Human fetal cell therapy in Huntington’s disease: a randomized, multicenter, phase II trial (MIG-HD)

Prof. Anne-Catherine Bachoud-Lévi¹²³, on behalf the MIG-HD-group

MIG-HD-group, list of authors:

- Bachoud-Lévi Anne-Catherine, MD PhD, National Reference Center for Huntington’s Disease, Neurology Department, Henri Mondor-Albert Chenevier Hospital, Assistance Publique - Hôpitaux de Paris, France
- Schramm Catherine, PhD, National Reference Center for Huntington’s Disease, Neurology Department, Henri Mondor Hospital, Assistance Publique - Hôpitaux de Paris, France
- Remy Philippe, MD PhD, Neurology Department, Henri Mondor Hospital, Assistance Publique - Hôpitaux de Paris, France
- Aubin Ghislaine, MD, University Hospital Center in Angers, France
- Blond Serge, MD, University Hospital Center in Lille, France
- Bocket Laurence, PharmD, Laurence.BOCKET@chru-lille.fr, University Hospital Center in Lille, France
- Brugieres Pierre, MD PhD, Neuroradiology Department, Henri Mondor Hospital, Assistance Publique - Hôpitaux de Paris, France
- Calvas Fabienne, MD, Inserm CIC 1436, University Hospital Center in Toulouse, Université Toulouse III Paul Sabatier, Toulouse, France
- Calvier Elisabeth, MD, University Hospital Center in Nantes, France
- Cassim François, MD PhD, University Hospital Center in Lille, France
- Challine Dominique, MD, Virology Department, Henri Mondor Hospital, Assistance Publique - Hôpitaux de Paris, France
- Cleret de Langavant Laurent, MD PhD, National Reference Center for Huntington’s Disease, Neurology Department, Henri Mondor Hospital, Assistance Publique - Hôpitaux de Paris, France
- Collier Francis, MD, University Hospital Center in Lille, France
- Cottencin Olivier, MD PhD, University Hospital Center in Lille, Lille University, F-59000 Lille, France
- David Philippe, MD, Erasmus University Hospital, Brussels, Belgium
- Damier Philippe, MD, University Hospital Center in Nantes, France
- Deliaux Marie, Master, University Hospital Center in Lille, France
- Delmaire Christine, MD PhD, University Hospital Center in Lille, France
- Delval Arnaud, PhD, University Hospital Center in Lille, France
- Démonet Jean-François, MD, INSERM U825, Hôpital de Purpan, Toulouse, France
- Descamps Philippe, MD PhD, University Hospital Center in Angers, France
- Gaura Véronique, MD, Molecular Imaging Research Center (Mircen), Atomic Energy Commission (CEA), Fontenay-aux-Roses, France ; Centre National de la Recherche Scientifique (CNRS), Université Paris-Sud, Université Paris-Saclay, UMR 9199, Neurodegenerative Diseases Laboratory, F-92260 Fontenay-aux-Roses, France ; Department of Nuclear Medicine, CHU Tenon Hospital, Paris, France
- Gohier Bénédicte, MD PhD, University Hospital Center in Angers, France
- Goldman Serge, MD, Erasmus University Hospital, Brussels, Belgium
- Haddad Basam, MD, Gynecology Department, Intercommunal Hospital Center of Creteil, France
• Izopet Jacques, PhD, University Hospital Center in Toulouse, France
• Jeny Roland, MD, Maternity Hospital Esquirol Saint Maurice, France
• Kerr-Conte Julie, PhD, University Lille, Inserm, CHU Lille, U1190 - EGID, F-59000 Lille, France
• Krystowiak Pierre, MD PhD, Service de neurologie, CHU d’Amiens, Amiens, France; CHIMERE, EA 7516, Universite de Picardie Jules Verne, Amiens, France
• Lalanne Christophe, PhD, Paris Diderot University, France
• Lavisse Sonia, PhD, Molecular Imaging Research Center (Mircen), Atomic Energy Commission (CEA), Fontenay-aux-Roses, France; Centre National de la Recherche Scientifique (CNRS), Universite Paris-Sud, Université Paris-Saclay, UMR 9199, Neurodegenerative Diseases Laboratory, F-92260 Fontenay-aux-Roses, France
• Lefaucheur Jean-Pascal, MD PhD, EA 4391, Faculty of Medicine, Paris-Est University, Creteil, France; Clinical Neurophysiology Department, Henri Mondor University Hospital, APHP, Creteil, France
• Lemoine Laurie, Psych, National Reference Center for Huntington’s Disease, Neurology Department, Henri Mondor Hospital, Assistance Publique - Hôpitaux de Paris, France
• Levivier Marc, MD PhD, Neurosurgery Service and Gamma Knife Center, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland; Faculty of Biology and Medicine (FBM), University of Lausanne, Lausanne, Switzerland
• Lotterie Jean-Albert, MD, University Hospital Center in Toulouse, France
• Lunel-Fabiani Françoise, MD, University Hospital Center in Angers, France
• Maison Patrick, MD PhD, Neurology Department, Henri Mondor Hospital, Assistance Publique - Hôpitaux de Paris, France
• Massager Nicolas, MD PhD, Erasmus University Hospital, Brussels, Belgium
• Massart Renaud, PhD, National Reference Center for Huntington’s Disease, Neurology Department, Henri Mondor Hospital, Assistance Publique - Hôpitaux de Paris, France
• Menei Philippe, MD PhD, University Hospital Center in Angers, France
• Montero-Menei Claudia, PhD, University of Angers, UMR 1066, 49045, Angers, France
• Neveu Isabelle, PhD, University Hospital Center in Nantes, France
• Parant Olivier, MD, University Hospital Center in Toulouse, France
• Pautot Vivien, MD, University Hospital Center in Angers, France
• Payoux Pierre, MD, University Hospital Center in Toulouse, France
• Pereon Yann, MD PhD, Reference Centre for Neuromuscular Diseases Atlantique-Occitanie-Caraïbes, FILNEMUS, University Hospital Center in Nantes, France
• Rialland Amandine, Master, Clinical Research unit, Henri Mondor Hospital, Assistance Publique - Hôpitaux de Paris, France
• Rosser Anne, MD PhD, Schools of Medicine and Biosciences, Cardiff University, UK
• Rouard Hélène, PharmD PhD, Cell Therapy Center (Etablissement Francais du Sang Ile de France – site Creteil), France
• Scherer Gagou Clarisse, MD, Neurology Department, University Hospital Center in Angers, 4 rue Larrey, 49933 ANGERS cedex 9, France
• Schmitz David, Master, Clinical Research unit, Henri Mondor Hospital, Assistance Publique - Hôpitaux de Paris, France
• Simonetta-Moreau Marion, MD PhD, University Hospital Center in Toulouse, France
• Simonin Clémence, MD PhD, University Hospital Center in Lille, France
• Slama Hichem, PhD, Erasmus University Hospital, Brussels, Belgium
• Sol Jean-Christophe, MD, University Hospital Center in Toulouse, France
• Supiot Frédéric, MD, Erasmus University Hospital, Brussels, Belgium
• Tanguy Jean-Yves, MD, University Hospital Center in Angers, France
• Tenenbaum Liliane, PhD, Department of Clinical Neurosciences, University Hospital of Lausanne, Lausanne, Switzerland
• Verny Christophe, MD PhD, National Reference Center for Huntington’s Disease, Neurology Department, University Hospital Center in Angers, UMR CNRS 6015, INSERM U1083, Institut MitoVasc, 49933 Angers, France
• Youssov Katia, MD, National Reference Center for Huntington’s Disease, Neurology Department, Henri Mondor Hospital, Assistance Publique - Hôpitaux de Paris, France
• Peschanski Marc, MD PhD, INSERM U861, I-Stem, Corbeil-Essonnes 91100 France
• Audureau Etienne, MD PhD, Clinical Research Unit, Henri Mondor Hospital, Assistance Publique - Hôpitaux de Paris, France
• Palfi Stéphane, MD, Neurosurgery Department, Henri Mondor Hospital, Assistance Publique - Hôpitaux de Paris, France
• Hantraye Philippe, PhD, Molecular Imaging Research Center (Mircen), Atomic Energy Commission (CEA), Fontenay-aux-Roses, France ; Centre National de la Recherche Scientifique (CNRS), Université Paris-Sud, Université Paris-Saclay, UMR 9199, Neurodegenerative Diseases Laboratory, F-92260 Fontenay-aux-Roses, France

**Corresponding author:**

Prof. Anne-Catherine Bachoud-Lévi\textsuperscript{1,2,3}, on behalf the MIG-HD-group:

\textsuperscript{1}Assistance Publique-Hôpitaux de Paris, National Reference Center for Huntington’s Disease, Neurology Department, Henri Mondor-Albert Chenevier Hospital, 94000 Créteil, France
\textsuperscript{2}Equipe neuropsychologie interventionnelle, Département d’études cognitives, École normale supérieure, PSL, Research University, Institut Mondor de Recherche biomédicale, Université Paris-Est, INSERM, 75005 Paris, and Créteil 94010, France
\textsuperscript{3}Faculté de Santé, Université Paris Est, 94000 Créteil, France

bachoud@gmail.com

Tel +33 1 49 81 23 15; Fax +33 1 49 81 23 26

**Manuscript word count:** 3696

**Running title:** MIG-HD, a phase 2 cell therapy trial in HD

**Key words:** Huntington’s disease, cell therapy, phase 2 trial, MIG-HD

**Relevant conflicts of interest/financial disclosures:** The authors have no conflict of interests to disclose.

**Funding sources for study:** AOM00139 and AOM04021 “Direction de la Recherche Clinique” (Assistance Publique – Hôpitaux de Paris) and AFM.

**ClinicalTrials.gov NCT00190450**
ABSTRACT

Background: Huntington's disease is a rare, severe, inherited neurodegenerative disease in which we assessed the safety and efficacy of grafting human fetal ganglionic eminence intrastriatally.

Methods: Patients with early-stage of the disease were enrolled in the Multicentric Intracerebral Grafting in Huntington’s Disease trial, a delayed-start phase II randomized study. After a run-in period of 12 months, patients were randomized at M12 to either the treatment group (transplanted at M13-M14) or the control group, secondarily treated 20 months later (M33-M34). The primary outcome was total motor score compared between both groups at 20-month post-randomization (M32). Secondary outcomes included clinical, imaging and electrophysiological findings, and comparison of pre- and post-graft total motor score slopes over the whole study period (M0-M52) regardless of the time of transplant.

Results: Of 54 randomized patients, 45 were transplanted; 26 immediately (treatment) and 19 delayed (control). Mean total motor score at M32 did not differ between groups (treated-controls difference in means adjusted for M12: +2.9 [CI95 -2.8 to 8.6]; P=0.31). Its rate of decline after transplantation was similar to that before transplantation. 27 severe adverse events were recorded in the randomized patients, 10 of which were related to the transplant procedure. Improvement of procedures during the trial significantly decreased the frequency of surgical events. We found anti-Human Leucocytes Antigen antibodies in 40% of patients.

Conclusion: No clinical benefit was found in this trial. This may have been related to graft rejection. Ectopia and high track number negatively influence the graft outcome. Procedural adjustments substantially improved surgical safety.
INTRODUCTION

HD is a rare inherited neurodegenerative disorder, which causes cognitive, behavioral, and motor deficits, often beginning in early adulthood. Genetic diagnosis is unequivocal for patients with more than 39 CAG repeats in the huntingtin gene. Despite intense pathophysiological research, disease-modifying treatments remain elusive, and patients have a mean survival, with considerable dispersion, of 20 years after motor onset. Gene-silencing therapies are promising, but will probably be more effective for prevention than restoration. Multiple therapeutic strategies would presumably be required, particularly for individuals already displaying striatal degeneration.

In HD, degeneration of neurons is particularly marked in the striatum, although not exclusive to this region. Striatal quinolinic acid (QA) lesions in experimental animals indicate that massive losses of striatal medium-sized spiny neurons, as occur in HD, can trigger progressive cortical projection neuron degeneration. Homotopic transplantation of cells derived from the ganglionic eminence (the fetal zone giving rise to the striatum) can replace the lost striatal neurons in rodent and non-human primate QA lesion models, partially restoring frontostriatal connections and striatal efferent links to output nuclei, and promoting recovery of cognitive and motor functions. Despite little neurodegeneration in R6/2 transgenic mice, modest improvement in locomotion was recorded after ganglionic eminence grafting. Functional improvement was also reported in transgenic models following stem cell-derived transplants. Since the 1990s, 70 HD patients have been enrolled in open-label, non-randomized, single-center trials (1-16 participants) of striatum-reconstructing treatments. These studies were too heterogeneous (different cell sources, tissue preparations, and surgical protocols) and underpowered to be conclusive or to drive improvements for future trials. Nevertheless, some patients showed clear signs of sustained improvement. Graft-host connection was demonstrated in post mortem samples, with structures resembling normal striatum in the grafted region, cortical and nigral afferents from the host, and efferent to downstream pallidal nuclei and substantia nigra. International guidelines consider cell transplantation into the brain to be safe despite some reports of overgrowth, graft tissues ectopic to the target area, and subdural hematomas (SDHs).

We set up a phase II randomized controlled trial: Multicentric Intracerebral Grafting in Huntington’s Disease (MIG-HD), to assess the safety and efficacy of human fetal cell intrastratal transplantation in patients with early-stage HD. This report summarizes the main study findings and key lessons learned during the course of the trial. We identified factors that may influence transplant functionality for consideration in future trials.
METHODS

Study design and oversight

MIG-HD was a multicenter randomized phase II study assessing the safety of intrastratal human fetal cell transplantation and its effect on motor function in patients with early-stage HD. The study was conceived as a delayed-start design, where active treatment is sequentially provided to all participants over time, so that all patients could eventually benefit from the transplantation procedure. The study was approved by the institutional review boards of Henri Mondor Hospital in France and Erasme Hospital in Belgium. It complied with the Helsinki Declaration, current Good Clinical Practice guidelines, and local laws and regulations. Written informed consent was obtained from patients at M0 or M1. An independent safety committee monitored the study conduct, the collected data and any severe adverse events (SAEs). The protocol was registered at ClinicalTrials.gov (NCT00190450). Methodological details are provided in the supplementary methods.

Participants

Consenting patients with genetic diagnoses of HD underwent transplantation at six French and Belgian hospitals between 2001 and 2010; their follow-up to M52 was completed in 2013. The main inclusion criteria were: having manifest HD for ≥1 year, >36 CAG repeats in the huntingtin gene, age 18-65 years, total motor score (TMS) >5 on the Unified Huntington’s Disease Rating Scale (UHDRS), and total functional capacity (TFC) score >9. The main exclusion criteria were: Mattis Dementia Rating Scale (MDRS) score <120, and contraindication for surgery or magnetic resonance imaging (MRI) (supplementary methods).

Randomization and masking

After a one-year run-in period, designed to verify patients’ compliance and exclude unusual patterns of clinical deterioration, patients were randomly assigned at month 12 (M12) in a 1:1 ratio either to treatment (receiving transplant at M13-M14) or to the (initially untreated) control group, which were subsequently grafted 20-months later (M33-M34) (Figure S1). Randomization was computer-generated, with centralized allocation concealment. A randomisation list prepared at the Henri Mondor Clinical Research Unit with Nquery software (Statistical Solutions Ltd., Boston, USA) was used. Participants and investigators responsible for clinical follow-up were not blind to treatment allocation. However, the validity of the primary outcome (UHDRS TMS excluding rigidity) was assessed by video recordings at M12, M32 and M52 and scored by specialists not involved in patient follow-up and recruitment and blind to treatment allocation (Figure S2).

Procedures

Small blocks of whole ganglionic eminences from one to three 8.5- to 12-week-old fetuses (mean ±standard deviation: 1.6±0.6) per grafting session were implanted stereotactically, within 48 hours of retrieval, into the striatum ipsilateral to the dominant hand. A mean of 2.45±3.03 months later, the contralateral striatum was grafted (supplementary methods). Cells were injected through six tracks (mean 4.91±1.46; range 3 to 6) within the head of the caudate nucleus (pre-commissural and commissural) and the putamen (one in each of pre-commissural and commissural, and two in post-commissural putamen). This totalled a volume of 206.0±43.1 µL unilaterally, distributed as 8 deposits per track (mean 5.1±1.0 µL by deposit) with significant variations across centres. Two tracks were omitted after the first 29 grafting sessions, to avoid SDH in patients with major striatal atrophy. Cerebrospinal fluid leakage was limited by confinement to bed and hyperhydration for 48 h after surgery.
Imunosuppression was achieved with cyclosporine A, beginning three days before surgery (400 mg/day, then adjusted to maintain blood concentrations between 100 and 150 mg/L), prednisolone (0.25 mg/kg per day) and azathioprine (0.75 mg/kg per day) both initiated on the day of surgery. Cyclosporine A was stopped six months after the second transplantation, and prednisolone and azathioprine were stopped six months later. After the occurrence of acute graft rejection and the identification of Human Leucocytes Antigen (HLA) antibodies in 30% of the patients tested,23 guided by international experts in immunology, we established a new immunosuppression protocol for the last 20 patients. This involved monitoring HLA antibodies at each centre and prolongation of full immunosuppression for up to one year after the second graft. Azathioprine and prednisolone were continued for six additional months, and prednisolone was withdrawn gradually. Plasma HLA antibodies were then monitored locally at each hospital, and treatment was modified (withdrawal of cyclosporine or of prednisolone) on occurrence of any unusual signs. Oral immunosuppressive therapy was withdrawn if no HLA antibodies against the grafts were detected.

Short and full assessments were alternated for clinical examination (Figure S1). We used the complete UHDRS, cognitive tasks,24 back-and-forth hand-tapping, and electrophysiological assessments. When surgery could not be done on the scheduled date due to lack of foetus availability, preoperative assessments were repeated if the interval between them and the transplant exceeded three months. Brain imaging included MRI, 18F-fluorodeoxyglucose PET (FDG-PET) and, in patients not on neuroleptics, with 11C-raclopride PET (supplementary methods).

Endpoints

The primary outcome was the UHDRS-TMS compared between treatment and control groups at 20-month post-randomization (M32). TMS is a composite score for chorea, dystonia, oculomotor movement, tapping, pronation/supination, palm/hand/fist sequence task, walking, tongue protrusion and rigidity, rated from 0 to 124 points, with higher scores indicating poorer performance. Secondary outcomes included clinical, imaging and electrophysiological findings, as well as comparison of pre- and post-graft TMS slopes over the whole study period (M0-M52) regardless of the time of transplant. Adverse events (AEs) were identified on clinical examination, according to the World Health Organization checklist, at all visits and between visits if spontaneously reported by patients (Table S1).

Statistical analysis

Sample size calculation relied on data from an observational cohort of early HD patients comparable to those included in the present trial and followed for up to 4 years,24 showing an average annual natural progression of +13.2±14.1 for the UHDRS-TMS. Hypothesizing a stable evolution as a clinically meaningful effect of the graft, inclusion of ≥18 subjects per group was required to achieve 80% power at a 2-sided 5% alpha level. To account for a prespecified subgroup analysis led in graft recipients with a metabolically active transplant based on FDG-PET imaging (60% expected like in 11), a sample size of 60 (30 per group) was targeted.

For the primary outcome, patients were assessed according to randomized group under the modified intent-to-treat principle, including all patients from the control group and patients from the treatment group having received a transplant. The main planned primary endpoint analysis relied on the comparison of the TMS at M32 between treatment and control groups using analysis of covariance (ANCOVA) of the score at M32 with the initial value at M12 as a covariate. Supportive sensitivity analyses of the primary endpoint included: i) ANCOVA with further adjustment for centre and other covariates at M12 with prognostic value or showing evidence of a potential imbalance between study arms at the time of randomization and/or transplant; ii) comparing the absolute change in TMS from M12 to M32 between the two randomized groups and iii) assessing the graft effect on the evolution of
TMS over time (M0-M52) regardless of the randomized group using a piecewise two-part (before-after the first transplant) linear mixed model.

Clinical and electrophysiological secondary endpoints were compared between randomized groups using ANCOVA of values at M32 with values at M12 as a covariate, adjusting for similar covariates as for the primary outcome, with the addition of the total motor score. Potential effect modifiers that could predict improved response to intrastriatal transplant were searched for from a preselected list of 21 variables relating to patients and intervention, by testing for interactions between time after first graft and the candidate predictors in a piecewise linear mixed model (supplementary methods).

All tests were two-tailed, with P<0.05 considered significant. Analyses were prespecified in the trial protocol and performed with Stata v15.1 (StataCorp, College Station, USA) and R-3.6.0 (R Foundation, Vienna, Austria).

Following the discovery of immune rejection, detection of antibodies directed against HLA class I and class II antigens expressed by donor tissues was assessed in each center, using the locally available technique.

**MRI Analyses**

MRI was planned as part of the study design for safety only. We conducted a retrospective volumetric segmentation analysis using the Freesurfer software in patients scanned on the same machine for PET-coregistration (supplementary methods).
Results

Between January 2001 and May 2006, 66 patients met the inclusion criteria (M0-M1), 54 were randomized (M12), and 45 underwent transplantation (treatment group: 24 bilateral and 2 unilateral; and controls secondarily grafted: 17 bilateral and 2 unilateral) (Figure 1). Unilateral implantsations were due to cancellation of the contralateral transplantation following serious surgical complications after the first transplant in two patients, and to the decision of two others not having a second transplant following several cancellations of surgery due to insufficient tissue collection. Demographic and baseline characteristics are shown in Table 1. Patient demographic and clinical characteristics were not significantly different between the two groups at the M12 randomization time point, except for a longer disease duration and a more severe 1-figure cancellation task for the treatment group. Median follow-up was 56.9 months (interquartile range [IQR] 54.5-64.1) for the treatment group, and 60.0 months (IQR 56.6-65.7) for controls.

Safety

We recorded 287 AEs from M0 to M52 in the 54 randomized patients over a period of 12 years (Table S1); 91% were not attributed to the procedure and 9% related to the procedure (immunosuppressant or transplant). Among those, there were 27 SAEs, of which 17 were considered unrelated to the procedure: one death by suicide, two suicide attempts, three fractures, one road accident, one acute fever, two gastrointestinal disorders, one pulmonary embolism, and six hospitalizations for psychiatric disorders. Ten SAEs were procedure-related: one intracranial empyema, three SDHs (two requiring surgical drainage), one putaminal hematoma resulting in hemiparesis and aphasia, one seizure, one graft rejection,23 and three intrastriatal cysts. Due to progressive cranial hypertension, one of these patients with an intra-graft cyst required cauterisation of aberrant choroid plexus within the graft. Following this, the patient improved clinically and in terms of his striatal metabolism (ipsilateral to the cyst) compared to pre-surgery. Surgical and postoperative procedures were modified to prevent further hematomas in the following 57 grafts, leading to significant improvement (Fisher’s test P=0.03).

Despite cyclosporine monitoring and dose titration, eighteen of the 43 patients tested (39 during the 52-month study and four subsequently) were positive for HLA antibodies. We did not find correlation between the clinical results and the presence of HLA antibodies.

Efficacy

M32 TMS scores did not differ significantly between treatment (50.8±17.3, N=26) and control groups (39.0±17.0, N=26; ANCOVA adjusted for M12: P=0.31, adjusted difference in means: +2.9 [CI95 -2.8 to 8.6]). This was confirmed by supportive analyses after adjustment for disease duration (P=0.54), center (P=0.30), or multiple adjustment for both and other potentially influent covariates (i.e. independence scale, functional assessment scale, 1-figure cancellation, categorical fluency (1 min.); P=0.68), and in comparisons of mean absolute TMS change from M12 to M32 (+10.3±standard error 2.3 [treatment] vs. +8.1±2.1 [controls], P=0.52, Table 2). A longitudinal analysis of graft effect on TMS, regardless of group randomization, found no difference between the pre-graft and post-graft progression slopes (piecewise linear mixed model, P=0.65; Figure 2A). The reliability of clinician-rated TMS, assessed by blind scoring on the 96 exploitable videos from M12 to M52, was excellent (intraclass-correlation coefficient=0.92 with 95% CI [0.88;0.94] and P<0.001) (Figure S2).

No significant striatal metabolic differences were observed in FDG PET-scans between M12 and M32 in either treated (N=26) or control (N=19; Figure 3) patients. At M32, eight treated patients showed a
non-significant lower number of hypometabolic striatal voxels compared to M12 (means M12: 1519.3±395.9 and M32: 1308.0±315.1). Their TMS (mean 49.8±10.7) was similar to that of control patients (ANCOVA adjusted for M12: P=0.46). As for clinical and electrophysiological secondary endpoints, no statistically significant differences were found between randomized groups between M12 and M32, adjusted for potentially confounding covariates (i.e. M12 values of total motor score, 1-figure cancellation, categorical fluency (1 min.), independence scale, functional assessment scale and disease duration), except for Stroop word showing a more severe decrease in the treated, than in the control group (Table 2).

Analyses of basal ganglia MRI volumes between M12 and M32 showed a significant increase of the striatal volume in treated patients (N=13) compared to controls (N=16, P<0.001) without correlation with clinical scores (supplementary methods).

Exploratory analyses were performed on 10 parameters characterizing the patients’ pattern and 11 procedural aspects to identify potential predictors of transplantation outcome (supplementary methods). Interaction analyses in the longitudinal linear mixed model detected two detrimental predictors of steeper decline in post-graft TMS: ectopia (interaction term -0.29 [CI95% -0.58 to -0.002], P=0.049) and a trend for high number of tracks per side ≤5.5 (-0.25 [-0.51 to 0.047], P=0.067) (Figures 2B and 2C).
Discussion

This randomized multicentre delayed-start phase II trial was designed to assess the safety and efficacy of the intrastratial transplantation of human fetal cells in 54 patients in early to moderate stages of HD, of whom 45 were eventually grafted. A comparison of the treatment (N=26) and control groups (N=19) at M32 showed no improvement in TMS, even after restricting the analysis to the treated patients identified as having an increased striatal metabolism on FDG-PET-imaging. TMS slope was unaffected by transplantation. No benefit for secondary outcomes was observed (Table 2). We observed no increase in raclopride binding, suggesting no/little increase in striatal-like tissue, and no metabolic improvement in the striatum or frontal cortex post-transplantation in 80% of the grafted patients. This may have been due to implantation of insufficient quantities of tissue or poor tissue survival for a range of reasons including graft rejection, the latter according with the demonstration of transplant alloimmunogenicity in 41% patients tested for HLA antibodies.

Human fetal cells dissected from the developing striatum are theoretically good donor cells for transplantation in HD patients, but their availability is limited. This limitation necessitated a long study period (2001-2013) but did not affect the planned analyses, with repeated assessments for the comparison of treated and control (secondarily transplanted) patients. The high degree of consistency of blinded and investigator-attributed TMS scores demonstrates robustness (but possibly also insensitivity) of TMS scoring. Of note, an imbalance in TMS values at M12 was apparent between controls and treated patients, despite randomization. This observation most likely did not affect our findings based on between-groups comparisons adjusted for M12 values, with comparable results found in the longitudinal analysis of TMS in all grafted patients, regardless of initial group allocation.

Deaths occurred even before randomization (Figure S1), highlighting the fragility of patients with HD. Where appropriate, protocol adaptations were made during the study to address AEs, improve patient safety and prevent transplantation-related SAEs (see methods), without modifying the statistical validity of the trial. The initial surgical procedure, which resulted in SDH or putaminal hematoma in 10% of transplant recipients, compared favourably with the 43% reported in some pilot studies of fetal cell transplantation in HD. This risk was eliminated by omitting the two posterior tracks in patients with marked atrophy, hyperhydrating patients and imposing 48 hours bed rest; no such events occurred in the subsequent 57 surgical implantations. We also successfully treated an expanding choroid cyst within the graft by endoscopic cauterisation of choroid cells. This strategy would likely be of value for future stereotaxic surgical trials.

Only a few studies have reported unequivocal long-lasting transplant success, and little is known about the factors underlying graft failure. Graft-host connectivity has been demonstrated, but previous studies in small cohort of patients were unable to identify the key factors influencing transplant outcome. The MIG-HD trial, with 45 grafted patients at six centres, will help to advance cell transplantation practices for HD by identifying some key factors that need to be considered in future studies. The transition from single-centre to multicentre settings resulted in greater variability between centres than anticipated, particularly for surgery-related factors, resulting in substantial graft variability across the study. For example, larger numbers of injection tracks were expected to improve graft function, but our results suggest in contrast that slower deterioration of the TMS was associated with lower number of tracks. This observation might result from a combination of the number of foetuses (from 1 to 2), presence of HLA antibodies, patients’ gender, and duration of surgery; even if not proven statistically in these few individuals. It was unclear in the study by Paganini et al. whether ectopic grafts had a negative impact on graft function. In a blind analysis of MRI images, we show here that TMS deteriorated more in patients with ectopic transplants. Whereas we did not find any correlation
between striatal volume change measured using MRI and clinical evolution, recent MRI techniques should constitute a key marker in future trials. \(^3\) In contrast, given the difficulty to avoid neuroleptic intake in HD, alternative tracers in future longitudinal long-term studies should replace \(^{11}\)C-raclopride PET imaging. The number of hypometabolic striatal voxels correlated with TMS on FDG PET-scans, without allowing us to detect clinically responsive patients. This lack of consistent correlation of imaging and clinical response reproduces the results of other studies also reporting alloimmunisation processes against the graft. \(^{20,31}\) It might be the case that chronic inflammation due to alloimmunisation and transplant variability blurred the picture. Alloimmunization \(^{23}\) was unpredictable and changes in detection techniques during MIG-HD made it impossible to model the impact of HLA antibodies. Compared to our pilot trial, \(^{11}\) the use of older fetuses, the pooling of ganglionic eminences from several fetuses to increase graft volume, and reducing the inter-graft interval from one year to about two months, may have increased the risk of alloimmunisation. Here, 40% of patients developed HLA antibodies against the graft. In contrast, none of our patients from the pilot trial, with one-year intervals between transplants, had antibodies against the transplant five years after surgery (unpublished data). In two studies with short inter-graft intervals (2-7 months), HLA antibodies were present in 50% of patients in the German branch of MIG-HD \(^{29}\) and 37.5% in the Firenze study. \(^{31}\) The results of the MIG-HD study suggest that better standardization and control of procedures, with improvements in atrophic structure targeting and cell injection methods, are required for future transplant studies. It should be possible to decrease the numbers of ectopic grafts and injection tracks, but it will be harder to control HLA antibody development. These antibodies were also present in patients on immunosuppressants despite a correct cyclosporine titration, suggesting suboptimal immunosuppression protocol. Yet, establishing the link between presence of HLA antibodies against the graft and its lack of functionality is difficult because, except in the case of acute rejection \(^{23}\) alloimmunisation appears to be a long process. However, functional impact of alloimmunization, reported in monkeys, \(^{32}\) justifies better procedures to avoid alloimmunisation in future studies. The future use of stem cell-derived neural precursors should resolve many of the critical issues highlighted here, improving surgical intervention planning and facilitating the use of well-defined homogeneous cell therapy products effectively matched with the patient’s characteristics in advance. There are also some factors not considered here, such as tissue preparation, \(^{33,34}\) which could be addressed in further studies. Besides, in retrospect, the outcome measures lacked sensitivity (see \(^{35}\)), which calls for new sensitive digitalised measures, as developed in the RepairHD program.

In summary, it could be concluded that grafts cannot restore the fronto-striatal circuits despite the positive abundant animal literature, \(^{18}\) but we think that it would be premature to conclude this based on the MIG-HD study, which has highlighted many important questions that need to be addressed. It would also be premature to disregard the results of our previous pilot study, in which striking clinical improvement was seen in three patients across multiple outcomes analysed blindly to each other (clinics PET, electrophysiology, and digitalized movement analysis), including an increase of the metabolism in the frontal cortex, \(^{13,25,27}\) together constituting a proof of concept. We thus believe that a rational approach is to return to the bench to solve the issues raised here; if that can be achieved there may be a place for intracerebral transplantation, which is the only approach currently available with the potential to reverse the loss of striatal tissue. We propose that the lessons learned from MIG-HD could guide future transplant trials, whether for HD or other neurodegenerative diseases.
Acknowledgments

The sponsor was Assistance Publique – Hôpitaux de Paris (Département de la Recherche Clinique et du Développement, Clinical Research and Development Department) and by delegation, the Clinical Research and Development Department (DRCD), which carries out research missions in accordance with Article L.1121-1 of the French Public Health Code. AC Bachoud-Lévi is the principal investigator of MIG-HD. The Association Française contre les Myopathies and the Fonds National de la Recherche Scientifique provided complementary grants for the hospital in Brussels. The work was supported by ANR-10-LABX-0087 IEC and ANR-10-IDEX-0001-02 PSL, ANR-11-INBS-0011 - NeurATRIS and by the National Reference Centre for Huntington’s Disease (French Ministry of Health).

We thank the members of the Data and Safety Monitoring Board: from 2001 to 2005: Jean-Thomas Vilquin (cell therapist, Institut de Myologie), Gilles Defer (neurologist, CHU Caen), Guido Nikkhah (neurosurgeon, Germany), Murielle Vray (methodologist – Institut Pasteur), and from 2005 to the end 2013: Henri Kreis: nephrologist/immunologist (Necker, Paris), Gilles Defer (neurologist, CHU Caen), Marie Vidailhet (neurologist, IHU-A-ICM, Paris), Jean-Thomas Vilquin (cell therapist, Institut de Myologie), Carole Dufouil (methodologist/statistician, Bordeaux), Emmanuel Cuny (neurosurgeon, Hôpital Pellegrin-Bordeaux) Anne Fagot-Largeault (ethicist; Collège de France). We wish to thank our colleagues from Angers for their participation in data collection or the procedure: Audrey Olivier (Research assistant), Anne Clavreul and Nicole Piard (cell therapist); Brussels: Corinne Liesnard (virologist); Créteil: Marie-Françoise Boissé (neuropsychologist), Catherine Bourdet (Psychiatrist); Lille: Kathy Dujardin (neuropsychologist), Eric Decorte (research assistant); Bruno Quesnel (Biologist), Bruno Lukowiak (cell therapy engineer), Gustavo Touzet (neurosurgeon); Nantes: Pierre Renou (neuropsychologist), Philippe Naveilhan (cell therapist), Séverine Le Dily (Research Assistant), Prof. Philip David (gynecologist), Jean-Marie Vanelle (psychiatrist), Prof. Youenn Lajat (neurosurgeon); Toulouse: Prof. Yves Lazorthes (PI Neurosurgeon), Suzanne Jozan (cell therapist). We thank Michel Golmann and Henri Kreis for their advice on the alloimmunization part of the study and Jean-Luc Taupin and Cristina Sampaio for their advices concerning the manuscript. Julie Sappa from Alex Edelman and Associates was responsible for English editing.

The MIG-HD group

Anne-Catherine Bachoud-Lévi, the principal investigator of MIG-HD, supervised all aspects of the study and was responsible for neurological and neuropsychological training. Drs. Catherine Schramm, Christophe Lalanne and Renaud Massart curated the data. They ran the analyses with Prof. Etienne Audureau. The PIs at the various centers were Prof. Christophe Verny, Prof. Philippe Menei (Angers), Dr. Clemence Simonin, Prof. Pierre Krystkowiak, Prof. Serge Blond (Lille/Amiens), Dr. Frédéric Supiot, Prof. Marc Levivier (Brussels), Prof. Jean-François Démonet, Prof. Jean-Christophe Sol (neurosurgeon co-PI) (Toulouse), Prof. Philippe Damier (Nantes); Dr. Marc Peschanski and Dr. Philippe Hantraye supervised cell therapy, Prof. Stéphane Palfi supervised surgery (Créteil, neurosurgeon PI), Prof. Philippe Remy, Dr. Véronique Gaura and Sonia Lavisse supervised the PET-scans, Drs. Pierre Brugières and Laurent Cleret de Langavant supervised MRI and analyzed the data obtained, Prof. Bassam Haddad and Dr. Roland Jeny were responsible for obstetric supervision, Dr. Patrick Maison was responsible for methodology, and Prof. Jean-Pascal Lefaucheur supervised the electrophysiology studies. Amandine Rialland and David Schmitz participated in data curation and administrative supervision, Dr. Dominique Challine oversaw the viral work, and Prof. Anne Rosser was responsible for blind videoscoring.

The following participated in data collection and/or in the procedure:
Angers Hospital: Dr. Clarisse Scherer Gagou (neurologist), Ghislaine Aubin (neuropsychologist), Dr. Bénédicte Gohier (psychiatrist), Dr. Claudia Montero-Menei (cell therapist), Dr. Francoise Lunel-Fabiani (virologist), Prof. Philippe Descamps (gynecologist), Dr. Vivien Pautot (neurophysiologist), Dr. Jean-Yves Tanguy (brain imaging).
Brussels Hospital: Dr. Nicolas Massager (neurosurgeon), Hichem Slama (neuropsychologist), Liliane Tenenbaum (cell therapist), Serge Goldman (PET imaging), Philip David (brain imaging).
Créteil Hospital – co-ordinating center: Laurie Lemoine (neuropsychologist), Dr. Hélène Rouard (cell therapist).
Lille/Amiens Hospital: Dr. Arnaud Delval (neurologist), Ms. Marie Delliaux (neuropsychologist), Prof. Olivier Cottencin (psychiatrist), Ms. Julie Kerr-Conte (cell therapist), Dr. Laurence Bocket (virologist), Dr. Francis Collier (gynecologist), Dr. François Cassim (neurophysiologist), Dr. Christine Delmaire (brain imaging).
Nantes Hospital: Dr. Isabelle Neveu (cell therapist), Prof. Yan Péréon (neurophysiologist), Dr. Elisabeth Auffray-Calvier (brain imaging).
Toulouse Hospital: Dr. Fabienne Calvas (CIC physician), Dr. Olivier Parant (gynecologist), Dr. Marion Simonetta-Moreau (neurophysiologist), Dr. Jean-Albert Lotterie (brain imaging), Prof. Pierre Payoux (PET imaging), Prof. Jacques Izopet (virologist).

**Author’s roles**

Conceptualization: ACBL MP PH PMa
Methodology: ACBL PMa MP PH SP PR JPL DC PB RJ BH
Software: CSc CL LCL VG SL
Validation: CSc CL ACBL EA
Formal analysis: CSc CL RM EA
Investigation: ACBL CV PMe CSi PK SB FS ML JFD JCS PDam MP PH SP PR VG SL PB LCL BH RJ PMa JPL ARi DS DC ARo LL HR KY CSG GA BG CMM FLF PDe VP JYT NM HS LT SG PDav IN YP EAC AD MD OC JKC LB FCo FCas CD FCaL OP MSM JAL PP JI
Resources: ACBL
Data curation: CSc DS AR RM ACBL
Writing (original draft preparation): ACBL CSc CL SP PR LCL JPL PH RM EA
Writing (review and editing): ACBL CV PMe CSi PK SB FS ML JFD JCS PDam MP PH SP PR VG SL PB LCL BH RJ PMa JPL ARi DS DC ARo LL HR KY CSG GA BG CMM FLF PDe VP JYT NM HS LT SG PDav IN YP EAC AD MD OC JKC LB FCo FCas CD FCaL OP MSM JAL PP JI CSc CL RM EA
Visualization: CS ACBL
Supervision: ACBL
Project administration: ACBL DS ARi
Funding acquisition: ACBL MP
Financial disclosures (for the preceding 12 months)

- Bachoud-Lévi Anne-Catherine
  Consulting and Advisory Board Membership with honoraria: Roche
  Grants and Research: investment for the future ANR grant (Neuratris, Front EUR), national center of reference for Huntington’s disease (DGOS, ministry of Health), PHRCs (DRCI grants).
  Intellectual Property Rights: Cognitive assessments (SelfCog, CATEX, CALAP)
  Salary: University Hospital
  - Schramm Catherine
    - Grants: postdoctoral fellowship FRM
  - Remy Philippe
    No financial disclosure for the preceding 12 months.
  - Gaura Véronique
    No financial disclosure for the preceding 12 months.
  - Lavisse Sonia
    No financial disclosure for the preceding 12 months.
  - Massart Renaud
    Salary: Foundation AP-HP
  - Aubin Ghislaine
    No financial disclosure for the preceding 12 months.
  - Blond Serge
    No financial disclosure for the preceding 12 months.
  - Bocket Laurence
    No financial disclosure for the preceding 12 months.
  - Brugieres Pierre
    No financial disclosure for the preceding 12 months.
  - CalvasFabienne
    No financial disclosure for the preceding 12 months.
  - Calvier Elisabeth
    - Consultancies: Roche France
  - Cassim François
    - Honoraria: Biogen
  - Chaline Dominique
    No financial disclosure for the preceding 12 months.
  - Cleret Laurent
    - Grants: IRESP-INSERM (APP Prévention et Promotion de la Santé) LI-CLERET-AAP18-PREV-003
  - Collier Francis
    No financial disclosure for the preceding 12 months.
  - Cottencin Olivier
    - Honoraria : Speaker for Janssen, Indivior, and Bouchara
    - Grants : DGOS (1 PHRC) & ARS (1 AO Fonds Addiction)
  - Damier Philippe
    - Stock Ownership: CurePark
      - Honoraria for lectures: Teva, Novartis
  - David Philippe
    No financial disclosure for the preceding 12 months.
  - Delliaux Marie
    No financial disclosure for the preceding 12 months.
• Delmaire Christine
No financial disclosure for the preceding 12 months.
• Delval Arnaud
No financial disclosure for the preceding 12 months.
• Démonet Jean-François
- Advisory Boards: Vifor Pharma (Switzerland)
- Grants: EU Eurostars, Synapsis Stiftung (Switzerland), Fondation Leenaards (Switzerland), Fondation Empiris (Switzerland), Swiss National Foundation, Vifor Pharma (Switzerland)
• Descamps Philippe
No financial disclosure for the preceding 12 months.
• Lunel-Fabiani Françoise
- Partnerships: governmental institutions (INSERM, ANRS)
- Grants: ANRS
• Gohier Bénédicte
No financial disclosure for the preceding 12 months.
• Goldman Serge
- Employment: Université libre de Bruxelles
- Honoraria: NMEu
- Grants: Walloon Region, Fonds Erasme, AVN, FRS-FNRS
• Haddad Basam
- Consultancies: Roche diagnostics France
• Izopet Jacques
No financial disclosure for the preceding 12 months.
• Jeny Roland
No financial disclosure for the preceding 12 months.
• Kerre-Conde Julie
No financial disclosure for the preceding 12 months.
• Krystkowiak Pierre
No financial disclosure for the preceding 12 months.
• Lalanne Christophe
No financial disclosure for the preceding 12 months.
• Lefaucheur Jean-Pascal
- Salary: University Hospital
• Lemoine Laurie
Salary: AP-HP
• Levivier Marc
No financial disclosure for the preceding 12 months.
• Lotterie Jean-Albert
No financial disclosure for the preceding 12 months.
• Maison Patrick
No financial disclosure for the preceding 12 months.
• Massager Nicolas
No financial disclosure for the preceding 12 months.
• Menei Philippe
- Advisory Boards: Journal of neurosurgery
- Contracts: Expression santé
- Grants: Fondation de l’avenir
• Montero-Menei Claudia
- Grants: Région Pays de la Loire
• Neveu Isabelle  
No financial disclosure for the preceding 12 months.
• Parant Olivier  
No financial disclosure for the preceding 12 months.
• Pautot Vivien  
No financial disclosure for the preceding 12 months.
• Payoux Pierre  
No financial disclosure for the preceding 12 months.
• Pereon Yann  
- Advisory Boards: Avexis, PTC, Alnylam, Axelys  
- Honoraria: Novartis, Sanofi, Pfizer
• Rosser Anne  
- Consulting and Advisory Board Membership with honoraria: NMHD-UKRI MRC, UK KMP-UKRI MRC, Roche HD Advisory Board  
- Grants and Research: Medical Research Council (MRC), Healthcare Research Wales, CHDI Foundation, C.A.R.E., European Commission Horizon 2020  
- Honoraria: NMHD-UKRI MRC, UK KMP-UKRI MRC, Roche HD Advisory Board
• Rouard Hélène  
- Employment: paris Est University and Etablissement Français du Sang  
- Partnerships: ulm, Bergen University, UCM madrid, Universidad Autónoma de Madrid  
- Grants: H2020, ANR
• Schmitz David  
- Salary: AP-HP
• Simonetta-Moreau Marion  
- Honoraria: Allergan, Merz
• Simonin Clémence  
- Employment: CHU Lille  
- Honoraria: puntual expertises
• Slama Hichem  
- Employment: ULB ERASME HOSPITAL  
- Contracts: ULB ERASME HOSPITAL
• Sol Jean-Christophe  
No financial disclosure for the preceding 12 months.
• Supiot Frédéric  
No financial disclosure for the preceding 12 months.
• Tanguy Jean-Yves  
No financial disclosure for the preceding 12 months.
• Tenenbaum Lilane  
- Employment: Centre hospitalier universitaire vaudois.  
- Grants: Swiss national research foundation (SNF grant n°31003A_179527) ), Biosafety Advisory Council
• Verny Christophe  
- Grants: Fondation Maladies Rares GROUPAMA, AAP DGOS (ministère de la Santé)
• Scherer-Gagou Clarisse  
No financial disclosure for the preceding 12 months.
• Youssov Katia  
- Employment: AP-HP  
- Honoraria for consultancies: Roche
• Palfi Stéphane
- Consultancies: Yes
- Advisory Boards: Yes
- Contracts: Yes
- Honoraria: Yes
- Grants: Yes
  - **Audureau Etienne**
    No financial disclosure for the preceding 12 months.
  - **Peschanski Marc**
    No financial disclosure for the preceding 12 months.
  - **Hantraye Philippe**
    No financial disclosure for the preceding 12 months.
References

10. Bachoud-Lévi AC. From open to large-scale randomized cell transplantation trials in Huntington’s disease: Lessons from the multicentric intracerebral grafting in Huntington’s disease trial (MIG-HD) and previous pilot studies. Prog Brain Res. 2017;230:227–261.
2010;222:30–41.
Figure captions

**Figure 1: Participant flow chart**
At the end of the study, 41 patients had undergone bilateral transplantation and 4 had undergone unilateral transplantation.
DSMB: Data and Safety Monitoring Board; MRI: magnetic resonance imaging; HD: Huntington’s disease; TFC: Total Functional Capacity; MDRS: Mattis Dementia Rating Scale; UHDRS: Unified Huntington's Disease Rating Scale.

**Figure 2: Changes in UHDRS motor score in individual patients after the first transplant: results for the whole study population (A) and as a function of ectopia (B), and number of tracks per side (C)**
The black line shows the estimated progression of the MIG-HD cohort through the piecewise linear mixed model over the pre- and post-graft time periods.
UHDRS: Unified Huntington’s Disease Rating Scale.

**Figure 3. SPM analysis at M32 comparing the treated patients and the control not yet treated groups at FDG scans**
Regions in which changes in metabolism relative to the M12 baseline differed significantly between the treated group and control not yet treated group at M32 (P<0.001). These regions, overlaid on a T1-weighted brain MRI scan, correspond to the right angular gyrus and precuneus. Left: higher metabolism in the right angular cortex and precuneus in the treated patients. Right: lower metabolism in the left insula in the treated patients. No significant difference was observed in the striatum.

List of tables

**Table 1. Demographic and baseline characteristics of patients**

**Table 2. Comparisons between randomized groups in adjusted changes from M12 to M32 for the primary and secondary endpoints.**