] Most of the trials for PD used heterotopic injections; some evidence now indicates that homotopic grafts or cograft may be possible or preferable.[38]

EVALUATION OF RESULTS

Pilot trials

Pilot trials need to demonstrate the survival of the implanted neurons and the effectiveness of the postulated mechanism of action of graft itself. Several imaging techniques, mainly PET imaging, are available at our disposal. Because of lack of appropriate tracing of grafted neurons in HD patients, regional glucose imaging has been used.[39] For neurodegenerative diseases, unilateral graft may help to ensure better demonstration of the effect, on the basis of an assumed asymmetric motor improvements.

The clinical evaluation needs first to demonstrate a clinical effect, without the need for direct demonstration of the improvement in the quality of life. Timed tests were designed to achieve this goal for the example of PD. In HD patients, timed tests also showed rich promise to achieve this goal. It is of critical importance, in a given disease, to build a complex protocol to ensure a multiparametric evaluation of the results. In HD patients, CAPIT-HD included many neurological examinations (unified Huntington's disease rating scale), neuropsychologic testing, psychiatric follow-up, and electrophysiological recordings.[40] Each team performing a trial on the same disease may want to compare, or to associate their own results, and common evaluation criteria are mandatory.

The benefit-to-risk ratio has to be evaluated thoroughly in these trials. The collection of the adverse events in an open protocol may not be sufficient. Apart from collection of data under the possible consideration of side effects of surgery, immunosuppressive treatments, and the development of the cells in the host's brain, general scales of side effects have to be systematically used, such as the WHO criteria of severity, which were used in a pilot trial of indirect gene therapy in HD.[37]

Long-term follow-up is mandatory for CNS transplantation. Such aggressive treatments have to maintain therapeutic effects for a long time, and the real benefit to risk ratio will become apparent after several years.

Controlled trials

The main objective of controlled trials is to demonstrate the efficacy of the treatment. Published trials demonstrated that controlled trials, with parallel blinded cohorts, are now possible, but very difficult and expensive to build.[41] The French HD trial, designated to assess the graft efficacy, includes a control cohort, randomized but unblinded. This design was adopted on ethical grounds, and one main reason was that sham surgery did not seem acceptable for the French ethical committee. Such trials may also evaluate the dose of treatment given to different cohorts, e.g., one donor *versus* four donors per side in Olanow's trial.[25]

The scales that are to be used may differ for controlled trials; clinical global scales, activity of daily living, and quality of life scales may be useful. As for drug trial, the main endpoint has to be robust, sensitive, and already validated. For PD patients the evaluation may move from UPDRS III in defined "off" state to UPDRS in "on," UPDRS II, and drug requirements. When available, an alternate validated treatment (e.g., deep brain subthalamic stimulation) may be a convenient competitor in randomized cohorts. The same security evaluations, indicated for pilot trial, also apply in controlled trials.

Registry

Registry may help to collect larger cohorts of evaluated patients and allow meta-analysis, if the common evaluation criteria are shared by different teams. Anonymous data are to be collected in a registry that prospec-

tively includes all the possible cases. The collection of data must include adverse events. In view of feasibility, gross data may only be incorporated each year for a given patient. This may include demography, severity and evolution of the disease, drug intake, volume injected and dose of cells administered to the patient, major scales, and adverse events. Such registry has also been proposed to compare with alternative surgical treatments.

Postmortem studies

Postmortem examination of the brain is of critical importance in validating the clinical diagnosis (e.g., PD *vs* multiple system atrophy), the brain area involved by the transplant, the survival and development of the graft, the establishment of functional connections with the host's neurons, the presence of lesions arising from immune rejection and/or patient's pathology. In PD cases, postmortem studies have validated the preclinical experiments and the postulated dopaminergic reinnervation of the striatum by grafted neurons.[8,9] The possibility of postmortem examination of the brain should be included in the informed consent given to the patient and/or caregiver. It is essential to include a pathologist in the team.

Data analysis

As indicated before, the prospective follow-up of grafted patients has to be performed to validate the long-term effect of the graft. A methodologist helps to go beyond the classical ANOVA of the single cases or small cohorts. This is of particular importance for controlled trials, to calculate the minimal size of cohorts to reach the major endpoint.

The correlation analysis (e.g., PET results imaging *vs* clinical parameters) help to estimate the amount of grafted tissue needed. Nevertheless, the single case analysis is still of major importance; the "best results" collected from the pilot trials in PD or HD patients clearly indicate the theoretical goals to be reached, yet group results remain inconclusive. Single results can demonstrate that efforts are to be put forward to reproduce them, and to detect the reasons of "bad" results. These can be selection of patients (age and severity of disease) or technical procedures (the amount and the location of grafted tissue, etc.). The cohort analysis may indicate that a treatment can be introduced in therapy; single reports indicate that the tested treatment could be useful in the future.

GENE THERAPY AND THE LIMITS OF CELL THERAPY

Gene therapy raises specific technical as well as ethical concerns. In addition to many of the points analyzed before, the validation of the therapeutic gene, its vector and its administration to the patient are to be specifically addressed. The vector has to be standardized, its expression and the duration of expression must be checked, as well as the possibilities of specific side effects (e.g., tumor induction) are to be explored. As quoted before, indirect gene therapy has been introduced in pilot trials, using xenogenic cell lines implanted in semipermeable capsules.[37] The security concerns about the treatment must be compared with the expected survival for a specific disease, e.g., gene therapy for glioblastoma. Presently, a trial has been launched in PD including direct brain injection of a viral vector expressing enzymes of levodopa synthesis, following positive preclinical results.[42] Here, we reach the limits of cell therapy and go beyond, if we consider intrastriatal injections of glial-derived neurotrophic factor (GDNF) for PD.[43] Interestingly, the evaluation criteria used in this trial are the same as those considered for cell therapy.

CONCLUSIONS

Although initial excitement was raised in the medical and scientific communities by pilot trials using adrenal autografts (1980s) and fetal allograft (1990s), two controlled trials in PD patients demonstrated negative results, with the conclusion that today, cell therapy cannot be recommended for PD. Nevertheless, trials are still in progress for diseases like HD, stroke, MS, cord transection, epilepsy, and others. Human knowledge about the biology of stem cells and precursor cells is growing and we have more and more evidence that cell therapy could be useful in "repairing" brain lesions, not only by survival and integration of grafted neurons, but also by stimulation of resident precursors already present within the CNS.[30,31]

The knowledge gathered from animal experiments is increasing, and several new directions may be taken. They can lead us to implant pure cell lines, with a reproducible amount, to get predicable clinical results, or to implant cells which are able to stimulate the intrinsic regenerative properties of the brain. The latter may not apply to diseases which directly affect all resident cells in the CNS (e.g., HD) but may be of critical importance for traumatic or vascular brain lesions. Finally, some of the trophic factors which may improve the survival of grafted cells could represent, *per se*, a therapeutic alternative (e.g., GDNF, reelin[44]). In a sense, their use means "to teach the brain to graft himself with its own cells."

From a medical point of view, we have to transfer preclinical knowledge, ensuring a maximal security for the patients. This, as an ethical research, has to implement logical, comprehensive, highly controlled methods that need a complex organization. The transplantation teams should work as quickly as possible in the design of the trials, but must comply with, and not precede, the preclinical research.

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