# PROJECT FINAL REPORT


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## Executive Summary

The Repair-HD Consortium developed and optimized a GMP-compatible protocol to differentiate MSNs progenitors (Cell therapy product; CTP) from hESCs. Following careful evaluation of several research grade versions of GMP-hESC lines, RC9 was selected as the most suitable for further work. Using the RC9 cells, the Consortium's newly optimized protocol was then tested against existing MSN protocols and found to out-perform others protocols both *in vitro* and following transplantation into the rodent brain. Further optimized was then undertaken through an iterative process whereby systematic comparison of the survival, growth, phenotypic differentiation and preliminary functional screens of the lead protocol informed modifications, leading to a CTP protocol which was locked down and translation to a GMP environment. We have set-up *in vitro* quality-control assays, based on next generation sequencing, electrophysiology and calcium imaging technologies, to identify, quantify and characterize the appearance of the CTP. Using rabies viral tracing and slice electrophysiology we have shown that grafted CTP can connect to the host brain. We are testing the capacity of grafted CTP to influence behavioural recovery in a rodent model of HD (assessments ongoing), and have preliminary evidence of improvement on apomorphine rotation. A dedicated GMP bank of RC9, sufficient for the planned first-in-man trial, has been generated.

The locked-down protocol has also been tested on several iPSC lines and shown to be accurate (yield and purity of MSN cells), reliable (consistency of CTP production across a range of different hPSC lines), and reproducible (consistency of CTP production between replicate experiments run in same or different labs). We have also accessed a GMP-compliant human induced pluripotent cell line (iPSC), representing a back-up target.

In parallel a quinolinic acid model of HD was established in a macaque (m) model to allow grafting of miPSC CTP, in order to mimic as far as possible the clinical scenario (allotransplantation). This model has yielded information which will be key to the establishment of clinical protocols and systematic follow-up post-transplantation in HD patients: it allowed us to validate suitable imaging follow-up using PET and MRI; to develop a behavioural testing strategies using tactile screens and motor videos; and to generate assays to look for peripheral surrogate markers of immune rejection. We demonstrated that allografts can be immunogenic even in an "immune privileged" site as the brain, and demonstrated that matching MHC I and II is not sufficient to avoid immunogenicity of the grafted CTPs. Thus, peripheral immunosuppression is likely to be needed in the long term.

We have undertaken a full revision of CAPIT-HD; the existing battery for assessing patients with HD undergoing surgical interventions. We analysed published CAPIT-HD data and existing unpublished data (previously generated by the partners) to allow us to select the most useful tasks from CAPIT-HD. We combined these with novel objective assessment tools of motor, cognitive, behavioural and functional outcomes, all created and validated by the consortium, to create a preliminary revised battery (CAPIT-HD2), which we have beta tested in HD participants in 4 clinical centers. 12 month longitudinal assessment of all participants is nearing completion and we have performed preliminary analyses to guide the final integrative analysis and final task selection, which will occur after closure of the database in autumn 2018. We have also generated a fully specified GCP and EMA compliant eCRF for data collection. In readiness for a future clinical trial, we have written a fully documented trial protocol, which can be the template for a future clinical trial of hESC-derived grafts in HD, and we reviewed the post-operative rehabilitation literature to guide post operative management. In order to understand and to start addressing the key ethical and regulatory challenges associated with the design of a trial of hESC derived cells in HD, we held a workshop of experts in cell transplantation, surgical trials, and ethics, and have prepared a report addressing the ethical and regulatory challenges. With our international partners, we have established a global network of experts to enable further work towards this goal.

In conclusion the Repair-HD programme has yielded a major impact on the creation of new knowledge and the development of new techniques controlling differentiation and proliferation of human stem cells and reprogrammed cells for therapeutic purposes. It has also developed a framework for the safe and effective clinical delivery of cells into the brain in HD. Together, these advances will facilitate progression to 'first in man' trials of stem cell-based therapy in HD.

## Summary description of project context and objectives

There are compelling reasons for considering cell replacement therapy in a wide range of, currently untreatable, neurodegenerative conditions. For most of these conditions targeted pharmacological treatments are a long way off, as the detailed pathogenesis is not yet described, thus making targeted treatments difficult or impossible. However, even if pathogenesis is obscure, a condition can still be amenable to cell replacement therapy if the anatomy and distribution of neuronal cell loss is characterised. There is clinical 'proof of principle' that primary foetal precursors that are specified through normal development can repair circuitry in both Parkinson's (PD: specified to become dopaminergic neurons) and Huntington's disease (HD: specified to become medium spiny neurons: MSNs), and with preliminary evidence of sustained alleviation of some of the functional deficits. Nevertheless, although transplantation of primary foetal cells is important to establish the validity of cell replacement therapy in a particular condition using appropriately specified "gold standard" cells, the scarcity of foetal tissue of sufficient quality and the impossibility of standardisation of such grafts severely limit clinical application. Human pluripotent stem cells (hPSCs) present an attractive potential alternative source, as they can be expanded indefinitely in vitro, can be stored by cryopreservation, and can be differentiated into mature somatic cells. However, controlling their proliferation and differentiation sufficiently to provide cells suitable for clinical transplantation is key and has been challenging.

Repair-HD proposed to establish all the components necessary to take human pluripotent stem cell-derived neuronal cells through to the point of 'first-in-man' clinical trial in Huntington's disease (HD). The concept is to use HD as a 'model' disease to test the principle that an in vitro differentiated stem cell product can yield clinically useful structural and functional repair, and that this will pave the way for clinical translation of hPSC-based products in a broad spectrum of neurodegenerative conditions. Given the complexities inherent in both the hPSC-derived donor cells and neurodegenerative conditions it is important to use a single well-defined target condition to provide a pathway to clinical translation. HD provides an excellent test-bed for several reasons, not least that, donor cells must be placed homotopically into their normal position within the striatum, in contrast to PD where they are placed ectopically, thus allowing restoration of normal anatomical circuitry and restoring striatal synaptic plasticity at the host-graft interface.

The specific objectives of Repair-HD were:

- Select and standardise the optimum protocol for in vitro differentiation of hESCs to an authentic MSN phenotype whilst controlling the production of non-neuronal and non-striatal populations.
- Test and rank the lead protocols selected from the in vitro work for reliability of MSN differentiation and lack of tumour formation in rodent HD animal models according to behavioural profile of the animals, electrophysiological assessments, and anatomical parameters.
- Identify surrogate markers of the safety and therapeutic potential of hPS-MSNs for HD cell therapy
- Determine parameters for neurosurgical scale-up, to optimize in vivo imaging protocols, to extend sophisticated functional analysis, and to determine tumorigenic potential of the existing differentiation protocols in the primate HD lesioned brain using macaque iPS-MSNs (induced pluripotent stem-MSNs) allografted into macaque as the closest match to allografting in man.
- Adapt and scale up the most promising hES-MSNs for medicinal (GMP) grade production.
- Test the ability of GMP-compatible hiPSCs to differentiate to MSNs using the optimized Consortium ptrotocol, as an alternative donor cell source should the ethical and/or regulatory hurdles associated with hESCs prove prohibitive.
- Establish all elements for the first in man proof of concept clinical trial of hPS-MSNs. This will be designed to assess safety (in particular absence of overgrowth/tumour), and functional efficacy and will require the prior development and validation of a sensitive assessment battery, an effective trial design, and ethical and regulatory permissions ready to commence a first in man clinical study.

## Main S&T results

Repair-HD set out to put in place all the elements that would be necessary for a first-in-man study of pluripotent stem cell derived cells for neural transplantation in Huntington's disease. In broad terms, the work packages addressed issues around the generation of donor cells and tools required for a first in man clinic trial.

***Generation of donor cells for neural transplantation in HD***

A key requirement of a neural transplant therapy for HD is to generate cells capable of replacing the ones lost in the disease process. The cell type predominantly lost in HD, especially in the earliest stages, are the medium spiny neurons of the striatum. Thus, MSNs were the target cell type for the Repair-HD project; also referred to as Cell Therpay Product (CTP). One of the earliest tasks of the Consortium was to perform extensive comparisons of existing MSN differentiation protocols; some of which had been developed by the Consortium Partners and some were published protocols from other groups. Protocols were compared across the human embryonic stem cell (hESC) lines: RC9, RC17 and H9. Key outcomes measures were the proportion of medium spiny like neurons (MSNs, as indicated by MSN markers such as DARPP-32) differentiating in culture and following transplantation into animal models of HD, and the absence of continually proliferative cells in transplants or overgrowth of transplants. These comparisons revealed that a combination of the two Consortium protocols were superior to other published protocols, and also allowed selection of RC9 as the hESC that responded best to the Consortium combined differentiation protocol.

A considerable amount of work was then undertaken to optimize the new joint protocol. In particular, modifications were instituted to the protocol constituents, timings, and processes to further increase the yield of MSNs both *in vitro* and following transplantation into the brain, and to eliminate cells with the capacity to continually divide (tumourogenic potential). In addition, a cryopreservation step was tested at the MSN progenitor stage and was demonstrated not to detrimentally impair yields of MSNs following thawing and transplantation into the brain of HD rodents. Importantly, we demonstrated consistency of the protocol between labs, with yields of CTIP2-positive cells (CTIP2 is a marker of both differentiated and progenitor MSNs) at 22 days *in vitro* and DARPP32-positive neurons at 45 days *in vitro*. These findings confirmed the accuracy (yield and purity of MSN cells), the reliability (consistency of CTP production across a range of different hPSC lines), and reproducibility (consistency of CTP production between replicate experiments run in same or different labs).

The principle animal model used for rodent transplantation studies within the Consortium was the Quinolinic acid lesioned rat striatum. Injection of quinolinic acid into the striatum produces a selective loss of MSNs, sparing interneurons, and thus mimicking the histology of the striatum in HD more faithfully than has been seen to date in transgenic HD rodent models. It also has the advantage that it is a relatively stable lesion. Unilateral transplantation into the lesioned striatum of *adult* rats was routinely used to test the survival, differentiation potential, and safety (absence of tumourgenic cells) in CTP produced by the consortium. Prevention of rejection of the xenogeneic human donor cells was achieved by daily cyclosporine injections or by using genetically immunosuppressed rats as the hosts. With optimization of the protocol, we have been able to show reliable long-term survival, differentiation into striatal-like cells, and safety of the donor cells following transplantation into the rodent brain.

Using a monosynaptic retro-tracing system based on a modified rabies virus, we have obtained proof of principle that implanted hESC-CTP can make synaptic connections with both cortex and thalamus. We have, also worked to optimize and establish standard operating procedures/protocols for extracellular electrophysiological recordings of neuronal activity in brain slices containing striatal grafted NPCs/neurons, and have recorded small excitatory post-synaptic potentials when stimulating within the graft itself. Although these results are preliminary and requiring replication, they suggest that the hESC-CTP has the capacity to reconnect damaged neural circuitry.

However, ultimately, only behavioural studies can demonstrate whether the implanted cells survive and differentiate appropriately in sufficient numbers to influence brain function. A study is underway to comprehensively assess the ability hESC-CTP to influence a range of motor and cognitive behaviours in the rodent brain, and to compare this with the "gold standard" of human whole ganglionic eminence (WGE: the area of the fetal brain from which the striatum, containing MSNs, will develop). To date, we have seen a trend

towards improved motor bias on the apomorphine test for rats grafted hESC-CTP and human WGE although final analysis will have to await the availability of the full data set.

Although evidence of efficacy is awaited, the robustness of the *in vitro* analysis and *in vivo* evidence of differentiation potential and safety was considered sufficient to lock down the protocol and to proceed with translating the optimised protocol to a Good Manufacturing Practice (GMP) compatible protocol, suitable for eventual human application. GMP involves ensuring that the final product can be consistently produced and controlled according to quality standards, and seeks to minimize the risks to the patient.

One of the first steps for GMP translation was to generate a target profile for a hESC CTP by drafting of a living Cell Therapy Product Profile (CTTP) for future engagement of Regulatory Authorities. A working draft of the RepairHD CTTP has been initiated as a living document to guide future drafts, cross-reference current information, capture new information generated by the programme going forward, engage Regulatory Authorities and incorporate their feedback. Another important early step in the GMP process was to identify surrogate marker to permit quality Control (QC) of GMP-grade batches of CTP. Eight assays were developed: QRT-PCR based assay, Immunostaining based assay, Ampliseq based assay, Glutamate-evoked neuronal death assay, Glutamate-evoked Ca2+ dyshomeostasis assay, Electrophysiological recordings and analyses, Calcium-imaging assay, and Ampliseq hotspot cancer panel v2 assay.

Some further, relatively minor, amendments to the cell production protocol were necessary to generate a GMP-compliant protocol and the amended protocol was evaluated in 3 independent trials on the RC9 hESC line and demonstrated the capacity for consistency of in in vitro production. In addition, we establish a Repair-HD dedicated GMP bank of RC9 hESC to serve as source material for future preclinical research development and clinical evaluation.

Although most of the activities in Repair-HD have been focused on hESCs, we have also explored human induced pluripotent cells (iPSCs) as an alternative cell source to hESCs, as the destruction of blastocycts to create hESCs is viewed as ethically challenging in some European countries, whereas hiPSCs are generally created from adult somatic tissue. We have demonstrated that the Consortium protocol works equally well in several hiPSCs and have demonstrated its efficacy in monkey iPSCs, hence providing further support for this as the founding protocol for making GMP-grade CTPs. We have secured access to a GMP grade iPSC for future translational studies and have preliminary evidence of its responsiveness to the Consortium protocol, albeit that further amendments may be required for optimization.

***Tools for future preclinical assessment in rodents***

As a function of the work undertaken in Repair-HD, we have generated/improved methodology that will facilitate more efficient future preclinical assessment of donor cells. One example is imaging tools to allow imaging of grafts in living rodents and brain slices, which will be valuable for future behavioural and electropysiological studies by permitting selection of animals with viable adequately sized grafts for detailed analysis. Small animal MRI is available for this but is expensive and currently labour intensive, so we explored the use of bioluminescence for this purpose. hESC-CTP was treated with a virus containing firefly luciferase and was then transplanted into the striatum of rats. Luciferase can act on certain luciferin substrates to cause them to fluoresce. Nineteen weeks after being transplanted with luciferin-containing hESC CTP, we injected rats with one of two different substrates; Akalumine-HCl and D-luciferin, and demonstrated that the luciferin expressing cells were able to cleave the substrate resulting in a bioluminescent signal which was confirmed then confirmed in brain slices.

***Large animal studies***

Large animal models, such as primate and pigs have much larger and more complex brains than rodents and so provide an important transition to human studies by allowing questions to be addressed that cannot be addressed in small animal models. In Repair-HD we developed and used a monkey (macaque) model of HD to validate *in vivo* follow-up protocols and address issues of graft immune rejection. This involved detailed experimental plan involving baseline imaging and quinolinic acid lesions in France and then transferring the HD primates to Italy, where expertise in peripheral immunosuppression was available, to perform transplantation experiments. Animals were trained to perform complex behavioural tasks on tactile screens, which were conceived to assess fronto-striatal connectivity. Anatomical MRI was performed and PET imaging, using 18F-FDG to quantify glucose metabolism and 18F-Fallypride to asses D2 receptor density, was also undertaken. Donor cells were

iPSC derived from monkey peripheral blood mononuclear cells from several individuals. The cells were differentiated to CTP using the consortium differentiation protocol and tested for safety (i.e not over proliferative) in vivo in QA rodents (when possible i.e with frozen CTP, as for the human counterpart CTP), and for their commitment CTP to a striatal fate (i.e capable of differentiating into MSN). The production of three types of CTP batches was undertaken: CTPs suitable for (a) autologous grafting, (b) MHC I&II "matched" allogenic grafting and (c) MHC I&II "mismatched" allogenic grafting in three groups of monkeys genotyped for their MHC class. This allowed us to test 3 different MHC conditions (fully matched/autologous; matched, mis-matched) and assessed the impact on the host's the immune response as well as cell survival and differentiation. In agreement with some of the clinical literature, the results demonstrate that allografts can be immunogenic even in an "immune privileged" site as the brain. Interestingly, they also showed that matching MHC I and II is not sufficient to avoid immunogenicity of the grafted CTPs, and peripheral immunosuppression is most likely needed in the long term. This information is key to the establishment of clinical protocols and a systematic follow-up post-transplantation in HD patients. The analysis of peripheral blood and CSF surrogate markers to assess the possible activation of the immune system as a consequence of cell transplantation in the brain has yielded some insights into the underlying mechanisms and a full analysis is underway.

We also confirmed the ability of longitudinal MRI and PET imaging and behavioural tests to detect the survival, maturation and functional integration of the grafted cells at 3 and 6 months. In particular, we demonstrated that longitudinal MRI scans can be used to monitor grafts over time in terms of anatomical local, graft size and potential adverse events, such as cavitation of the grafts (probably due to immune rejection), which could be detected as a bright hypersignal within the grafted region and corresponded to a CSF cyst within the tissue in histological sections.

***Preparation for a future clinical trial***

In addition to the generation and assessment of donor cells, Repair-HD undertook the groundwork to prepare for a future clinical trial. The starting point was to develop and validate new patient assessment battery. A published assessment tool, the *Core Assessment Protocol for Intrastriatal Transplantation in HD* (CAPIT-HD) has been widely used in previous transplantation studies, but comprises mostly semi-quantitative assessments, with few objective assessments, and has not been updated for 25 years. Thus, we aimed to generate an updated version of the battery: CAPIT-HD2. We started by analyzing a collection of existing unpublished CAPIT-HD data (using the original battery). This data was collected in all but one of the sites within the CAPIT-HD Consortium and also additional UK sites outside the consortium who agreed to contribute their data. In addition, further data from Creteil coming from newly created patient databases has been merged with the existing data, in order to increase as much as possible the statistical power. The dataset includes 173 patients with a follow-up of at least 12 months, 159 patients with a follow-up of at least 24 months, and 146 patients with a follow-up of at least 36 months. The aim of the analysis was to identify motor, cognitive and psychiatric tests showing significant decline within the follow-up period. Using two different statistical analyses, we identified a number of test scores for which the slope of decline was significant. Secondly, we performed a complementary analysis of the literature, of longitudinal studies on cognitive outcomes in HD patients focused on cognitive measures as these are known to appear early in HD (often before motor signs) and appear to be particularly sensitive to change early on. A total of 33 studies were returned and produced complementary findings to the analysis of our own data, demonstrating that some cognitive measures are able to detect decline in HD over a short time period.

However, the analyses also confirmed that new more objective tasks are required in order to more reliably measure decline in HD. We therefore designed more objective assessment tasks, most of them digitalized, to replace existing tasks, which are heavily questionnaire-based and subject to rater bias. It was important for the tasks to be adapted for use by participants speaking English, French and German, which required a process of translation and validation. Although many of the tasks contained small components of language only (mostly instructions to be delivered to participants by the tester) some, in particular some of the cognitive task, directly test components of language.

Novel tasks were assessed in HD positive individuals in the Centres in which they were developed. Through a face-to-face workshop, we collectively assessed both the analysis of CAPIT-HD data as described above and analysis of the novel task data and selected tasks for inclusion in the provisional CAPIT-HD2, which contains core and optional components. We made this available in each clinical center (provision of task materials, software, translations and training on administering the tasks). We then beta tested the provisional battery

longitudinally (baseline, + 1 months to assess and wash out practice effects, and +12 months), starting in March 2016 with completion of all 12 month (M12) follow ups at all centres projected for October 2018. Statistical analysis plans have been written to provide guidelines for the final analysis of the provisional CAPIT-HD2 battery. They include a full description of the tests, and of their scoring systems, as well as statistics to used to answer primary and secondary objectives. A workshop will be convened to use this data to make the final selections for the definitive CAPIT-HD2 battery.

***Summary of tasks included in the provisional CAPIT-HD2 battery***

The new cognitive tasks were all found to be highly efficient in discriminating between controls and patients or between stages of the disease. While no firm conclusion can be drawn concerning their sensitivity to changes over time, the preliminary analyses performed using M12 assessment data collected in Creteil, provided highly promising results. Indeed, performance decline in most of the cognitive functions assessed could be detected over a period of 11 months with a population as low as 20 patients and 18 controls.

Moreover, a novel computed composite score was designed in Creteil from 6 individual cognitive measures (3 old and 3 new measures), showing a much better longitudinal Signal/Noise ratio than individual measures. These results are highly promising; a large Signal/Noise ratio indicating a greater reliable variance, which is a desirable characteristic for the general use of a clinical endpoint.

A preliminary analysis of baseline the two dual task assessments: step & stroop (lower limb) and C3T (upper limb) was performed on data pooled from the CAPIT-HD2 data from the four clinical centres. These were available for manifest HD (n=53) and gene-negative controls (n=47). The Mean function score from the UHDRS (out of a maximum of 25) was 23 in the manifest HD group and 25 in the gene negative control group. Machine learning was used to assess the discriminative capacity of the tasks to distinguish gene positive and gene negative participants and to discriminate across disease stages. The initial results suggest that both the C3t and Step & Stroop functional assessments are well suited for discriminating between gene-negative controls and manifest-HD groups, and maximal information about disease state appears to be encoded in only two baseline task variables. Ongoing analysis incudes inclusion of additional variables to explored whether they can lead to increase accuracy, and analysis of longitudinal data.

A preliminary cross-sectional analysis of the baseline psychiatric data replicated previous studies, suggesting that measures of apathy and perseveration tend to correlate strongly with other (functional, motor and cognitive) measures of disease progression, while disorders of mood (depression and anxiety) and irritability are much more variable over time, and from person to person. This may be a reflection of the complex aetiology of depression and anxiety, encompassing psychological and environmental variables, and even individual variation in genetic susceptibility to affective disorders, as well as the contribution of the neurodegenerative process in HD. Affective disorders and irritability are also responsive to symptomatic treatments such as antidepressants, which may also mask any relationship with disease progression. It is likely that measures of apathy and perseveration will prove more sensitive to disease progression than semi-quantitative scales which measure mood disorders. The next step will be to repeat this analysis on the entire longitudinal dataset when the data cleaning process has been completed, and to examine correlations between some of these measures and those from other clinical domains (motor, cognitive and functional).

It was possible to assess a subset of the digital Psychiatric data only, in part because automated methods to extract the relevant data efficiently are still under modification. The analysis of ta task of persistence demonstrated that it was able to discriminate between gene positive HD individuals and gene negative controls at baseline and month 1, but numbers at M2 were too small to give sufficient power to make a conclusive statement. It also predicted apathy as measured by the PBA apathy assessment. A task of spontaneous thought; he maze task, was also able to discriminate HD positive and negative cases at baseline, but in this cohort, did not predict apathy, and again, numbers were too low to make a conclusive statement about its value in longitudinal assessment at this stage.

A digital assessment of motor function; The Q-Motor assessment battery, has been designed to quantitatively measure motor deficits and progression for use in clinical studies (Reilmann and Schubert 2017). The assessments have shown to be able to detect subtle motor changes reliably and with absence of rater induced bias (Reilmann et al. 2015). The Q-Cog assessment battery adds an assessment with an increased cognitive share, with the goal of achieving a comparable level of sensitivity for cognitive deficits of participants. Highly significant differences between control and manifest groups could be found for various variables in all

performed tests. The longitudinal performance, robustness and possible redundancy of many variables is however yet to be explored.

We have explored the use of quantitative eye movement assessments using a digital eye tracker and have preliminary evidence of meas**u**rements that can distinguish between control and gene positive individuals and measurements which may be useful for longitudinal follow-up.

***Data base***

We generated a fully specified GCP and EMA compliant eCRF to allow centralization of data entry. Paper test data of 135 participants have been entered so far onto the eCRF. Final modifications of the eCRF to allow proper data entry of the digitized cognitive tasks and an easy export of the data for statistical analysis are almost complete.

***Post operative rehabilitation***

We generated a report, based on the existing literature, making recommendations for post-operative rehabilitation of patients who have undergone neural transplantation surgery.

***Ethics and trial design***

Discussion of the ethical and practical issues that will be important for a first in man clinical trial of stem cell therapy in Huntington's disease have been undertaken with an independent Advisory Board and through contact with regulatory agencies. We also held a workshop on Ethics and Trial Design in London in 201, during which several themes emerged, two of which have led to two substantial packages of work led by Repair-HD partners:

First, it became clear that there is substantial overlap between the issues critical to performing a first in man trial to transplant hESC-MSNs and transplanting human fetal cells into HD participants. Thus, alongside Repair-HD, we raised funding to undertake a study of the safety and feasibility of transplanting human fetal cells into HD recipients in Cardiff (TRIal designs for DElivery of Novel Therapies for Neurodegeneration; TRIDENT), funded through *Health And Care Wales.* We have used this opportunity to develop a fully documented protocol for TRIDENT, which will provide a template that can be readily adapted once hESCs are ready for the clinic. This protocol has now passed through multiple revisions and levels of review and has recently been approved by the UK Ethics board for use in the TRIDENT trial in Cardiff. Thus, this protocol will be tested in an ongoing clinical trial of neural transplantation in HD, which will further provide the opportunity for revisions, enabling us to be in the best possible position to progress rapidly to clinical trials once the hESC-MSNs are suitable for human use.

Secondly, we identified a number of trial design, ethical, and regulatory issues which are complex and controversial; for example the issue of whether sham surgery is ethically acceptable for control participants, the selection of a trial design suitable for transplant surgery in which trial designs incorporating multiple small cohorts may be more suitable for safety reasons, and the new surgical devices regulation which will impact on the cell delivery device. Many of these issues will be the same as those faced by any group planning to transplant cells from any source in HD. As there are several groups world-wide working towards transplantation therapies for HD, it was decided that the most efficient way to solve these issues would be to form a global alliance in order to provide a platform for discussion, the first meeting of which took place in May 2018.

## Potential impact and the main dissemination activities and exploitation of results

The Repair-HD consortium has made substantial progress towards a future first-in-man trial of human pluripotent stem cell (hPSC) derived grafts in Huntington's disease (HD), and in doing so has delivered increased understanding across a range of related areas.

***Progress towards first-in-man trial of hPSC neural transplantation***

Although we are not yet at the point of being able to confidently deliver a safe clinical trial of hPSCs in HD, through Repair-HD, we have made substantial direct progress towards this goal. In particular, we now have a robust differentiation protocol that results in approximately 40% of MSN-like neurons in vitro, which is substantially higher than seen in other published protocols. Following the modifications to the Consortium protocol, we have demonstrated reliable survival and lack of overgrowth in a rodent model of HD. We also have preliminary evidence of graft connectivity, both anatomically and electrophysiologically, and preliminary evidence of behavioural improvements. We have translated the differentiation protocol to a GMP-compatible version, ready for manufacture of donor cells for human application. Using a primate HD model, we have demonstrated that MRI and PET can be used to track the implanted cell grafts, and in particular, that structural MRI reliably identifies graft-related adverse events, such as graft necrosis and cyst formation. We demonstrated features of inflammation and/or immune rejection, not only in MHC mis-matched grafts, but also in matched grafts and, in some cases, even in autografts. Although this process does not necessarily lead to complete loss of the graft, it is almost certainly responsible for graft damage, which we would expect to lead to graft under-functioning. This is important because, up to this point, the impact of immune damage of grafts has been under-recognised. Indeed this work has had a direct impact on a (separately funded) trial of primary fetal cell transplants in HD (TRIDENT trial), which is currently in set-up in Cardiff University. Specifically, because of these results, the immunosuppression regimen has now been amended to extend it for as long as the patient can tolerate it, in contrast to previous trials in which immunosuppression was discontinued by a year post transplantation.

We have made excellent progress towards having the necessary elements of the clinical trial in place. We have designed and tested a range of new tests to more objectively assess the deficits in HD and have combined these with the most effective published tests to produce a new battery of assessments. We have tested this battery in patients with HD across the four clinical Centres (two in the UK, one in France and one in Germany), the results of which should be analysed by the end of 2018. This will allow us to make the final selection of assessment tools for the definitive CAPIT-HD2 battery, ready for use in a clinical trial. In addition, we have developed a "live" clinical trial protocol for the TRIDENT trial in which the only difference to a first-in-man hPSC trial is the source of donor cells. Using the protocol in a real transplant trial in HD means that will have the opportunity to assess it's suitability and make any necessary adjustments, ready for a first in man study of hPSC. Furthermore, our discussions of the ethical and trial design issues (both during regular Repair-HD meetings and through specific workshops as outlined below) have led to us establishing an international network (Stem Cells for HD: SC4HD) to ensure that such issues are discussed by a range of international experts in order to achieve the best outcomes for patients.

***Additional impacts of the Repair-HD study***

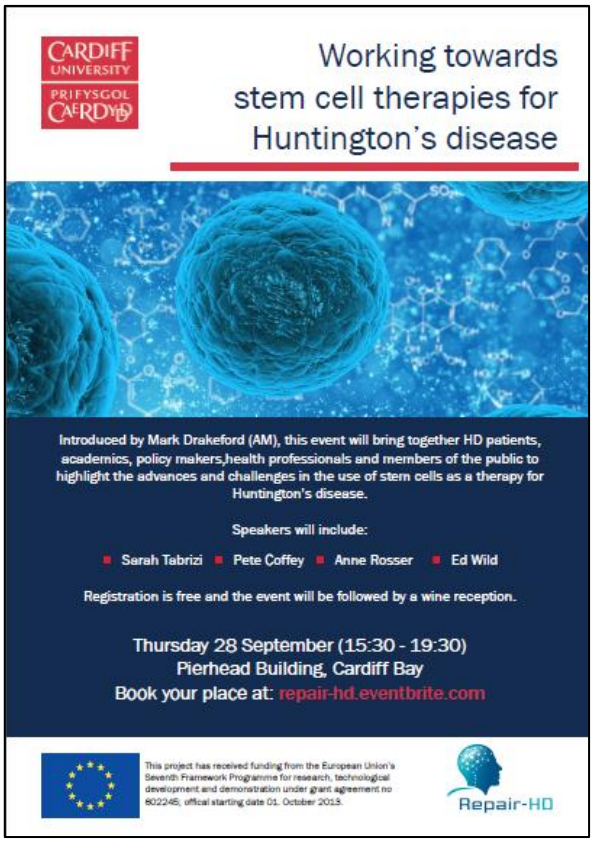
Repair-HD has led to a large range of additional impacts, not directly related to achieving a first-in-man hPSC-derived transplant trial. These include (but are not restricted to):

- An improved MSN differentiation protocol is important for producing neural cells for modelling both normal and disease carrying iPSCs in vitro.
- The work on the MSN differentiation protocol has led to an improved understanding of normal MSN development.
- Elements of the Quality Control and GMP development work can be applied to other donor cell systems.
- The development of a macaque model of HD along with the behavioural test battery will be available for testing other potential therapeutics.

- The findings related to the rejection of matched and mismatched allografts and autografts has the potential to reveal fundamental mechanisms underlying immune and inflammatory reactions in the brain.
- The finding that structural MRI can be used to track graft health is applicable to grafts studies in other neurological diseases.
- CAPIT-HD is likely to be a valuable assessment battery for any potential therapy in HD that requires sensitivity in relatively small numbers of patients over a period of at least a year. It may also be applicable to some other neurological conditions. With respect to this, the C3T is already being tested in other neurological conditions, such as PD and MS.
- The novel HD outcome measures will provide new insight into mechanisms underlying the symptoms and signs in HD. For example, the development of novel objective measures to explore apathy in HD has led to the finding that the neuropsychological constructs undrelying apathy in HD are not the same as in other neurological conditions explored to date (submitted for publication).
- The Database can be adaptable for other HD applications
- The novel trial design developed in Repair-HD, which will be tested in TRIDENT, will be applicable to other therapies in which delivery requires direct placement of the therapeutic agent into the brain (e.g. a variety of gene therapies).

***Repair-HD Dissemination activities***

During the lifetime of the Repair-HD project the consortium has organised several workshops to exchange with patients, representatives of patient organisations, clinicians and scientists in the field in particular on the ethical issues related to the trial design. These workshops were generally very well received we would like to exemplify by the heartfelt feedback we received from one of the participants: "*It was a privilege to be invited as a public/patient representative to attend the Repair-HD workshop and to meet so many eminent people in the field of HD research internationally. It was exciting and encouraging to see at first-hand their enthusiasm, dedication and commitment to finding a way forward for the proposed trial. I would particularly like to thank them for welcoming me as a member of the public to be part of the workshop and for listening to my comments and considering them. I am grateful that my experience of living with my husband with HD for over 30 years, was accepted to be of some value in certain areas that need to be considered from all aspects, when setting up a research trial, particularly some of the ethical, consent and participation issues. From a public point of view, it was very reassuring to see the depth and thoroughness of consideration given to the whole project and its safety, and the areas of expertise involved. I can now understand far better the difficulties and constraints, which necessitate a lengthy timescale in setting up such a trial, although the public will of course always wish things could happen quickly! However, I came home very excited and optimistic for the future and on behalf of HD families everywhere, may I thank everyone at the workshop (and their colleagues) from the bottom of my heart for all they are doing and wish them every success in their endeavours to make this major breakthrough for us.*"




A highlight of the dissemination programme was the final meeting of the network which was organised as a public symposium on September 28th 2017 in Cardiff UK, bringing together HD patients, academics, policy makers, health professionals and members of the public to highlight the advances and challenges in the use of stem cells as a therapy for Hungington's disease. The meeting aimed to promote public awareness of the Repair-HD programme, HD research in the UK and Europe and also community support and care available to families affected by HD.

## Partners involved and coordinator's contact details

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| 2B | | Anne-Catherine Bachoud-Lévi | | |
| 3 | Commissariat à l'énergie atomique | Philippe Hantraye | CEA | France |
| 4 | Consorzio per la ricerca sul trapianto di organi tessuti cellule e medicina rigenerativa consorzio | Emanuele Cozzi | CORIT | Italy |
| 5 | Roslin Cells Ltd | Paul de Sousa | RC | UK |
| 6 | The University of Manchester | David Craufurd | UNIMAN | UK |
| 8 | Inserm Transfert SA | Christiane Dascher-Nadel | IT | France |
| 9 | George-Huntington-Institut GmbH | Ralf Reilmann | GHI | Germany |
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## Project logo and public website

Repair-HD

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