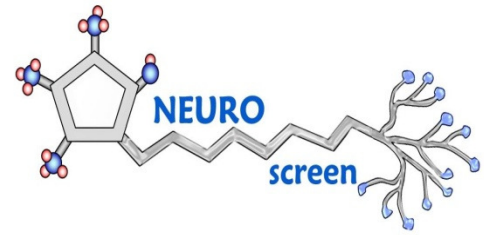

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NEUROscreen

The Discovery of Future Neuro-therapeutic Molecules

Specific Targeted Project

LIFESCIHEALTH

Publishable Final Activity Report

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Project coordinator name: Dr Paul Bello
Project coordinator organisation name: Stem Cell Sciences UK Ltd

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1. PROJECT EXECUTION

Project Objectives

NEUROscreen built on a significant European discovery made within the EU-FP6 project EuroStemCell. In 2004 a new type of mammalian neural stem (NS) cell was discovered and NEUROscreen is focussed on the successful utilisation of this stem cell type in chemical genetic screening.

Described more explicitly, the NEUROscreen project aimed to maximise the utility of new rodent and human (NS) cell lines in the design of unique assays for the tractable automated discovery of small molecules that modulate the biological function of neural stem cells and derivative neurons and glia. Such a chemical genetics approach applied to scalable cell lines was expected to yield molecular discoveries with inherent attributes of value for their prospective development as future regenerative medicines to treat neurological and neurodegenerative diseases.

NEUROscreen aimed to endeavour to reset the limits of neural stem cell potency in generating neural fates *in vitro* using small molecule modulation. The project used novel neural stem cells as a tool, not out of scientific curiosity, to design new technology for the drug discovery industry.

A principle objective of the project was to blend fledgling and well established European commercial partners in a pre-competitive, collective drive to translate fundamental stem cell biology onto a state of the art discovery platform and successfully demonstrate the potential exploitability of the concept while simultaneously strengthening European bio-industry. As human cells were used as *in vitro* discovery tools with prospects for commercialisation, the project had an important ethical dimension and the activities were guided and advised by a dedicated European ethicist.

Translational objectives of NEUROscreen were:

- Establishment and development of an ethically resourced, quality controlled, dedicated repository of rodent and human stem cell lines
- Development of bioassays – stem cell in origin – apt in providing highly germane neurons and glia expressing therapeutic targets associated with major unmet medical needs such as Alzheimer's and Huntington's diseases and stroke
- Identification of tractable, chemical modulation of the regulatory pathways governing neural cell specification from stem cells
- Exploration of the intrinsic, genetic basis demarcating the limits of sustained potency of neural stem cell lines and the potential resetting of these limits via chemical modulation to generate greater neuronal diversity *in vitro*
- Defining a set of prospective medicines for ageing and neurodegenerative disease treatment based on the optimisation of molecules with functional tissue restorative or regenerative capacity

- Development of reagents and processes adequately to harness the biology of neural stem cells when said are grown and manipulated on multi-array, automated sample handling platforms
- Design and testing with representatives of the European pharmaceutical industry, a model to achieve the successful dissemination and commercialisation of the technology platform.

Contractors Involved

The nine contractors in the NEUROscreen consortium came from three EU countries and comprised five SMEs, three leading Universities and a major research institute. They were:

1. Stem Cell Sciences (UK) Ltd
2. Dialectica srl -- left the consortium during the second year owing to insolvency
3. BioRep SrL
4. Life & Brain GmbH
5. The Chancellor, Master and Scholars of the University of Cambridge
6. Università degli Studi di Milano
7. Rheinische Friedrich Wilhelms Universität Bonn
8. Medical Research Council – National Institute for Medical Research
9. Summit plc

Project Website

www.stemcellsciences.com/Neuroscreen/introduction.html (currently for partners only)

State of the Art

Stem cells are capable of generating identical progeny through unlimited cell divisions (self renewal) whilst retaining the ability to respond in a homeostatic manner to stress via the production of progeny committed to a particular tissue function (differentiation). It has proven a problem to grow homogenous cultures of stem cells *ex vivo* (other than ES cells). For example neural stem cells isolated from the mammalian brain can be expanded *in vitro* (*Reynolds & Weiss. 1992. Science 255:1707-1710*) as neurospheres but differentiation is a concomitant feature of routine propagation under conditions thought to favour self-renewal. The artificial, mixed cellular environment of the sphere is likely to provide a microenvironment that permits the renewal of only a sparse number of stem cells. Although the neurosphere culture has been developed into an assay of utility for research and screening purposes, in that the aggregates derived from developing or adult rodent and primate CNS can yield mature neural cells (*Sanai et al. 2004. Nature 427:740-744*), the significant limitations of the system make its utility problematic in major applications such as stem cell based discovery. For example, a recent chemical genetic screen using neurospheres identified 'hits' active against the neural precursor pool, rather than the neural stem cells, precursor cells constituting the bulk of the mass in spheres (*Diamandis et al 2007 Nat. Chem. Biol. 3:268-273*). Culture heterogeneity is undesirable from a number of fundamental perspectives; principle ones being that the

basic regulatory biology of neural cell specification is difficult to comprehend and the direct consequences of manipulation of the cells to generate more neurons *in vitro* in technical applications is almost impossible to decipher, as their exists considerable variation between samples (wells) within a single culture.

Adherent, neural stem/progenitor cells can be propagated via support from growth factors, but without genetic transformation neuronal differentiation potential is usually extinguished in these conditions**.** Recently, significant progress has yielded the discovery of a neural stem cell type (NS cell) that undergoes sustained symmetrical self-renewal with complete suppression of differentiation in adherent culture in response to the growth factors, FGF-2 and EGF (*Conti et. al 2005. PLoS 3:1-13*). The human NS cells (not artificially transformed) are stably neurogenic after extensive passage and display a uniformity of marker expression such that cultures are homogeneous. The NS cells can be derived from ES cell lines, foetal or adult CNS material and offer for the first time an unlimited resource - propagating robustly in the laboratory - from which can be generated a continuous supply of neural cells for disease study. The cells not only provide a directly accessible system for fundamental research into how neural cells are specified, but also are a highly attractive technical resource for applications in stem cell biotechnology. The patent claims for the technology are granted in the UK and the extensive claim set has obtained a favourable, international patent examiner review. This indicates that the technology has a strong prospect of commercial success.

Chemical genetics is the study of biological systems using small molecule ('chemical') intervention. A combination of chemicals and cell-based (phenotype) pathway screens have enabled significant inroads into the analysis of complex cellular processes. Small molecules have been identified in such a manner that promote the neuronal differentiation of mouse ES cells, but the mechanism of action of such molecules remains to be elucidated (*Ding et al. 2003. Proc. Natl. Acad. Sci. 100:7632-7637).* A screen has also been performed using high content imaging and a library of chemicals on primary neural stem cells isolated from adult rat hippocampus and small molecules have been discovered that direct neuronal differentiation with reasonable efficiency (*Ding & Schulz 2004. Nat. Biotech. 22:833-840*). This approach did not furnish clear details of those genes and mechanisms following small molecule exposure, required for the induction of neuronal fate in either acute or long term established, stably neurogenic cell lines. It also does not provide temporal resolution in defining how and on what genes the molecules were acting to exert their effect. Clearly a more systematic approach is required to identify from additional chemical diversity, molecules that can act to affect neural cell specification, while more accurately defining the mode of action via changing patterns of gene expression.

As it is now technically possible to derive and propagate stably neurogenic populations of neural stem cells, NEUROscreen has derived and characterized human lines from a range of samples, including embryonic and induced pluripotent stem cells (*Koch et al 2009. Proc Natl Acad Sci USA 106:3225-3230; Danovi et al 2010, Biochem Soc Trans 38:1067-1071*) and neoplastic lesions (*Pollard et al 2009. Cell Stem Cell 4:568-580*) with a view to building a unique abundant resource for understanding more clearly how stem

cells generate neural cell fates. Clonal, adherently growing neural stem cell lines derived from neoplastic lesions are of great relevance for anti-cancer drug discovery and more amenable to high-throughput screening than the currently used aggregate sphere cultures of brain cancer stem cells. A recent screen on breast/epithelial cancer stem cells identified a number of compounds with selective toxicity (*Gupta et al 2009. Cell 138:645-659*). Such success was replicated with chemical screens for, and identification of, compounds affecting proliferation and cell death of brain tumour stem cells and the live image-based screening method enabled the discrimination between cytotoxic and cytostatic effects (*Danovi et al 2010, Biochem Soc Trans 38:1067-1071*). Europe has been in the vanguard of post-genomic technology, medicinal chemistry development and the consortium contains a European based commercial partner with high expertise in chemical genetic screening. In the first year of the consortium, a landmark publication by Ying and colleagues led to a paradigm shift of thinking in the field of chemical screening (*Ying et al 2008. Nature 453:519-523).* Whereas previously such screens focused on influencing cell proliferation and survival or directed differentiation towards a specific lineage, this paper demonstrated that it was possible -- in this case for mouse embryonic stem cells -- by knowledge of the pathways involved, to use small molecules to dispense of protein growth factors with promiscuous effects (and thought to be essential for cell expansion for decades) thereby leading to a dramatic improvement of the quality, pureness and stem cell state of the cultures. It is, therefore, ian attractive proposition to pursue specific small molecule compounds that could replace the requirement for mitogenic protein growth factors for the expansion of neural stem cells in the hope to circumvent the utility limiting long-term effect of restricted differentiation potential exerted by these factors. This project has allowed a first glimpse of pathways and structures which are promising and warrant further examination in the pursuit of this goal.

Neurodegenerative diseases, regenerative medicine and chemical genetic screens are currently in the international focus, with intense research activities ongoing principally in the USA, Asia and Canada, but a unique forward advantage for Europe is provided by the novel neural stem cell resources which came together in this project and where considerably expanded during its duration and rapid translation of the advances made is an absolute necessity. Maintaining a clear focus on tools and technology development, while also researching basic comparative biology of different stem cell populations, has aided NEUROscreen in achieving meaningful progress towards this aim.

Work Performed

The work performed was divided into six work packages, each with one Strategic Deliverable objective. Each Strategic Deliverable was divided into individual Deliverables, which, in turn were divided into Milestones. As work was complete on each Milestone or Deliverable, the lead PI responsible submitted a formal report on the work performed, and on exploitable or publishable results. The following summarises the work performed in each of the Project's Strategic Deliverables.

SD1: A repository of annotated QA/QC lines & product release criteria
Deliverable 1.1.2 - Partner 3

Report on and deposition of fully characterised and QA/QC NS cell line derived from different mouse CNS regions, human and mouse fetal and adult neoplastic brain lesions

Neural stem cells were successfully derived from both mouse and human tissue and propagated in serum free culture medium containing animal-derived supplements on an adherent substrate without spontaneous differentiation or cell death. Such conditions are not entirely devoid of animal protein-containing components and it is unclear as to what effect there might be on the consistent performance of human cell lines that have been chronically exposed to such supplements. For this purpose a further refinement to humanize culture methodology for human stem cells was developed. The resulting cell lines appeared similar in all characteristics tested, such as morphology, growth rate and potency, demonstrating that the humanised culture medium version had no apparent detrimental effect on hNS cell derivation and initial growth. Furthermore, four adherent cell lines from malignant gliomas that display stem cell properties have been successfully derived. Human brain tumours appear to have a hierarchical cellular organisation suggestive of a stem cell foundation. Thus, glioma neural stem (GNS) cell lines from different tumours exhibit divergent gene expression signatures and differentiation behaviour that correlate with specific neural progenitor subtypes. Cell lines have also been derived form a series of tumours, including those classified as giant cell glioblastoma, anaplastic oligodendroglioma, glioblastoma multiforme. Cell lines could not be established from lower grade tumours, such as ependymomas and lower grade astrocytomas, suggesting some specificity in the culture conditions for the propagation of brain cancer stem cells from higher grade tumours. To evaluate the relationship between each GNS cell line and their correspondence to foetal NS cells a global mRNA expression profiling was carried out using microarrays. Genes located on chromosome 7 were significantly overrepresented within this set. The top 100 differentially expressed genes (excluding those on chromosome 7 or 19q) provide a set of candidate markers that distinguish foetal NS cells from GNS cells. The most significantly down-regulated gene in GNS cells relative to foetal NS cells is the well studied tumour suppressor PTEN. All the cell lines have been controlled as to contaminations from bacteria, fungi and mycoplasma; additional screen for viral contamination has been performed on human neural cell lines. Every test is highly standardized to reach the most stringent level of accuracy and detection. In addition, the neural stem and progenitor cells lines available within the consortium were evaluated for apoptosis, pluripotency, differentiation and chromosomal stability as a function of the cryopreservation technique adopted. Specification sheets were provided summarizing the main characteristics of the cell lines generated.

Deliverable 1.2 - Partner 3

Release of cryopreservation & recovery procedure for screening purposes

Current methods of freezing, storage and recovery may trigger apoptosis and spontaneous differentiation of stem cells with consequent loss of pluripotency. Furthermore, freezing/thawing techniques may lead to alteration in DNA replication or, possibly, chromatin structure. The combined use of DMSO and cooling seems to reduce much of these alterations, but the high concentration of cryoprotectant needed is known to cause unexpected changes in cell fate and adverse effects and toxicity to patients. In addition

the use of components or additives of animal origin in the freezing medium exclude the application of these newly generated cell lines into therapy. Several studies showed that the freezing procedure displays its detrimental effects mainly at 24 hours after thawing, due to failure to recover from the freezing damages. Identifying the optimal cooling rate, that permits some cell shrinkage (dehydratation) without the formation of significant amounts of intracellular ice, is essential for reducing cellular stress that irreversibly triggers apoptosis and cell death. Within this project, new strategies have been developed to improve recovery, reduce apoptosis and eventually DNA damage. Using fully defined freezing medium protein-free (GMP manufacturing and FDA compliant) containing 10% DMSO (CryoStor™) and a highly controlled cooling rate (programmable rate freezer), it was possible to achieved an optimal cell viability and a low apoptotic level at 24 hours after thawing from neural stem cell lines made available by the consortium. However, important considerations should be taken in using non-toxic levels of DMSO on stem cells during preservation processes since several studies reported a diminished pluripotency capacity and an induction of cell quiescence in a dose dependent and reversible manner. For this reason, the freezing medium has been modified to decrease DMSO concentration and substitute it with trehalose, an alternative non-toxic cryoprotectant, able to reduce ice crystals formation and dimension, preserve plasma membrane integrity and stabilize intracellular proteins. Contrary to DMSO, this sugar has no effect on DNA methylation and acetylation. Cells cryopreserved in trehalose exhibited a differentiation potential comparable to the standard DMSO technique proving that this non toxic cryoprotectant can be safely used to store neural stem cells without affecting their pluripotency status

Deliverable 1.3 - Partner 3

Detailed annotation of genomic variations occurring in NS cells

The use of in vitro cultured stem cells in cell therapy, must meet at least two requirements: the preservation of a normal genetic karyotype and maintenance of integrity during long term-culturing and storage in liquid nitrogen tanks. Despite the wide use of both mESCs and adult derived stem cells as model system for mammalian development and cell differentiation, there is lack of systematic studies aiming in characterizing karyotype changes during *in vitro* culturing and storing conditions. Systematic cytogenetic analysis of mouse adult, fetal and ES-derived NSC lines at different culturing passages showed: (i) various chromosomal abnormalities including polyploidy, mono and trisomy, translocations and duplications in all the cell lines considered; and (ii) the engineering procedures applied to the established NS lines exacerbate the accumulation of the chromosomal defects independently of the source of origin of the NS cells.

To investigate the onset and progression of karyotypic abnormalities observed in long-term expanded cells, new derivation of NS has been performed from mouse ESCs. Karyotype analysis revealed a broad genetic stability and integrity of the starting mESC culture; during NS conversion, a consistent fraction (about 50%) of Neural Progenitors (NP) in culture exhibited the presence of extra-chromosomal chromatin fibres. These fibres appear early in the NP generation phase and gradually disappear after complete NS conversion and subsequent *in vitro* culturing. We hypothesize that these fibres might be the result of a change of chromatinic status occurring during the transition from

pluripotent ESCs to somatic stem cell population or of a gradual adaptation of the cells to the serum-free conditions they were exposed to during the neuralization process. With *in vitro* passaging, NS cells progressively showed signs of chromosomal instability, gaining clonal numeric aberrations of chromosomes 1 and/or 19 in about 50% of the metaphase analysed. On the other hand, data on cryopreserved NS cells indicated that when clonal aneuploidy occurs, the same chromosomes are maintained in the cells independently of the cryopreservation steps.
On the contrary, all human foetal NS cell lines showed a normal karyotype after expansion and after cryopreservation. To assure genomic stability we performed a comparative genomic hybridization (CGH) analysis on the recovered culture after cryopreservation in Cryostor™ with a programmable rate freezer. Most of the copy number variations (CNVs) observed were probably constitutively expressed in the genome of the original patient, while those on chromosome 6, 10, 12, 14, 17 and X could be acquired during the *in vitro* manipulation or after the cryopreservation technique. However, important modifications in the behaviour of NS cells (human and mouse) we never observed, even after prolonged growth in culture and the chromosomal abnormalities observed in the cell lines tested did not lead to any alteration in their differentiation capabilities.

Deliverable 1.4 - Partner 3
Set of standards and procedures for safe: collection, transportation, and banking and the establishment of NS cell
To assure the proper handling of the received material, neural stem cells cultures need to meet high quality standards. These include the assessment of bacterial, fungal and mycoplasma contamination-free cultures. For all human neural cells a certified lab performs an additional screen for viral contamination (HIV, HCV, HBV, EBV, HCMV, HSV1-2 and HPV). Viral contamination needs particular attention because infection may be without cytopathic effect for the cell culture or may be latent (e.g. herpes virus) and hard to detect. Human and non-human primate cultures are more likely to harbour viruses that are highly pathogenic to humans. Of particular concern are the blood-borne viruses such as Hepatitis B Virus (HBV), Human Immunodeficiency Virus (HIV) and Hepatitis C virus (HCV), but they are easily detected even at low viral charges by NAT testing.
These highly controlled NS cell lines are then cryopreserved (according to a standardized DMSO based protocol) and banked in liquid nitrogen tank equipped with independent temperature sensors that are connected to an alarm Central Monitoring System, which generates a complete temperature status reports. Cells are then assessed for viability and maintenance of pluripotency, differentiation capability and stable chromosomal asset.
All the cell culture information on passages, number of viable cells, sub cultivation method, freezing medium and cryoprotectant are recorded in **Partner 3**'s database that allows tracing each individual sample. Testing results are then used to build a cell line datasheet and consultable through **Partner 3**'s online catalogue, accessible by protected registration.
Upon request, cells are distributed following the International Air Transport Association (IATA) recommendations for the transport of biological substances, category B. All shipments must comply with all applicable local, state and federal laws and all packages are appropriately marked with the proper labels (diamond shaped UN3373 mark and the

text “BIOLOGICAL SUBSTANCE CATEGORY B”). **Partner 3** checks all local import regulations with the recipient and includes a “Material Transfer Agreement” (MTA) that needs to be signed by the recipient scientist before distribution. In this document, all the terms and conditions for the use of the biomaterial are explained to assure intellectual rights to the scientists that generated the cell lines. Furthermore a custom declaration form and a material datasheet are included in the shipment to assure proper clearance and handling of the material upon delivery.

SD2: A set of tools for assay development
Deliverable 2.1 - Partner 8
A region specific signature of differentiation gene expression for mouse & human NS cells
Mouse NS cells originating from different brain regions have been analyzed for their expression of a panel of markers, mostly transcription factors, that are known to mark the positional identity of progenitor cells in the embryonic brain. The profiling of these cells has shown that they retained some, but not all, of the positional markers of their regions of origin.
Human NS cells originating from foetal brains and NES cells originating from ES and iPS cells for profiled for expression of a large (190) panel of progenitor and cell type-specific markers, transcription factors, cell cycle regulators, *etc*. This analysis showed that NS and NES cells have markedly divergent molecular profiles and that NES cells adopt a caudal positional character.

Deliverable 2.2 - Partner 8
Novel bioassays for neuronal differentiation of NS cells
Several fluorescent reporter constructs specific for different stages of differentiation of neural stem cells into neurons have been generated and stably transfected NS cell lines for some of these constructs have been generated. These lines displayed faithful recapitulation of expression of the reporter gene and can now be used for real-time read and quantification of neuronal differentiation for drug screening purposes.

Deliverable 2.3 - Partner 8
Novel bioassays for the subtype specification of NS cells
Several fluorescent reporter NS cell lines have been generated that can be used to easily visualize and quantify the differentiation of NS cells into different types of neurons, namely GABAergic telencepphalic neurons, glutamatergic neurons and striatal projection neurons.

SD3: Validation of cellular systems & protocols for optimal screening procedures
Deliverable 3.1 - Partner 5
Report of the potency of human neural stem cells from neoplastic versus normal tissue samples
Activity performed in the context of this Deliverable has been focussed on two main themes. First, to characterise the competence of human NS cells for differentiation into mature neurons and oligodendrocytes. Human neural stem (NS) cells can be derived from

foetal forebrain and spinal cord tissue donated for research following elective termination of pregnancy. **Partners 5** and **6** have investigated the ability of expanded NS cell lines to differentiate into neurons. It was found that after long-term expansion and clonal expansion, human NS cells can reproducibly generate neuronal cells characterized by morphology and expression of various neuronal antigens, although no specific neurochemical identity was acquired. These neurons mature over 6-8 weeks in culture to acquire, although with an exceptionally low efficiency, voltage gated Na channels and generate spontaneous action potentials. In parallel, **Partners 1** and **7** have evaluated the potency of human foetal NS cells to generate oligodendrocytes. In particular, it has been tested whether an established protocol for oligodendroglial differentiation of mouse ESC-derived NS cells (previously developed by **Partner 7**) could be translated to other mouse ESC-derived NS cell lines, mouse primary cell-derived NS cells and human fetal NS cells. The obtained results indicate that whereas oligodendroglial differentiation could be translated to other mouse ESC-derived NS cell lines, primary mouse and human fetal NS cells largely failed to differentiate into oligodendrocytes. Overall, these data suggest that not only the species (mouse versus human) but also the cell origin (ESC versus primary cells) of NS cells can influence their multipotency and/or the amenability to established oligodendroglial differentiation paradigms. A patent application is currently in preparation by **Partner 7** on the optimization of oligodendroglial differentiation protocol.
The second main aspect of this Deliverable was to apply the NS cell derivation and culture system to establish glioblastoma stem cell lines and to establish their in vitro "differentiative" potential. Human brain tumours comprise a cellular hierarchy consistent with a stem cell foundation. Derivation of stem cell lines with patient tumour-specific character would present individualised analytical, diagnostic and therapeutic opportunities. In collaboration with a laboratory in Toronto (Dr Peter Dirks), **Partner 5** has demonstrated routine derivation of NS cell lines from malignant glioma. Four lines have been characterised in detail: G144, G166, G179 and GliNS2. Glioma neural stem (GNS) cells recapitulated the human disease in xenografts. However, individual GNS cell lines exhibit divergent gene expression signatures and differentiation behaviour. The diversity of gliomas may therefore originate from distinct cancer stem cell phenotypes. G144 and GliNS2 display features of oligodendrocyte progenitors or type C cells, while G179 has more similarity to adult SVZ astrocytes (type B cells). G166 seems more restricted in differentiation capacity, but can generate GFAP+ astrocytes. **Partner 5** has filed a patent application on the generation and use of these cells.

Deliverable 3.2 - Partner 6

Report on the extrinsic regulation of neuronal subtypes differentiation from mouse neural stem cell lines

The generation of cell diversity in the nervous system involves multiple developmental mechanisms, including anterior-posterior and dorsal-ventral patterning, temporal specification, and the generation of secondary or intermediate progenitor populations. Recent studies have begun to reveal the mechanisms that generate regional specification of progenitors and diversity of the neuronal differentiated progenies. In the telencephalon, the two main subdivisions (the dorsal telencephalon and the ventral telencephalon) generate very different types of neurons. The germinal zone of the dorsal telencephalon gives rise to the excitatory glutamatergic projection neurons of the cerebral cortex, which

sequentially reach the different layers of the cortex by radial migration. Progenitors of the ventral telencephalon generate GABA-ergic inhibitory neurons, including basal ganglia neurons, as well as interneurons that migrate tangentially to contribute to the formation of the cortex. In this framework, efforts have been devoted to investigate the possibility to generate regional specific neuronal populations during neuronal differentiation of NS cells of murine and human origins. Investigating this issue is crucial when considering the use of neural stem cells for drug screening approaches aimed at identifying molecules that could direct or favour the differentiation towards specific neuronal subtypes. The neuronal populations considered have included cortical and striatal neurons, with more specific interest on glutamatergic pyramidal neurons and striatal GABAergic neurons. Several human and mouse NS cell lines have been tested. During the course of the project, **Partner 6** developed and published different protocols for mouse NS cells neuronal differentiation based on modulation by extrinsic stimuli such as exposure to different growth factors, such as FGFs and neurotrophins. Overall, these cells exhibit a pronounced ability efficiently to generate mature neuronal cells although with a marked restriction to the GABAergic subtype. **Partner 6** has also investigated the potential role of small molecules on the biochemical maturation of GABAergic mouse NS-derived neurons. Results obtained indicated a consistent effect exerted by some of these small molecules in increasing the expression of neuronal markers and the neurite elongation capacity. **Partners 6** and **1** have investigated the possibility to generate neuronal subtypes from NS cells of human origin. Overall, these cells displayed a poor responsiveness to acquire any specific neuronal subtype identity. The results obtained indicate that these cells need a long period (more than 4-6 months) in order to exhibit a specific neuronal subtype identity restricted to the GABAergic lineage -- although the maturation is very poor in terms of efficiency. Nonetheless, foetal hNS cell lines after six months of *in vitro* maturation clearly acquired neuropharmacological hallmarks of neuronal cells. In parallel, **Partner 8** investigated the impact of genetic manipulation by forced expression of specific transcription factors, *i.e.*, Mash 1 and Ngn 2, as a means to induce specific telencephalic dorsal (glutamatergic) or more medial-ventral (GABAergic) neuronal populations. The results indicated that Mash1 is able to activate a GABAergic neuronal programme, as expected from what is known of Mash1 function in the embryonic forebrain. The type of neurons induced by Ngn2 expression is currently under investigation (by analogy with Ngn2 function in the embryonic forebrain, it is expected that Ngn2 confers a glutamatergic phenotype to NS-derived neurons). In addition, **Partner 6** has generated new populations of mouse NS cells, named pp3 NS cells, that do express transcription factors normally not expressed in NS cells, *eg.,* neurogenin 2. These cells, when exposed to specific developmental cues, can generate glutamatergic-like neurons. These results indicate that mouse NS cells may exhibit a certain degree of developmental plasticity when exposed to specific developmental cues or to defined genetic determinants.

Deliverable 3.3 - Partner 1

Validation of leads generated from screening procedures for use in expansion media.

The aim of Deliverable 3.3 was to validate the leads generated from screenings. Active leads from the screens performed in other parts of the project (SD6) were assessed for

their potential effects on proliferation, toxic effect, stability and then evaluated as media additives for use in expansion media. The evaluation indicates that these compounds are soluble and stable, and therefore have potential for use as cell culture media additives. As yet, however, the specific format of use for these compounds has not been defined because under the conditions so far tested, there were no simple growth advantages observed using the compounds as media additives for the expansion of hNES cells. Further studies would be required in order to investigate whether the compounds confer any other advantages, for example hNES cell phenotypic improvement or culture longevity or enhanced differentiation potential.

SD4: A set of assays to evaluate functional characteristics of developing NS cells and other stably proliferating neural precursors

Deliverable 4.1 - Partner 7

Defining functional characteristics of neural stem cells progeny expanded in cell culture

Partner 7 has developed a population of human embryonic stem cell-derived neural stem cells, which are characterized by extensive proliferation and, eventually, stable neuro- and gliogenic differentiation both *in vitro* and *in vivo*. In this study, **Partner 7** characterised the functional properties of neurons derived from these long-term self-renewing human embryonic stem cell-derived neural stem cells (lt-hESNSC). In a current clamp, these neurons were able to fire single or multiple action potentials during long-lasting depolarisation. Analysis of whole cell currents in the voltage clamp revealed both inward sodium currents and outward potassium currents. Human embryonic stem cell (hESC)-derived neurons have a tremendous potential for drug screening applications. Knowing the type of transmitter and receptors expressed in a neuron is a pre-requisite for any drug screening application. **Partner 7** characterized the expression of functional receptors in these neurons. The cells were voltage clamped at -60 mV and GABA or glutamate agonists were applied onto the cell by a computer controlled drug application system and the responses of the voltage clamped cells were monitored. Application of GABA or glutamate induced inward currents. Similarly, application of another GABA agonist muscimol also elicited an inward current. The results show that these cells express both GABA and glutamate receptors.

Thus, neurons derived from lt-hESNSCs are functional and have normal electrophysiological properties including the ability to fire action potentials, expression of ion channels and receptors.

Deliverable 4.2 - Partner 7

Parameters of functional neuronal networks formed by hNS cells and other stably proliferating human neural precursors maturing in monolayer culture

To characterise the neurotransmitters responsible for spontaneous activity of neurons derived from lt-hESNSCs, voltage clamp recordings were performed on neurons differentiated from these cells after two months of differentiation *in vitro.* Spontaneous post synaptic currents mediated by glutamate or GABA were observed. Thus, both GABA and glutamate contribute to the observed spontaneous activity in these neurons.

Neurons derived from hESC require long periods of differentiation to mature and exhibit physiological properties. In an effort to shorten the time needed by these neurons to show functional properties, lt-hESNSC-derived neurons were differentiated on multielectrode

arrays (MEAs) for four weeks in the presence of nerve growth factor (NGF) or N-[(3,5-Difluorophenyl) acetyl]-L-alanyl-2-phenyl] glycin e-1,1-dimethylethyl ester (DAPT). After four weeks of differentiation, no enhancement of spontaneous network activity in the presence of NGF or DAPT was observed. Furthermore, no action potentials could be evoked by treating cell differentiated for isx weeks with L-glutamate (100-500 µM) or the GABA receptor agonist muscimol (100 µM). These results indicate that lt-hESNSC-derived neurons plated on glass MEAs, despite treatement with DAPT or NGF, may require longer time periods of differentiation to mature and exhibit physiological properties.

Electroporation of individual identified cells is a more recent technique, and has been applied *in vitro* and *in vivo* to inject genetic markers, histological dyes, siRNA, etc. in to cells. Single cell electroporation has also the advantage that it can be used to deliver microscopic amounts of molecules not only to the soma of neurons, but also to their compartments such as individual neurites and growth cones. In addition, this technique also enables the delivery of compounds at arbitrarily chosen time points sequentially into a pre-selected individual cell. In this study, a protocol was optimized for the successful electroporation of neurons derived from lt-hESNSCs with Alexa Fluor 594 hydrazide, which is a cell membrane-impermeant molecule that can be used as a cell tracer. This technique can be used for axonal tracing at different time points in culture. With appropriate trans-synaptic markers, this technique can also be used to study synapse formation between neurons derived from hESCs.

Deliverable 4.4 - Partner 8

A brain slice culture system to assess endogenous neurogenesis

The expression of the proneural genes Mash1/Ascl1 and Ngn2/Neurog2 and particularly of the reporter miceMash1-GFP and Ngn2-GFP in neurogenic regions of the adult mouse brain was characterized. This found that that Mash1 is expressed extensively by dividing progenitors in the subependymal zone (SEZ) of young and adult mice and that GFP expression in Mash1-GFP mice reflex this expression. Mash1 is also expressed by progenitors in the subgranular zone of the dentate gyrus (DG), the second neurogenic region of the brain. Mash1 expression, and GFP in Mash1-GFP mice, is more limited. This reflects the more limited proliferation potential of progenitors in the DG than in the SEZ. However, analysis of Mash1 function in adult neurogenesis by conditional deletion using the Cre-LoxP technology shows that Mash1 is essential for the generation of dividing progenitors in both the DG and the SEZ. Ngn2 is like Mash1 expressed in the SEZ, but in a smaller subset of progenitors located mostly in the dorsal part of the SEZ. Again, GFP expression in Ngn2-GFP mice reflects this expression. Finally Ngn2, and GFP in Ngn2-GFP mice, is also expressed by a subset of progenitors in the DG and double labeling experiments have shown that Mash1 and Ngn2 are expressed sequentially by DG progenitors with only limited overlap of expression of the two proneural factors. Analysis of the expression of the GFP reporters in adult brain slices is still in progress and will continue after the formal end of the project.

SD5: Processes for the production of NS cells and other stably proliferating human neural precursors for screening purposes

Deliverable 5.1 - Partner 4

Scale-up procedures for stably proliferating human neural stem / precursor lines in bioreactor systems

The employed human and mouse neural stem and precursor cell populations must be produced in large quantities by processes that guarantee a consistent production of high quality cells. To validate the quality of the produced cells reliable quality control (QC) criteria were defined that ensure absence of bacterial, viral, mycoplasma and endotoxin contamination as well as confirmation of cell identity as defined by standard phenotypic and biochemical assays.

To further exploited the amenability of neural stem and progenitor cells to bioreactor and suspension culture systems **Partners 1** and **4** evaluated different suspension culture systems. In a comparative study, cultivation of long-term self-renewing human embryonic stem cell-derived neural stem cells (lt-hESNSC) on microcarriers (Cytodex-3) and as neurospheres was performed. **Partner 4** found that the microcarrier-based expansion in petri-dishes was as efficient as conventional 2D cultures regarding growth and cell yield. The cells maintained their typical neural stem cell marker expression and grew as an adherent monolayer. Due to their extremely high surface area to volume ratio, microcarriers are an attractive alternative to conventional monolayer cell culture methods. In contrast to the adherent expansion of neural stem cells, the neurosphere culture system showed major limitations, *i.e.,* regions of differing cell densities within the neurosphere, which alters the microenvironment and therefore affects both proliferation capacity and positional cues that determine the properties of the cells within the neurospheres. **Partner 4** observed slower growth and heterogeneity as indicated by pronounced size variation of the neurospheres. Microcarrier techniques are therefore a logical choice for following scale-up applications. However, the translation of those microcarrier-based suspension cultures to a scalable stirred vessel reactors failed most likely due to high sheer forces induced by the use of impellers within the utilized spinner flasks. This led to the replacement of stirred bioreactors by the BioLevitator™ system by **Partner 4**, who reported an efficient method for the expansion of lt-hESNSCs on Global Eukaryotic Microcarriers™ (GEM™) utilizing Hamilton's BioLevitator™. The BioLevitator™ takes advantage of a magnetic microcarrier (Global Eucaryotic Microcarrier GEM™) for cell culture that maintains the cells in the liquid phase without the use of impellers. As a magnetic microcarrier, the GEM™ can be controlled throughout medium exchange or culture collection during manual or automated handling. **Partner 4** showed that the BioLevitator™ mediated expansion of lt-hESNSCs neither affected their neural stem cell state, differentiation capability nor their metabolic activity as determined by glucose consumption and lactate production. Confirmation of the typical rosette-like structure of lt-hESNSCs on GEMs™ by a luminar ZO-1 expression pattern demonstrated the supportive effect on growth and high biological compatibility of the GEM™ substrate. Enabling undifferentiated cell growth and high cell yields in small media volumes, the BioLevitator™ system was shown to be particularly suited for the expansion of lt-hESNSCs.

Deliverable 5.2 - Partner 1

Protocols for automated scale up of mouse and human NS cells and other stably proliferating human neural precursors

Employing the automated platforms, **Partner 1** and **Partner 4** established cultivation of neural stem and precursor cells of human and mouse origin. Technical specifications emulating manual cultivation of neural stem cells were complemented by biological validation of the processed cells after short and long-term automated cultivation. **Partner 1** automated long-term cultivation and scale-up of murine neural stem (mNS) cells employing the SelecT CompacT system. MNS cells maintained their multi-lineage potential after automated long-term cultivation and can be automatically plated into microplate formats for screening purposes. **Partner 4** established critical cell culture steps for the automated cultivation of lt-hESNSCs using the CellHOST system (Hamilton). Biological validation of automatically processed lt-hESNSCs proofed feasibility of automated cultivation and completed the process design for the automated long-term cultivation of those cells. **Partner 4** then established the fully automated long-term cultivation of lt-hESNSC. The CellHOST system was shown to perform all crucial steps of lt-hESNSC culture. Growth analysis of automatically cultured cells revealed an initial delay of proliferation, pointing to a potential adaptation phase required for transition to automated culture conditions. However, fully automated long-term cultivation (60 days) of those cells was demonstrated neither to compromise neural stem cell marker expression nor differentiation potential after growth factor withdrawal. Successful automated cultivation of lt-hESNSC achieved by **Partner 4** with the CellHOST was relevant for the production and standardization of high quality human neural stem and progenitor cells enabling subsequent scale-out of those cells for screening applications. To broaden potential screening applications conducted by the automated cell culture systems, **Partner 1** established protocols towards the automated cultivation and scale-up of other relevant human neural stem and progenitor cells, among those human primary NS cells (Hind05). First results obtained by **Partner 1** demonstrate that Hind05 primary hNS cells can be expanded with the SelecT CompacT automated cell culture system for multiple passages. In a biological validation process those cells maintain normal doubling times, neural marker expression and differentiation potential. Along the same line, **Partner 1** established protocols towards the automated cultivation and scale-up of glioblastoma-derived neural stem cells (gNS). Preliminary data from **Partner 1** suggest that transferring manual culture of the gNS line G179 to automated conditions is feasible in principle and underline the need to directly optimise the protocols in automated cell culture conditions. Current calculations indicate that with uniform expansion and operation of the SelecT-CompacT system at maximum capacity, sufficient cell numbers for screening applications should be achievable for both the H05 and G179 cell line.

To take into account the latest developments in the field of reprogramming the application of human induced pluripotent stem cell (hiPSC)-derived neural stem cells was implemented by **Partner 1.** The employed hiPS cell-derived neural stem cell line AF22 has the same biological and morphological properties as the hES-cell derived lt-hESNSCs and therefore enables comparison of human neural stem cell performance on the two automation platforms used: CellHOST and CompacT SelecT. **Partner 1** developed protocols for the automated maintenance and scale-up of the AF22 cell line on the CompacT SelecT automated platform. Automation-friendly protocols have been tested and transferred indicating that the cells, which have been passaged seven times with the CompacT SelecT exhibited a morphology similar to those passaged using the routine

manual method. Together with the work performed by **Partner 4**, the long-term self-renewing neural stem cell lines generated either from hES or hiPS cells were shown to be expandable on both cell culture automation platforms, classifying them as an ideal cellular model for industrial use on state of the art automation platforms. Such human pluripotent stem cell (hPSC)-derived neural cells, will constitute an important source for cells as clinical and research tools. These approaches, however, will critically depend on the purity of the *in vitro* differentiated cell populations, also for other tissue specific cell types derived from hPSCs. In particular, remaining undifferentiated hPSC in a transplant can induce teratoma formation. In order to address this challenge, the additional work performed by P**artner 4** toward therapeutic application of such hPSC-derived somatic cells was implemented. In this, **Partner 4** developed a laser-based method for the ablation of hPSC from differentiating cell cultures. These studies, which have now been published, showed efficient elimination of hPSC while co-treated hESCNP maintained their normal proliferation and differentiation potential. Laser-assisted photothermolysis thus represents a novel automatable, contact-free method for the efficient elimination of hPSC from *in vitro* differentiated hPSC-derived somatic cell populations.

Deliverable 5.3 - Partner 1 – SCS

Protocols for automated scale out of mouse and human NS cells and other stably proliferating human neural precursors

Automated scale out of neural stem and progenitor cells represents an integral part within the process of industrializing cellular products for screening applications. **Partners 1 and 4** set the stage for the production of human and murine neural stem and progenitor cells in microtiter plate formats (96- and 384-well plates) requested for large compound screenings. **Partner 1** established protocols for automated scale-out of mouse neural stem cells (mNS). The established workflow for the automated scale-up and scale-out platform could provide over 200 plates every two days, which would be sufficient for a high-throughput screening application. In order to upgrade the CellHOST System for scale-out procedures of lt-hESNSCs, **Partner 4** integrated a 96 channel pipetting head in the existing automated cell culture system, thereby enhancing the flexibility and throughput of the system by facilitating cultivation of lt-hESNSCs in six-well- and 96-well-plate formats. Subsequently, **Partner 4** optimized and validated protocols for the dispersion of human embryonic stem cell-derived neural stem cells (lt-hESNSC) into 96-well microplates in the CellHost system. In order to validate the established scale-out protocol, cell distribution across each 96-well plate and growth in the presence or absence of growth factors after automated scale-out were evaluated. The protocols for automated scale-out have been proven to be effective. Lt-hESNSCs remain fully viable and responsive to growth factors after scale-out into 96-well microplates on the CellHOST automated cell culture platform. The CellHOST automated cell culture platform also has the ability to perform full and partial media changes on 96 well microplates after cells have been scaled-out. This functionality renders this system particularly suitable for long-term assays.

Concurrently, **Partner 1** developed scale-out protocols using the human induced pluripotent stem cell-derived neuroepithelial stem cell (hPSNES) line AF22. Protocols for the maintenance, scale-up and scale-out of AF22 cells were successfully developed on the CompacT SelecT automated cell culture platform. The AF22 cell line was extremely

amenable to automation, thus **Partner 1** evaluated automated procedures for plating of partially differentiated AF22 cells into 96-well microplates. Due to the long time periods needed for differentiation of human neural stem cells, the ability partially to differentiate human neural stem cells in T175 flasks prior to transfer into 96 well microplate may offer significant advantages for the development of high-throughput human neural differentiation assays.

Deliverable 5.4 - Partner 4
Protocols for the handling & dispensing of cells in generic formats
Effective long-term storage is critical to the successful application of human and murine neural stem and progenitor cells as clinical and research tools. **Partner 4** addressed this challenge by establishing conditions for freezing and thawing undifferentiated, adherent lt-hESNSCs in microwell (96) plates. The established freezing and thawing procedures neither compromised neural stem cell marker expression, nor differentiation into mature neurons. Considering the well-to-well variability regarding freezing and thawing efficacies throughout a 96-well plate, integration of technologies that facilitate rapid and reliable reproducibility, efficacy, high throughput and quality control are essential. **Partner 4** therefore implemented a computer-controlled rate freezer (IceCube) and an automated image analysis platform (Cellavista^{TM}), thereby gaining sufficient throughput and quality control for processing 96-well microplates. The analysis showed good inter-day and inter-plate reproducibility for the spotted 96-well microplates with homogeneous cell distribution and percentage CV values of about 6% before freezing and about 10% after thawing as determined from three independent cell batches processed at different times. A slight increase of the measured percentage mean cell confluence was detected 24 h post-thaw, indicating fast recovery of the cells. In order to develop a protocol for partially differentiated human neural stem cells (AF22) to be frozen into 96 well plates, **Partner 1** tested either DMEM/F12 containing 10% DMSO or the freezing medium CryoStorTM CS10, which was supplied by **Partner 3**. Under the conditions tested, freezing medium CryoStorTM CS10 was proven to be effective although further optimization would be necessary including the adjustment of cell density prior to scaling out in order to compensate for a certain degree of cell loss during the freezing and recovery process.
The work performed in this section is pivotal to develop a competitive and customized cellular product for screening applications.

SD6: A defined set of bioactive compounds validated as affect on neural stem cell fate
Deliverable 6.1 - Partner 9
Completion of 1,000 compound screens and analysis of subsequent hits
Deliverable 6.1 was designed to assess a medium sized collection of compounds in various bioassays to establish proof of principle for screening small molecules as modulators of stem cell fate. This was successfully achieved and laid the foundation for the successful screening carried out in the remainder of WP6. Assays were optimised for high-throughput screening with the initial focus on the utility of first generation rodent engineered stem cell reagents. Knowledge was developed on aspects of assay development, such as identification of reference compounds, determination of toxicity

standard, assay reproducibility ('z' factor). Further, several small molecule screens were conducted in assays monitoring the fate of various neural stem cell lines. In particular, successful PoC screens against glioblastoma cell lines and mouse NS lines were completed.

Deliverable 6.2 - Partner 1
Screening protocols for neural stem cells
Several novel assays as reported were developed that demonstrate the breadth of cell types and assay technologies that have been employed within the consortium project. Assays have been developed using multiple cell types including: mouse neural stem cells derived from foetal tissue (mNS cells) and human neural stem cells that had been derived from sources including: (i) induced pluripotent stem (iPS) cells (hNES cells), (ii) embryonic stem (ES) cells (lt-hESNSC), (iii) neural stem cells that had been derived from human glioblastoma samples (GNS cells). Most of the assays described have been miniaturized to 96 well plate format (wpf) and of those the majority would be amenable to further miniaturization to at least 384 wpf. Several of the assays have gone on to be screened within the consortium, using a 1000-compound collection.

Deliverable 6.3 - Partner 9
A defined set of NCEs modulating glioblastoma cells in vitro
Glioblastomas are the most common form of brain tumours and are associated with rapid and aggressive development with only a 12-14 month mean survival term following diagnosis. Treatment options are extremely limited and of limited efficacy and, as such, there is a pressing need for new agents to treat brain tumours. **Partner 5** developed and published a live image-based method for the isolation of compounds affecting proliferation/cell death of GNS cells. This was successfully used to screen libraries of compounds as potential start points for the development of drugs for an indication with significant unmet medical need. Numerous hit compounds were identified and represent medicinal chemistry start points for the future development of novel therapies for primary brain tumours. In addition, the hits from the tool compound collection have highlighted several modes of action of interest.

Deliverable 6.4 - Partner 9
A defined set of NCEs modulating human neural stem cells in vitro
The 1,000 compound library (supplied and prepared by **Partner 9**) was screened by **Partner 1** in an ATP viability assay using a neural stem cell line. The cell line used as part of this deliverable is an iPS derived human neural epithelial stem (hNES) cell line (AF22). Use of human, rather then rodent, cell lines offers greater therapeutic relevance.
The screen successfully identified 24 hit compounds that show an increase in survival or proliferation.

End Results

The list below provides a summary of the main impact on the industrial and research sectors of the work performed in NEUROscreen. This is then broken down in more detail from each of the project's six Strategic Deliverable (SDs).

- The establishment of a set of procedures to check the quality of the neural stem cells and an effective commercial storage facility
- Development of an animal component-free medium suitable for the derivation and culture of human neural stem (NS) cells
- Establishment of a reproducible cryopreservation technique that guarantees the purity, cell viability and functional integrity of the sample and has potential in clinical practice to generate ready-to-use, safe and effective stem cell lines for therapeutic application
- Demonstration that human and mouse neural stem cell lines are valid models to study neural stem cells *in vitro* by showing that human NS maintain a normal karyotype after long-term expansion and that chromosomal abnormalities observed in mouse NS did not lead to detectable changes of their biology
- Production of several reporter neural stem cell lines that can be used to screen for molecules that direct the differentiation of stem cells into particular types of neurons, which have additional potential applications for *in vivo* tracing of transplanted and terminally differentiated neurons within the host brain
- The derivation of adherent glioblastoma (brain tumour) stem cell (GNS) lines that recapitulate the tumour phenotype in xenografts -- this provides a valuable resource for biomedical and biopharmaceutical research in brain cancer
- Development and publication of a live image-based method for the isolation of compounds affecting proliferation/cell death of GNS cells, which has been successfully used to screen libraries of compounds as potential start points for the development of drugs for primary brain tumours
- Establishment of robust protocols for deriving human neural stem cells with a very high propensity for oligodendrocyte differentiation with potential therapeutic application for demyelinating diseases and spinal cord injury
- Development of highly efficient protocols for neuronal differentiation of mouse neural stem cells, including previously not obtainable neurotransmitter subtypes, confirming their differentiation potency and appropriateness for an array of screening purposes
- Discovery of small molecule compounds with almost immediate commercial potential as more specific and cheaper substitutes for biological signalling factors in cell culture media
- Characterisation of the functional physiological properties of neurons derived from human embryonic stem cell-derived neural stem cells (lt-hESNSCs)
- Establishment of a protocol for plating and maintaining lt-hESNSC-derived neurons on multi-electrode arrays (MEAs) for the detection of extracellular potentials , which permits monitoring of the neuronal activity from a large number of neurons at the same time

- Implementation of a protocol for electroporation of single lt-hESNSC-derived neurons, a technique which used to study synapse formation between neurons derived from human stem cells
- Development of automated procedures for handling, dispensing and freezing of adherent undifferentiated or partially neuronal differentiated human neural stem cells in 96-well microplates, this enables large batch production and long-term storage for commercial-scale applications
- Development of protocols for the long-term automated culture and scale-up of mouse and human neural stem and progenitor cells of both tissue and pluripotent cell origin; this facilitates the generation of sufficient numbers of high quality cells for drug screening and clinical applications in the field of regenerative medicine
- Establishment of techniques for the automated derivation of neural stem cells from pluripotent sources and the elimination of residual pluripotent, potentially tumorigenic cells in such neural stem cell cultures by laser-assisted thermolysis
- Development of numerous neural stem cell-based assays, four of which have already gone on to be screened within the consortium on a collection of 1000 compounds
- Many neural stem cell-based assays developed to a stage where they could be progressed into screening campaigns with minimal additional work
- Development of assays using either human ES cell-derived or human iPS cell-derived neural stem cells, which have a proven capacity for rapid and consistent expansion

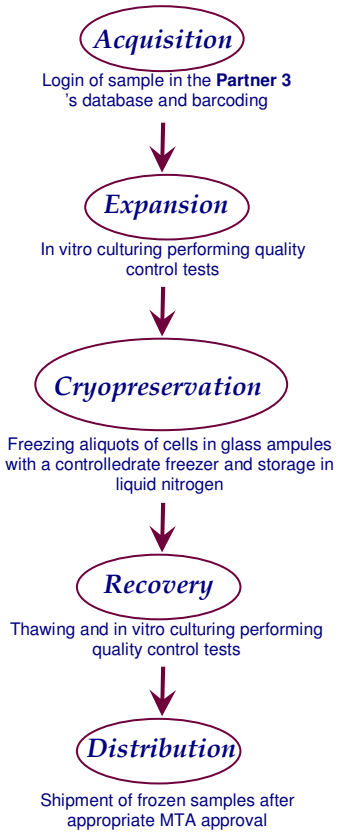
SD1

The exploitation of stem cells for the discovery of new therapeutics is having a strong impact in regenerative medicine. This requires the banking of highly controlled cell lines that fulfil the most stringent standards of the European Community. Joining the stem cell biology expertise of academia with the technology standards of industry has allowed the development of reagents and processes to generate safe and effective stem cell lines. This includes standardised animal component-free stem-cell-culture media and the setting of standards for generation, expansion and banking of these cells. In this way, several neural stem cell lines derived from a diversity of resources, including neoplastic samples from surgery, have been established and compared with respect to their growth characteristic and phenotype. All these data should establish a common set of characteristics of the normal stem cell phenotype and how this might be crucially different in tumour-derived lines. Definition of neural potency from homogenously growing human lines and comparison with tumour derived neural stem cells will provide benchmark information for the research and industry community. This work has built the basis for the successful application of highly appropriate neural stem cells in chemical genetics screening with the ultimate aims to understand the mechanism of stem cell differentiation and to develop new molecules for therapy.

Neural stem cells are acquiring a great deal of attention for their potential to expand and differentiate under appropriate condition. Standardization of *in vitro* culture and development of effective cryopreservation protocols are essential for their application in

regenerative medicine. Within this project, a reproducible cryopreservation technique that guarantee the purity of the sample, cell viability and functional integrity was established. Neural stem cells require great care in maintenance, handling and preservation in order to ensure that their stem cells characteristics and capacity for differentiation are retained. It is therefore important to establish proper cryopreserved stocks to avoid subjecting cells to substantial and irreversible changes due to variable or inappropriate culture condition. Using a programmable rate freezer and an intracellular-like solution as a freezing medium it was able to achieve optimal results to cryopreserve neural stem cells and maintain intact their characteristics with the aim to provide researchers with high quality material, which is fundamental to good scientific practice and for maximising reproducibility. Furthermore, in order to be able to move forward clinical application, an alternative non toxic cryoprotectant was successfully developed and tested to freeze several neural stem cell lines. Using trehalose, a natural sugar, preserved and recovered neural stem cells maintained intact their behaviour and potential. This technique has the potential to have a strong impact in clinical practice since it could be used to generate ready-to-use, safe and effective stem cell lines for therapeutic application.

Given the huge potential of NSCs as model systems to study molecular events in normal and pathological processes, it is critical to monitor cell-line identity to prove that the line is unique, *i.e.,* not switched nor cross-contaminated. For this purpose, different genotyping techniques are available to "profile" samples at the earliest stage possible and once the cell stocks are established. Karyology should be performed as a routine genotyping technique to detect the appearance of chromosomal aberrant clones and repeated testing is recommended to confirm the findings. Further investigation would be required to monitor genomic changes in cultured cells and eventually to determine the impact of such changes, thus identifying the best culturing conditions to decrease chromosomal alterations in long-term cultures. Nevertheless, this should encourage researchers to perform regular high-resolution molecular and cytogenetic studies to verify chromosome integrity in NSCs using more precise techniques to be able to detect any biologically relevant variants. Cell line identification and authentication should routinely be applied to diminish misidentification -- a routine procedure that individual researcher would find impracticable to support. Biobanks are the ideal infrastructures for actively working towards establishing procedures to collect, quality control, store and distribute cell lines. It is extremely important to perform such testing and share the results to safely identify cell lines, assure their stability and control



their release for the purpose of preventing any serious long term effects on the validity of research data performed with such lines.

The main achievement is the transfer of knowledge from science base to application. "*If Europe is to benefit, excellence in the science base is not enough: it is essential to have the capacity to translate knowledge into new products, processes and services, which in turn will generate benefits to society, skilled jobs and prosperity*" (from LifeSciHealth priority, "Lifesciences and biotechnology: a strategy for Europe"). The success of any knowledge-based economy rests upon the generation, diffusion and application of new knowledge and the work here described moves strongly from the traditional academic focus on understanding stem cells to a comprehensive commercial focus, an essential move for the transfer and application of new knowledge. The establishment of a set of procedures to check the quality of the neural stem cells and a proper storage facility can be the starting point to generate a consistent source of biological material. The standardisation of all processes, the tracking system and the study of the administrative/legal aspects helped identify a specific and successful workflow to be applied to the management of a stem cell bank and to the distribution of highly validated cells. Without proper visibility and diffusion, information and reagents originated by the NEUROscreen project will be of limited use. The integration of a biorepository such as **Partner 1**'s in the development of new strategies, which have a strong potential in regenerative medicine, it will help the sharing of valuable information and materials generated by this novel project and it will place Europe at the forefront of a commercial stem cell application.

SD2

Recent years have seen rapid progress in the ability to isolate and expand in culture neural stem cells. Two main methods have been used: (i) isolation of neural stem cells from different regions of the embryonic nervous system, and (ii) derivation of neural stem (NS) cells from pluripotent stem cells, *i.e.,* embryonic stem cells (ES) or induced pluripotent stem cells (iPS). In the mouse, neural stem cells generate through these two routes are globally very similar and are collectively termed NS cells. It was, however, not known whether NS cells derived from different regions of the neural tube and having presumably different positional identities *in vivo*, conserve these differences after extensive culture as NS cells. The results from this project confirmed that NS cells derived from different regions of the brain and spinal cord have very similar cellular properties of proliferation and differentiation potential, but they differ in their molecular marker expression (in particular transcription factors marking the antero-posterior identity of progenitors), reflecting, in part, their distinct origins. In contrast with the mouse, human neural stem cells in culture differ whether they originate from the embryonic brain or from pluripotent stem cells. Human-brain-derived neural stem cells are similar to their mouse counterparts and to radial glia progenitors *in vivo*, and are also termed NS cells. In contrast, human ES or iPS-derived neural stem cells have a phenotype that is similar to a more immature neuroepithelial progenitor, and have thus been called neuro-epithelial stem (NES) cells. We have systematically examined the expression of a large panel of genes chosen for their documented expression *in vivo* in different categories of progenitors and at different stages of development. Our results

confirmed that NS and NES cells are very different, not just in their rate of division and differentiation potential but also in their molecular phenotypes, expressing markers of radial glia and neuroepithelial cells, respectively. In addition, they showed that NES cells express more markers of a caudal identity, whereas NS cells, which were derived from brain regions, express more markers of a rostral identity. Together, the data from this project demonstrate that human and mouse neural stem cell lines are valid models to study neural stem cells *in vitro*, including how they acquire and maintain their positional information (for both mouse and human cells) and how they mature from a mostly neurogenic state to a mostly glial state (for human cells).

NS cells derived from mouse or human foetal brains or from pluripotent stem cell lines have been have been available for a few years, and are widely considered to have a huge potential for both basic science and the pharmaceutical industry as tools to screen for molecules that can modify the properties of neural cells in a predictable manner. For this potential to reach fruition, NS cells must be engineered in such a way that specific outcomes can be easily monitored in the context of large scale screens. In this project, several NS cell lines were generated that express fluorescent reporter molecules, which become activated when NS cells reach distinct stages along the pathway of differentiation into neurons. These new reporter NS cell lines can collectively detect the onset of neurogenesis and discriminate between a GABAergic and a glutamatergic mode of neurogenesis (Mash1 and Ngn2 reporters), and early and relatively late stages of neuronal differentiation (Dcx and Tau reporters, respectively). Together, the established and future (for the part of the work still in progress) mouse and human reporter lines enables real-time read and quantification of neuronal differentiation under various culture conditions. Some of these lines will also extend potential applications to *in vivo* tracing of transplanted and terminally differentiated neurons within the host brain.

Several reporter neural stem cell lines that can be used to screen for molecules have been produced in the project, which can direct the differentiation of stem cells into particular types of neurons. Cell replacement therapies of neural lesions require that the exact cell types that are lost in patients are used for repair. Efficient methods are now available to differentiate embryonic stem cells and other cell types into neurons. However, there is a severe lack of understanding of how to induce the differentiation of stem cells into particular types of neurons, including neurons of great medical relevance such as striatal projection neurons or cortical projection neurons. Two types of approaches are being used to address this problem: (i) characterisation of the regulatory pathways that control the generation of these particular types of neurons during embryonic development, and (ii) unbiased screens for molecules that promote the differentiation of stem cells into appropriate types of neurons. The reporter NS cell lines generated in this deliverable will be valuable for such screens.

SD3

The robust protocols for neuronal and oligodendrocyte differentiation of human neural stem (NS) cells developed in this research confirms their differentiation potency and consequent suitability for a variety of screening applications. The derivation of adherent glioblastoma stem cell lines that recapitulate the tumour phenotype in xenografts is an

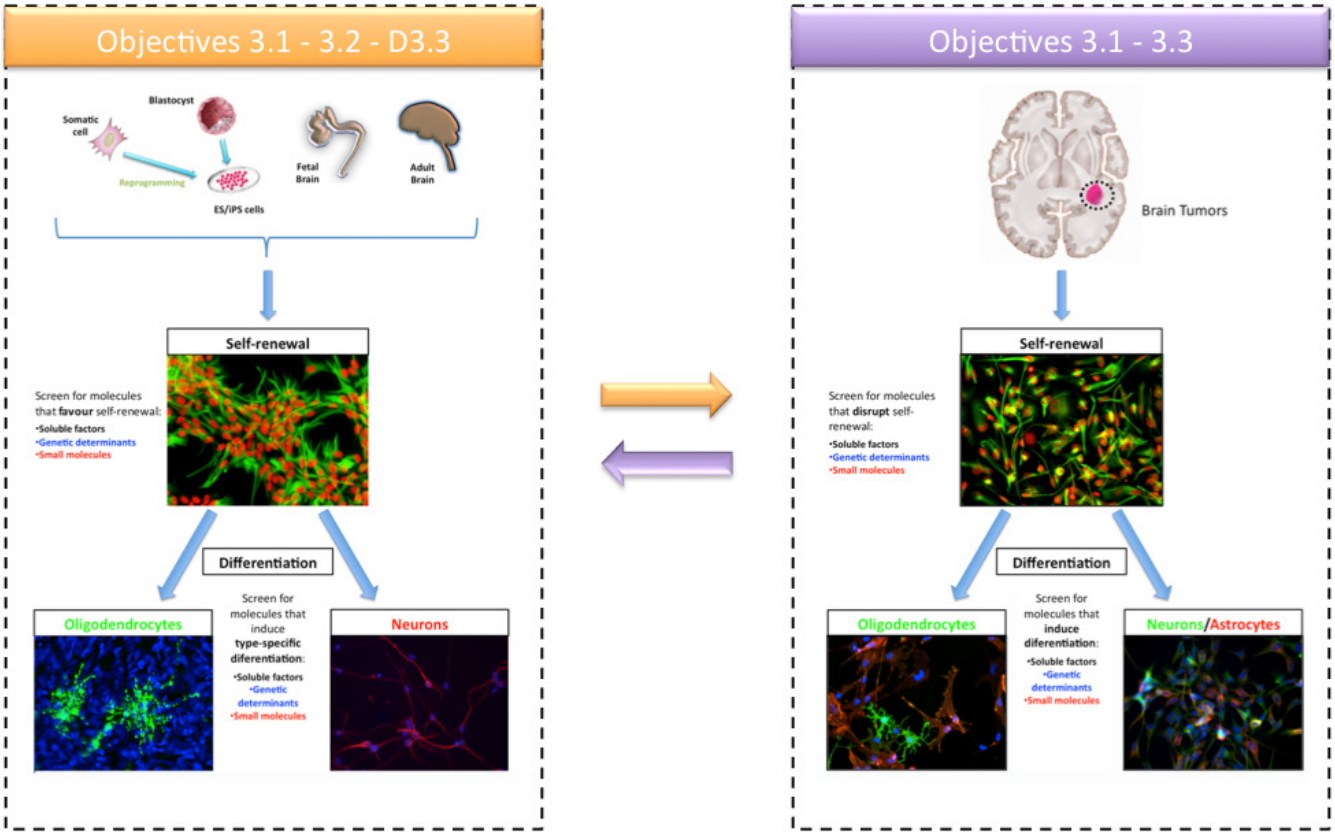
advance that provides a valuable resource for biomedical and biopharmaceutical research in brain cancer. In this respect the tumour stem cell lines have been placed in a repository for distribution to the community (in collaboration with **Partner 3**). Significantly, the glioblastoma stem cell lines exhibit varying differentiation behaviour, which may correspond to differences between tumours relating either to cell of origin or to specific molecular lesions. The availability of the tumour stem cell lines may therefore facilitate disease stratification and targeted therapeutic screening.

The highly efficient protocols for neuronal differentiation of mouse neural stem (NS) cells developed in this research confirm their differentiation potency and consequential appropriateness for an array of screening purposes. Understanding the competence of mouse NS cells to respond to specific environmental cues and small molecules and how the latter can improve their neuronal maturation can lead to neuronal differentiation protocols applicable to NS cells and their use in high-throughput screening (HTS) systems. The intrinsic poor plasticity of these cells to generate non-GABAergic neuronal subtypes might be the basis to develop screening approaches to identify new chemicals that may act as strong inducers of neurochemical phenotypes alternative to the "default" GABAergic identity. The work performed has also shown that foetal-derived NS cell lines might not represent, because of the very long and expensive differentiation process, the ideal system for screening processes aimed at identifying compounds acting on neuronal maturation in human neural cells. Nonetheless, they can be fully exploitable for screening purposed aimed at identifying new compound that might favour or modulate their self-renewal capability.

Further results generated suggest that there is commercial potential for compounds to be used as cell culture media additives based on demonstrated compound stability. This potential represents the most immediate commercial outcome for the consortium since only a modest amount of further development work would be required to validate commercial utility. This is in contrast to the extensive efforts that would be required to progress compounds through the drug discovery process. A key challenge in stem cell research is the maintenance and modulation of stem cell populations. In most cases, this is currently achieved through the use of complex and expensive cocktails of biological reagents. The use of small molecules has already been demonstrated to substitute for biological signalling factors for the maintenance of mouse embryonic stem cell populations and so it remains a commercially attractive proposition to pursue the chemical compounds that, either singularly or in unique combinations, may confer similar maintenance properties to the numerous other stem cell populations. In addition to increasing reproducibility and standardization across research groups, the development of new cell culture media using small molecules to substitute for biological signalling factors is also likely significantly to reduce the costs of cell culture reagents, thereby serving the international research community as a whole.

The results of this SD are summarised in the diagram, below, and have been mainly to: (i) find molecules (growth factors, genetic determinants and small molecules) that can be effective in favouring normal (nonpathological) NS cells expansion and improved differentiation capability toward the desired cell type (left box, in diagram), and (ii)

create new NS systems from brain tumour samples that can be exploited in order to find molecules with anti-cancer effect (right box). The two activities share aspects that can communally cross-fertilize each other.



SD4

These studies characterised the functional properties of the neurons derived from lt-hESNSC. It showed that the neurons are functional and have normal electrophysiological properties including the ability to fire action potentials, expression of ion channels and receptors. hESC-derived neurons have considerable potential for drug screening applications. Knowing the type of transmitter and receptors expressed in these neurons is a pre-requisite for drug screening applications.

The detection of extracellular potentials by means of multi-electrode arrays (MEAs) is a useful technique for monitoring the neuronal activity from a large number of neurons at the same time. For this reason, measuring activity of neurons plated on MEA is a method of choice for the screening of drug candidates. A protocol was established for plating and maintaining lt-hESNSC-derived neurons on MEAs. After four weeks of differentiation spontaneous spikes in this system could be observed.

For further refining manipulation of this system, a protocol for electroporation of single lt-hESNSC-derived neurons was implemented. This technique can be used for axonal tracing at different time points in culture. With appropriate trans-synaptic markers this

technique can also be used to study synapse formation between neurons derived from hESCs.

SD5
Enabling undifferentiated cell growth and high cell yields in small media volumes, the BioLevitatorTM system is particularly suited for the expansion of our lt-hESNSCs, which maintain self-renewal, multipotentiality and their typical rosette-like growth pattern. This technology could facilitate parallel scale-up of such stem cells for further downstream applications.

The work has also led to significant progress towards the long-term automated culture and scale-up of cell supply for mouse and human neural stem and progenitor cells of both tissue and pluripotent cell origin. This will facilitate the generation of sufficient numbers of high quality cells for drug screening and clinical applications. Such automated systems have the advantage of being more reproducible than manual culture and to be less labour intensive and more cost effective. The use of robotic cell culture platforms has been demonstrated to be applicable to numerous types of adherent neural stem cell lines and is well suited for the production of cells in small well format for drug discovery applications, where the whole process from scale-up, plating out of cells and screening can be automated. With future development this automated cascade can be even further extended to automated derivation and purification for neural stem and progenitor cells, as has been shown in two instances. The provision of proven human neural stem cell lines and of validated procedures and protocols for their bioprocessing will therefore present a significant opportunity for biotechnology companies to prosper in partnership with pharmaceutical industry clients and advance the field of regenerative medicine.

The work also demonstrated successful scaling out of a range of mouse and human neural stem cell populations -- either in the undifferentiated state or in a partially differentiated state. An immediate commercial impact of these applications is the high throughput capability that automation provides for neural stem cell screening applications. While the pharmaceutical industry has remained cautious in terms of the degree to which it will invest in stem cells as a biological source of screening material; perceptions are changing and the provision of robust supplies of stem cells for screening is key to the continuing derailment of those perceptions. One possible model for commercialisation is a technology transfer package where a pharmaceutical industry partner would license a neural stem cell automated culture platform technology in order to furnish cell supply for internal drug discovery programmes. Methods and protocols utilizing either of the two automated cell culture platforms have commercial potential. Given that protocols have now been developed for both the maintenance of mouse and human neural stem cells and also the scale out of both populations, it is now possible to maintain and supply cells for screening in a fully automated fashion.

Automated procedures were developed for handling, dispensing and freezing of adherent human neural stem cells in 96-well microplates enable large batch production and long-term storage for further downstream applications on demand. This facilitates rapid and

reliable quality control, reproducibility, efficacy and high throughput and thus provides a basis for future industrial use of human neural stem cells in screening applications.

SD6

Overall, the work in this SD has clearly demonstrated that high throughput screens with small molecules can identify compounds which have a clear effect on stem cell fate. The identification of such compounds has clear commercial output in two main areas.

1. Novel Therapies: The ultimate aim is to develop small molecule therapies for neurodegenerative conditions such as Alzheimer's or Parkinson's disease. These agents could be either *ex vivo* or *in vivo* therapies used in conjunction with cell therapies or as standalone drugs. However, regenerative medicine applications are a long-term goal and beyond the immediate remit of the project, which aimed to develop suitable screening assays and identify start points for drug discovery programmes.

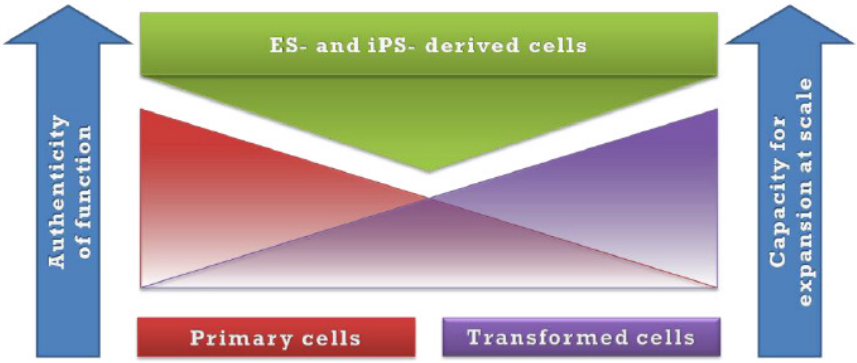
2. Research Reagents: This represents the most immediate commercial outcome for the consortium since minimal development of any hits is required, unlike the extensive efforts required in the drug discovery process. A key challenge in stem cell research is the maintenance and modulation of stem cell populations and this is currently achieved through the use of complex and expensive cocktails of biological reagents. The use of small molecules should be able to substitute many of the components of these cocktails, significantly reducing the associated cost of goods and providing a cheaper, versatile and tuneable tool kit of reagents for researchers.

Numerous neural stem cell-based assays have now been developed within the project, four of which have already gone on to be screened within the consortium on a collection of 1000 compounds. Many of the remaining assays are at a stage where they could be progressed into screening campaigns with minimal additional development work. Collectively therefore, a significant additional screening resource is now available to the pharmaceutical and biotechnological company drug development community. These assays are made available with added value because many of the cell lines used for the assays have already had protocols developed for automated production. This enables the expansion of cell lines on an industrial scale allowing for high-throughput screens to now be performed. Of particular importance in terms of potential healthcare benefit is the fact that a number of the assays have been developed using human cell lines that can be automated and expanded for extended periods of time and this will offer a significant advantage to high throughput screening efforts because it ensures reproducibility over time. In particular, development of assays using either human ES cell-derived or human iPS cell-derived neural stem cells has filled a previously inaccessible space in terms of cell utility.

Historically, primary cells have generally displayed the most authentic functions in the tissue culture dish and have most resembled cells as they should function in the human body. However, primary cells are difficult to maintain over extended periods of time making them less amenable to screening campaigns that run over a long time-frame. In

contrast, transformed cell lines (sometimes obtained from tumourigenic tissue) have the potential to be maintained in culture indefinitely, but have often failed faithfully to recapitulate the desired cellular functions. There is now another option and that is to derive cell lines of interest from either human ES cells or iPS cells. In this project, neural stem cell lines have been developed from both of these human cell sources and have gone on to be used for the development of assays that can be used for chemical compound screening.

Specification requirements of cell lines for screening include both authenticity of cellular function and capacity for expansion at scale are shown in the diagram, below.



Previously, primary cells and transformed cells each satisfied only one of these two requirements. Cell lines derived from either ES or iPS cells have been demonstrated to satisfy both of these requirements and therefore offer a new dynamic space from which to develop cell-based assays for screening. Embryonic stem (ES) and induced pluripotent stem (iPS) cells provide a new piece of the jigsaw in that they are a new cellular tool that can be used to derive cell lines for the development of neural stem cell-based assays for screening.

In addition to the work described above, **Partner 5** developed a live image-based method for the isolation of compounds affecting proliferation/cell death of GNS cells. This has been published and has been successfully used to screen libraries of compounds as potential start points for the development of drugs for primary brain tumours. Glioblastomas are the most common form of brain tumours and are associated with rapid and aggressive development with only a 12-24 month mean survival term following diagnosis. Treatment options are extremely limited. The only agents in clinical use are Temozolidine (a DNA alkylating agent) and Gliadel, a biodegradable polymer wafer delivering carmustine directly to the site of the tumour which is implanted into the resection cavity following tumour resection. Both agents show a poor level of efficacy with only a three- or four- month increase in median survival rates. As such, there is a pressing need for new agents to treat brain tumours.

A key issue in the development of novel therapies is the absence of adequate cell models of the disease from which novel drugs can be developed. In recent times, it has become clear that glioblastomas, as with other forms of cancer, contain a cellular hierarchy where only a fraction of the cells in the cancer mass are capable of forming and sustaining the

tumour and such cells have been dubbed tumour initiating cells or cancer stem cells. As such, the development of assays to monitor the effects of small molecules against these cells is of particular interest. Using this live image based assay, multiple chemical libraries were screened and compounds were identified that affect the survival of GNS cells and these represent potential start points in the development of novel therapies for the targeted treatment of primary brain cancers. Given the paucity of agents in development and the absence of suitable *in vitro* systems from which to develop drugs, this represents a significant advance.

2. DISSEMINATION AND USE

Section 1 - Exploitable knowledge and its Use

Overview table

A. Exploitable Knowledge (description)	B. Exploitable product(s) or measure(s)	Sector(s) of application	C. Timetable for commercial use	D & E. Patents or other IPR protection	Owner& Other Partner/s involved
1. Neural Stem cell culture	NS culture medium	biotech	2010	Trade Secret	Owner: Partner 1
2. New cryoprotection technique	Stem Cell Cryoprotection Medium	Biotech	2012	Trade Secret	Owner: Partner 3
3. Derivation of NS cell lines	Highly validated cell lines	Biotech and Medicine	2009	PCT/GB2005/002289.	Owner: Partner 5
4. Detailed annotation of cell lines banked	Web based database	Biotech	2011	N/A	Owner: Partner 3
5. Neural stem cells characterization	Protocols and Services for QA/QC testing	Biotech	2010	N/A	Owner: Partner 3
6. Derivation of NS cell lines from human brain tumors	Process of derivation and highly validated cell lines	Biotech and Medicine	2009	PCT/GB2005/002289.	Owner: Partner 5
7. Derivation of oligodendrocytes from human NSCs	Process of derivation and defined cell populations	Biotech and Medicine	2011	Under patenting	Owner: Partner 7

A. Exploitable Knowledge (description)	B. Exploitable product(s) or measure(s)	Sector(s) of application	C. Timetable for commercial use	D & E. Patents or other IPR protection	Owner& Other Partner/s involved
8. Derivation of neurons from mouse NS cells	Process of derivation and defined cell populations	Biotech	N/A	N/A	Owner: Partner 6
9. Pharmacology of ion channels and receptors expressed in human pluripotent stem cell-derived neurons.	Pre-requisite for drug screening application on human pluripotent stem cell-derived neurons	Biotech &Pharma	2010	none	Owner: Partner 7
10. Screening of drugs using human pluripotent stem cell-derived neurons cultured on MEAs	Pre-requisite for drug screening application with human pluripotent stem cell-derived neurons on MEAs	Biotech &Pharma	2010	none	Partner 7
11. Scalable process for culturing undifferentiated neural stem cells in suspension	Human neural stem cells	Biotech	2010	WO/2006/027229	Owner: Partner 4

A. Exploitable Knowledge (description)	B. Exploitable product(s) or measure(s)	Sector(s) of application	C. Timetable for commercial use	D & E. Patents or other IPR protection	Owner& Other Partner/s involved
12. Protocols for automated maintenance of neural cells	Human and mouse neural stem and progenitor cells, protocols & procedures for the automated production of cell products for screening	Biotech	2010	WO 2008/107695 PCT/CA2008/001741	Owner: Partner 1 Other partner involved: Partner 4
13. Protocols for automated scale-out of neural stem cells	Human and mouse neural stem and progenitor cells in 96 and 384-well plates; protocols & procedures for the automated production of cell products for screening	Biotech	2010	Trade secret	Owners: Partner 1 Partner 4
14. Frozen 96-well plates with neural stem cells	Ready-to-use 96-well plates for screening applications	Biotech	2010	Trade secret	Owners: Partner 1 Partner 4
15. Small molecule modulation of stem cells	Stem cell culture media additives	Biotech, Pharma and supporting industry	2012	Patents and trade secret	Owners: Partner 1 Partner 9
16. Small molecule modulation of stem	Novel therapies for glioblastoma	Biotech and Pharma	2014	Patents and trade secret	Owners: Partner 5 Partner 9

A. Exploitable Knowledge (description)	B. Exploitable product(s) or measure(s)	Sector(s) of application	C. Timetable for commercial use	D & E. Patents or other IPR protection	Owner& Other Partner/s involved
cells					
17. Small molecule modulation of stem cells	Novel therapies for neurodegenerative diseases	Biotech and Pharma	2014	Patents and trade secret	Owners: Partner 1 Partner 9

The following gives more information on each Exploitable Knowledge listed above:
Exploitable Knowledge Item 1: Joining the stem cell biology expertise of the academy (**Partners 5 and 6**) with the technology standards of the industry (**Partners 1 and 3**) has allowed the development of reagents and processes to generate safe and effective stem cell lines. This includes standardized animal component-free stem cell culture media that could be used both in academy and industry to safely work with neural stem cells. These products can be commercialized by **Partner 1** on an individual basis and can be suggested as preferred media to use together with the neural stem cells originated by the consortium.

Exploitable Knowledge Item 2: The development, by **Partner 3**, of new cryopreservation techniques for neural stem cells, generated by **Partner 5 and 6**, had the aim to provide researchers with high quality material, fundamental to all good scientific practice and for maximizing reproducibility. Furthermore, in order to be able to move forward clinical application, an alternative non-toxic cryoprotectant was successfully developed and tested by **Partner 3** to freeze several neural stem cell lines. This technique will have a strong impact in clinical practice since it could be used to generate ready-to-use, safe and effective stem cell lines for therapeutic application. Delays in commercialization could arise from certification issues in the use of these reagents in clinical practice and possible resistance to the use of new techniques.

Exploitable Knowledge Item 3: It is extremely important to perform regular quality controls on neural stem cells to assure their identity and control their release for the purpose of preventing any serious long term effects on the validity of research data performed with such lines. Biobanks are the only infrastructures which can actively work towards establishing procedures to collect, quality control, store and distribute cell lines. Thanks to the expertise acquired by **Partner 3** from **Partners 1, 5 and 6**, **Partner 3** became highly competent to handle delicate systems, as neural stem cells, and proved to be able to take its role as a centralized stem cell bank.

Exploitable Knowledge Item 4: **Partner 3**'s online database system stores all relevant information of all the cell lines deposited at **Partner 3** by other partners within the consortium and is accessible via secure website with specific username/password.

Without this proper visibility and diffusion, all information and reagent originated by the NEUROscreen project would be of limited use. The integration of a biorepository, such as at **Partner 3**, in the development of new strategies will help the sharing of valuable information and materials generated by this novel project. The link of **Partner 3**'s database with a centralized database such as STEMDB will help the dissemination of information and high quality material throughout the entire scientific community.

Exploitable Knowledge Item 5: The establishment of a set of procedures to check the quality of the neural stem cells can be used to provide researchers with valuable services routinely to control important parameters such as sterility, pluripotency, differentiation capabilities, expression profiling and karyotyping. Furthermore, cell line identification and authentication should routinely be applied to diminish misidentification. These are routine procedures that individual researcher cannot support. The use of a centralszed biorepository that has already developed the competence to perform these tasks (**Partner 3**) assures the validity and reproducibility of the research data generated.

Exploitable Knowledge Item 6: The establishment of robust protocols for oligodendrocyte differentiation of human neural stem cells developed in this research represents a significant advance in the field. This includes standardized procedures for generation of large numbers of oligodendrocytes, which could be used both in academia (for studying the molecular properties of this type of cell) and industry (screens for drugs). This know-how could have a strong impact in clinical practice as it could be used to generate ready-to-use, safe and effective stem cell lines for therapeutic application for demyelinating diseases and spinal cord injuries.

Exploitable Knowledge Item 7: Having established the know-how on the generation of adherent glioblastoma stem cell lines that recapitulate the tumour phenotype represents major progress that provides a significant resource for biomedical and biopharmaceutical research in the field of CNS cancer. Tumour stem cell lines generated in the context of NEUROscreen have been placed in a repository for distribution to the community (in collaboration with **Partner 3**). Significantly, the glioblastoma stem cell lines show evidence of varying differentiation, which may correspond to differences between tumours. The availability of the tumour stem cell lines may therefore facilitate disease stratification and targeted therapeutic screening.

Exploitable Knowledge Item 8: The establishment of robust protocols for high efficient neuronal differentiation of mouse neural stem cells developed in this research represents an important advance in the field. These standardized procedures allow for the generation of large numbers of neurons that can be used both in academia -- for studying the molecular and functional properties of neuronal cells -- and in industry, to screen for drugs with neuroprotective and/or regenerative actions.

Exploitable Knowledge Item 9: The data generated by **Partner 7** can be used for screening sodium or potassium channel blockers on human-embryonic-stem-cell-derived neurons. In addition, the finding that these neurons express GABA and glutamate receptors can be further exploited to identify appropriate modulators of these receptors.

There is significant commercialisation potential for the assays, especially in the pharmaceutical and biotech industries.

Exploitable Knowledge Item 10: Since MEAs allow the detection of extracellular potentials from a large number of neurons at the same time, this technique is useful for drug screening applications. **Partner 7** has established a protocol for plating and maintaining human embryonic stem cell-derived neural stem cell (lt-hESNSCs) -derived neurons on multielectrode arrays (MEAs). This technique can be further extended to develop a co-culture system where glutamatergic and GABAergic neurons are cultured on the same MEA to test enhancement or inhibition of the neural network with appropriate drugs. The implemented single-neuron-electroporation system can be used for further refining modulation of this system, *e.g.,* for tracing synapse formation. The results obtained can be further explored to provide a set of functional assays that are designed to extract information about intrinsic physiological parameters such as ion channels and transmitter receptors expressed by differentiating neuronal precursors and their ability to interact with CNS tissue. Such assays hold a potential broad application scope within both academic research and industrial pharmacology. In collaboration with academic partners, which provide distinct neuronal populations, the expression and function of different neurotransmitter receptors can be exploited. In collaboration with the biopharmaceutical industry such electrophysiological measurements of transmitter responses can be used to identify neuroactive molecules and downstream effector systems in drug screening approaches. At present, there are no immediate commercial applications or any given prospects on the patentability. However, established assays hold a great and promising potential to be commercialized. In particular, these assays and findings are of a high interest to the pharmaceutical and biotech industry.

Exploitable Knowledge Item 11: **Partner 4** demonstrated that human ES cell-derived neural stem cells (lt-hESNSCs) are applicable to microcarrier-based suspension cultures. They evaluated the BioLevitator™ system, a benchtop cell culture device that supports the culture of cells on microcarriers (GEM™), as a scalable suspension cell culture system. (The BioLevitator™ and GEM™ technology are proprietary to Hamilton Life Science Robotics and Global Cell Solutions Inc.) Enabling undifferentiated cell growth and high cell yields in small media volumes, the BioLevitator™ system is particularly suited for the expansion of lt-hESNSCs. This technology largely facilitates parallel scale-up of such stem cells for further downstream applications and thus was implemented by **Partner 4** as an enabling technology for the cell production. Part of the work is covered by an existing patent application of **Partner 4** and commercialization of the processed neural stem cell lines is covered by access to third party IP. For the development and validation of conventional therapeutics such as small molecules and recombinant proteins the biopharmaceutical industry widely utilises cell-based assays. Thus scalable technologies are a major pre-requisite for the production of homogeneous cell populations for any commercial use.

Exploitable Knowledge Item 12: Long-term automated culture, as shown by and scale-up of cell supply for mouse and human neural stem and progenitor cells, facilitate the generation of sufficient numbers and high quality cells for drug screening and clinical

applications. The use of robotic cell culture platforms has been demonstrated by **Partner 1** and **Partner 4** to be applicable to numerous types of adherent neural stem cell lines and is well suited for the production of cells in small-well-format for drug discovery applications, where the whole process from scale-up, plating out of cells and screening can be automated. Protocols for automated cultivation of stem cells are covered by a patent application of **Partner 1**. With future development this automated cascade can be even further extended to automated derivation (**Partner 1**) and purification (**Partner 4**) for neural stem and progenitor cells. Credible automation and scale-up with guarded fidelity will be a key factor in lowering the threshold for the pharmaceutical industry of embracing neural stem cell platform technology for large scale drug discovery with the purpose of finding effective medications for the treatment of neurological disorders ranging from neurodegenerative diseases, over acute injury and mood disorders to nervous system cancers. The provision of proven human neural stem cell lines and of validated procedures and protocols for their bioprocessing will therefore present a significant opportunity for biotechnology companies to prosper in partnership with pharmaceutical industry clients and will advance the field of regenerative medicine.

Exploitable Knowledge Item 13: Successful automated scale-out achieved by **Partner 1** and **Partner 4** of a range of mouse and human neural stem cell populations, either in the undifferentiated state or in a partially differentiated state, provides high throughput capability for screening applications. While the pharmaceutical industry has remained cautious in terms of the degree to which it will invest in stem cells as a biological source of screening material; perceptions are changing and the provision of robust supplies of stem cells for screening is key to the continuing derailment of those perceptions. One possible model for commercialisation is a technology transfer package where a pharmaceutical industry partner would license a neural stem cell automated culture platform technology in order to furnish cell supply for internal drug discovery programmes. Methods and protocols utilizing either of the two automated cell culture platforms have commercial potential. Small molecules identified through human neural stem cell-based screens have the potential to be used as research and development reagents or ultimately as novel *ex vivo* or *in vivo* therapies either in conjunction with cell therapies or as standalone drugs. In addition, now that neural stem cells can be efficiently and robustly scaled out into small well formats, numerous assays can be developed to investigate activities of chemical compounds in specific neural stem cell lines or systems. Such assays are of commercial value to other companies working in the field of regenerative medicine and collaboration or licensing of these assays to interested parties is also a commercial possibility.

Exploitable Knowledge Item 14: **Partner 4** established efficient cryopreservation protocols for automated freezing and thawing procedures of adherent neural stem cells with low batch-to-batch variations. In addition, **Partner 1** evaluated procedures for the cryopreservation of partially differentiated stem cells employing the CryoStorTM CS10 freezing medium supplied by **Partner 3**. The developed automated procedures for handling, dispensing and freezing of adherent human neural stem cells in 96-well microplates enable large batch production and long-term storage for further downstream applications on demand and facilitate rapid and reliable quality control, reproducibility,

efficacy and high throughput. The availability of cells at all times is a critical bottleneck in drug screening applications. Separating the production of cells from the actual screening process helps to avoid this bottleneck and increases the flexibility of screening schedule. Therefore, frozen cells that can be assayed without prior cultivation have become a valid and frequently used alternative to cells from a continuous growing culture. In particular, for secondary screening, which demands only a few assay plates per day over a longer period of time, it would be beneficial to provide frozen cells directly in assay ready plates. This would provide a basis for industrial use of human neural stem cells in screening applications.

General IP Considerations for next three Knowledge Items: Any IP generated by the project or future collaborations will be protected through either patents or trade secrets. The vast majority of the project outputs will be protected through chemical use patents covering use of the compounds against their intended therapeutic target or their use as tools. Any novel screening techniques and protocols developed during the course of the project will be most likely be protected as a trade secret since it is not particularly cost effective to patent screening techniques and protocols since the vast majority of a programme's value lies in the compounds and their utility. However, any screening technologies developed during the project that could have major commercial value will be assessed for protection under patent on a case-by-case basis. IP ownership and exploitation associated with any collaborations derived from the work carried out in NEUROscreen will be agreed prior to initiation of any work.

Exploitable Knowledge Item 15: A key challenge in stem cell research is the maintenance and modulation of stem cell populations and this is currently achieved through the use of complex and expensive cocktails of reagents. Work in this project has demonstrated that small molecules should be able to substitute many of the components of these cocktails, thus significantly reducing the associated cost of goods and providing a cheap, versatile and tuneable tool kit of reagents for researchers. This is an area of stem cell research and regenerative medicine that is rapidly expanding with numerous companies increasing their activities in this area. In 2007, the market for supporting technologies in stem cell research was estimated at $5bill and is expected to double by 2012. Therefore, this is a rapidly growing opportunity for outputs from the project in the short and medium term. Members of the NeuroScreen consortium are currently in discussions regarding further development and exploitation of this technology.

Exploitable Knowledge Item 16: The screen against the glioblastoma cell line has identified compounds as potential start points for the development of medicines for primary brain tumours. This form of cancer has limited therapy options with poor efficacy and, as such, there is a significant unmet medical need. Although proof of principle for the screens and approach has been established, significant work is required to develop these initial hit compounds. A formal drug discovery programme will need to be initiated to optimise the compound for both *in vitro* potency and *in vivo* activity and this can expect to take between two to four years. In addition to optimising for potency, significant hurdles, such as blood brain barrier penetration will also need to be overcome. After this period of medicinal chemistry, a candidate compound for formal preclinical

workup will have been identified and an IND/CTA filing can be expected after a further 12 months. Once the IND/CTA stage has been reached it is expected that a commercial partner for the programme will be sought. This would likely be a large pharmaceutical company more able to carry out the clinical development of the compound. It is expected that the programme would be out-licenced to under a standard deal structure (up-fronts, milestones and royalties). Following completion of the NEUROscreen project, members of the consortium are currently in discussions regarding further collaborations to develop these compounds further.

Exploitable Knowledge Item 17: There is a need to develop new medicines to treat neurodegenerative conditions such Alzheimer's and Parkinson's disease and regenerative medicine offers one of the most promising solutions to these debilitating conditions. As described above (Exploitable Knowledge Item 16), the compounds identified as part of the NEUROscreen consortium are only preliminary hits that can act as start points for a drug discovery programme. These compounds will need to undergo optimisation to identify candidate compounds followed by formal regulatory toxicology, manufacture, formulation etc. before IND/CTA filing. As detailed above, at this stage a partner will be sought for the programme to undertake the clinical trails and eventual marketing of any product. Members of the NEUROscreen consortium are currently in discussions regarding further development and exploitation of this technology.

Section 2 - Dissemination of knowledge

Partners have delivered 53 talks and presented thirteen posters to national and international academic, commercial or public audiences, which gave acknowledgement to NEUROscreen.

Overview table

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible/ involved
Aug-07	Press release	General public	UK, worldwide	large	1
Jan-08	Conference, invited talk	Industry (Biotech/Pharma)	International	100	1
Apr-08	Conference, invited talk	Industry (Biotech/Pharma)	US, worldwide	800	1
May-08	Conference	Research, Industry (Biotech/Pharma)	EU, worldwide	100	1
May-08	Conference	Research, Industry (Biotech/Pharma), General Public	Hungary, EU	100	1
Jun-08	Conference	Research, Industry (Biotech/Pharma)	Singapore, worldwide	500	1
Oct-08	Conference, invited talk	Research, Industry (Biotech/Pharma), UK Government	UK	75	1

Oct-08	Conference, talk	Research, Industry (Biotech/Pharma)	International	10	1
Jan-09	ESTools Winter Student School	Partners	EU	50	1
Feb-09	Conference, invited talk	Research, Industry (Biotech/Pharma)	International	40	1
Mar-09	Invited poster	Research, Industry (Biotech) and Funders (UK, Germany, EU);	UK, Germany, EU	65	1
Apr-09	Conference, invited talk	Research	International	150	1
Jun-10	Conference, poster	Research, Industry (Biotech/Pharma)	International	100's	1, 9
Aug-10	Conference, invited talk	Research, Industry (Biotech/Pharma)	International	150	1, 9
Jan-08	Conference, invited talk	Research	France, worldwide	100	2
Feb-08	Conference, invited talk	Research, Industry (Biotech/Pharma)	USA, worldwide	250	2
Apr-08	Conference	Industry (Biotech/Pharma)	Spain, worldwide	100	2
Nov-07	Conference	Research, Industry (Biotech/Pharma)	Germany, worldwide	300	4
Feb-08	Conference	Research, Industry (Biotech/Pharma)	France, worldwide	200	4
May-08	Conference	Research, Industry (Biotech/Pharma)	UK, worldwide	100	4
Mar-09	Conference, posters (x2)	Research, Industry (Biotech/Pharma)	International	100	4
May-09	Conference, talk	Research, Industry (Biotech/Pharma)	International	100	4
Jun-09	Conference, talk	Research, Industry (Biotech/Pharma)	International	100	4
Jul-09	Conference, posters (x3)	Research, Industry (Biotech/Pharma)	International	100's	4
Aug-10	Conference, invited talk	Research, Industry (Biotech/Pharma)	International	100's	4, 7
Oct-09	Conference, invited talk	Research, Industry (Biotech/Pharma)	International	100's	4, 7
Feb-10	Conference, invited talk	Research, Industry (Biotech/Pharma)	International	100	4, 7
Jun-10	Workshop, talk	Research	Germany	50	4, 7
Dec-08	Conference, talk	Research, Industry (Biotech/Pharma)	International	200	5
Apr-09	Conference,	Research, Industry	International	500	5

	invited talk	(Biotech/Pharma)			
May-09	Conference, invited talk	Research, Industry (Biotech/Pharma)	International	150	5
Jun-09	Conference, poster	Research, Industry (Biotech/Pharma)	UK	600	5
Jul-09	Conference, poster	Research, Industry (Biotech/Pharma)	International	150	5
Jul-09	Conference, invited talk	Research	International	80	5
Sep-09	Visit, poster	MRC (UK funders)	UK	10	5
Jan-10	Conference, invited talk	Research, Industry (Biotech/Pharma)	International	100	5
May-10	Conference, invited talk	Industry (Biotech/Pharma)	International	100	5
Apr-10	Poster	Research, Industry (Biotech/Pharma)	UK	100	5
Jun-10	Meeting, talk	Research	UK	30	5
Nov-10	Conference, invited talk	Research, Industry (Biotech/Pharma)	International	100	5
May-07	Conference, invited talk	Research	Italy, worldwide	300	6
Oct-07	Workshop, invited talk	Research	Italy, worldwide	120	6
Mar-08	Conference, invited talk	Research	France	100	6
Sep-08	Conference, talk	Research	International	250	6
Apr-09	Seminar	Research	Italy	100	6
Apr-09	Seminar	Research	Italy	200	6
May-09	Conference, talk	Research	International	100	6
Jul-09	Conference, talk	Research	International	150	6
Jul-09	Conference, posters (x2)	Research, Industry (Biotech/Pharma)	International	100's	6
May-10	Seminar	Research	Italy	50	6
Nov-09	Seminar	Research	Italy	150	6
Nov-09	Workshop, talk	Research	Italy	200	6
Oct-09	Seminar	Research	Italy	100	6
Oct-07	Conference	Research	Germany	500	7
Oct-07	Conference	Research, Industry (Biotech/Pharma)	Germany, worldwide	500	7
Oct-07	Conference	Research	Germany	500	7
Mar-08	Conference	Research	Germany	550	7
Jul-08	Conference	Research, Industry (Biotech/Pharma)	Germany, worldwide	600	7

Aug-08	Conference, talk	Research, Industry (Biotech/Pharma)	International	100's	7
Sep-08	Conference, talk	Research, Industry (Biotech/Pharma)	International	100's	7
Nov-08	Conference, talk	Research, Industry (Biotech/Pharma	International	100's	7
Jan-09	ESTools Winter Student School	Partners	EU	50	7
Mar-09	Conference, talk	Research	International	100	7
Jun-09	Conference, talk	Research, Industry (Biotech/Pharma)	International	100's	7
May 08 and ongoing	Support for EuroStemCell website	Research, Industry (Biotech/Pharma)	worldwide	large	1 [2-9]
Nov 07 and ongoing	NEUROscreen website	Partners	Germany, Italy, UK	Tens	1 [2-9]

The following gives more details on the talks listed in the above table:

Partner 1

- *Stem cell technology: from resource to platforms*, First International Symposium on Human Embryonic Stem Cell Research, Evry-Paris, January 31 – February 2, 2008
- *'Stem cells: From Assay to Screen'* Annual meeting of the Society for Biomolecular Sciences, St. Louis, USA, April 2008
- *Stem Cell Technology: From Resource to Platforms ,* EuroSciCon 02 May 2008
- *Platforms For Biomedical Discovery using Human ES Cells,* ESTOOLS public conference, Hungary, May 2008
- *Scalable Stem Cell Technologies for Pathway & Drug Discovery,* 4th Annual Stem Cell Asia Stem Cell Research & Application 26-27 June 2008
- BRIC meeting on Bioprocessing Advanced Therapeutics,government audience, Warwick, UK, October 2008
- International meeting on Stem Cell Commercialization, London, UK, October 2008
- ESTOOLS Winter Student School, Jan09, Saarielska, Finland, January 2009
- Stem Cells for Regenerative Medicine: Scalable Platform Technologies, SMi Conference, London, England, 16-17 February 2009
- UK-German Regenerative Medicine Workshop and Poster Exhibition, British Embassy Berlin, Germany, 2-3 March 2009
- Symposium on Molecular and Functional Properties of Human Stem Cell-derived Neurons *in vitro*, 20th British Neuroscience Association Annual Meeting, Liverpool, England, 19-22 April 2009
- ISSCR 8th Annual Meeting, San Francisco, USA, June 2010
- Stem Cells Europe, Edinburgh, UK, August 2010

Partner 2

- First International Symposium on Human Embryonic Stem Cell research, Paris, January 31 - February 2 2008
- CHDI, 3rd Annual Huntington Disease Therapeutics Conference, Palm Springs, CA, USA, February 4-7 2008
- BioEurope Spring 2008, Madrid, Spain, April 7-9 2008

Partner 4

- *Human ES cell-derived neural stem cells*, Functional Genomics with Embryonic Stem Cells, EMBL Heidelberg, November 24-26, 2007
- *Stable neural stem cells from human ES cells*, First International Symposium on Human Embryonic Stem Cell Research, Evry-Paris, January 31 – February 2, 2008
- 5th Stem Cell Conference, KNW, Aachen, Germany, March 24-25, 2009 (x2)
- *From pluripotent stem cells to purified human neurons: Biological and technological requirements*, European Stem Cells & Regenerative Medicine Congress 2008, 13 - 15 May 2008, Royal Horseguards Hotel, London, UK
- From Pluripotent to Somatic Stem Cells - Biological and Biotechnological Challenges iStem, Paris-Every, France, May 14, 2009
- From Pluripotent to Somatic Stem Cells - Biological and Biotechnological Challenges iStem, Paris-Every, France, June 11, 2009
- ISSCR Meeting, Barcelona, Spain, 7-11 July 2009 (x3)

Partners 4 & 7

- 7th Seoul Stem Cell Symposium, TERMIS 2nd World Congress, Seoul, Korea, August, 2009
- World Conference on Regenerative Medicine, Congress Center Leipzig, Germany, October 2009
- DZNE/UKB Basic Neuroscience Initiative, German Center for Neurodegenerative Diseases, Bonn, Germany, February 2010
- Max-Planck Society Workshop 'Disease-specific iPS cells', Ringberg Castle, Tegernsee, Germany, June 2010

Partner 5

- Brain Tumour 2008, Berlin, Germany, December, 2008
- UK National Stem Cell Network Meeting, Oxford, UK, April 2009
- Terrapinn World Stem Cells and regenerative medicine congress 2009, London, England, May 2009
- ERBI/LTN 11th BioPartnering Conference, Cambridge, UK, June 2009
- Models and Mechanisms of Cancer symposium, Cambridge, UK, July 2009
- EPFL Lausanne, Lausanne, Switzerland, July 2009
- MRC (UK funding agency) visit to Partner 5, Cambridge, UK, 2 September 2008
- Revolutionizing drug discovery with Stem Cell Technology, Stevenage, UK, January 2010
- Terrapinn World Stem Cells and Regenerative Medicine Congress, London, UK, May 2010

- LTN Biomarker discovery and validation, London, UK, April 2010
- Internal neuroncology meeting UCL, London, UK, June 2010
- Stem Cells in Drug Discovery and Development, San Diego, USA, November 2010

Partner 6

- Invited Speaker at III Meeting on the Molecular Mechanisms of Neurodegeneration. Milano, Italy, May 2008
- Invited Speaker at Meeting of the French Society of Neurosciences, Brain Diseases and Molecular Machines: Spotlights from Evolution, Development and Network biology. Paris, France, March 2008
- Invited Speaker at FEBS Workshop – Generating Neural Diversity in the Brain. Capri, Italy, October 2007
- 21st ECNP Congress, Barcelona, Spain, September 2008
- Seminar at the Molecular Biotechnology Center, University of Torino, Turin, Italy, April 2009
- Seminar at the Pharmacological Department, University of Padova, Italy, April 2009
- Symposium on Neurogenesis and Neural Differentiation of Stem Cells, Verona, Italy, May 2009
- Symposium on Molecular basis of nervous system differentiation and related pathologies, Rome, Italy, July 2009
- ISSCR Meeting, Barcelona, Spain, 7-11 July 2009 (x2)
- Seminar at the Centre for Integrative Biology, University of Trento, Italy, May 2010
- Seminar at the Pharmacological Department, University of Padova, Italy, November 2009
- Workshop on Neurodegenerative Diseases: from biology to clinic, National Institute of Biostructures and Biosystems, Rome, Italy, November 2009
- Seminar at the Centre of Cell Oncology and Ultrastructure, National Institute for Cancer Research, Genova, Italy, October 2009

Partner 7

- *From ES cells to neural stem cells to functional neurons*, SFB 497 Symposium 'Signalling Pathways in Cellular Differentiation', Ulm University, Ulm, Oct 11-13, 2007
- *Human ES cell-derived neural stem cells*, 3rd World Congress of Regenerative Medicine (Opening Lecture), Leipzig, October 17, 2007
- *Generation of stem cell-derived oligodendrocytes*, Retreat of the Institute for Multiple Sclerosis Research Göttingen and the Cambridge Centre for Myelin Repair, Göttingen, October 18-19, 2007
- *Fate restriction and plasticity of in vitro generated neural stem cells*, 31st Annual Meeting of the German Society for Cell Biology, Marburg, March 12-15, 2008
- *Generation of a primitive long-term self-renewing human ES cell-derived neural stem cell with potential for in vitro instruction and synaptic integration*, 2nd

International Congress on Stem Cells and Tissue Formation, Dresden, July 6-9, 2008

- Royan International Twin Congress (9th International Congress on Reproductive Biomedicine and 4th International Congress on Stem Cell Biology & Technology), Teheran, Iran, August 27-29, 2008
- 10th International Conference on Neural Transplantation and Repair, Freiburg, Germany, September 10-13, 2008
- International Titisee Conference on Differentiation, Reprogramming and Regeneration, Boehringer Ingelheim Fonds, Titisee, Germany, November 5-9, 2008
- ESTOOLS Winter Student School, Jan09, Saarielska, Finland, January 2009
- Cellules souches pluripotentes humaines, Atelier de formation #192, INSERM Saint-Raphael, France, March 8-10, 2009
- 3rd International Stem Cell Meeting; Stem Cells: Biology and Clinical Applications, The 8th National Life Science & Technology Week, ILSI BIOMED 2009, Tel Aviv, Israel, June 15-17, 2009

Section 3 - Publishable results

In total, members of the Consortium have published 14 scientific papers, and have submitted an additional four for publication, which legitimately relate to the NEUROscreen project output:

1. Garavaglia, A et al. *Adaptation of NS cells growth and differentiation to high-throughput screening-compatible plates* BMC Neuroscience 2010, 11:7

2. Diaferia, G et al. “Is stem cell chromosome stability affected by cryopreservation conditions? Cytotechnology. 2008 Sep;58(1):11-6

3. DeBlasio, P et al. “BioBanking for research on Alzheimer's disease: a translational infrastructure useful in the analysis of genes, transcripts and proteins”. Chapter 7 from A Multidisciplinary Approach to Dissect the Alzheimer’s Pathology, 2008 (in Press).

4. Riegman, P et al. “Biobanking for better Healthcare” Review. Molecular Oncology, 2008 (in Press).

5. Goffredo, D et al. “Setting the conditions for efficient, robust and reproducible generation of functionally active neurons from adult subventricular zone-derived neural stem cells”. Cell Death Differ. 2008 Dec;15(12):1847-56

6. Pollard, S et al. *Glioma Stem Cell Lines Expanded in Adherent Culture Have Tumor-Specific Phenotypes and Are Suitable for Chemical and Genetic Screens*. Cell Stem Cell 2009 Jun 5;4, 568–580

7. Spiliotopulos, D et al. *An optimized experimental strategy for efficient conversion of Embryonic Stem (ES)-derived mouse Neural Stem (NS) cells into a nearly homogeneous mature neuronal population* Neurobiology of Disease 2009 34 (2), 320-331.

8. Danovi, D. et al. *Live imaging-based chemical screens using normal and glioma-derived neural stem cells.* Biochemical Society Transactions. 2010 Aug; 38(4): 1067-71.

9. Danovi, D. and Wiskow, O. *Revolutionizing drug discovery with stem cell technology*, 18-19 January 2010, GlaxoSmithKline, Stevenage, UK 2009, Thomson Reuters (Scientific) Ltd

10. Terstegge, S et al. (2010) *Laser-assisted photoablation of human pluripotent stem cells from differentiating cultures*. Stem Cell Rev. 6(2):260-9

11. Terstegge, S et al. (2009) *Laser-assisted selection and passaging of human pluripotent stem cell colonies*. J Biotechnol 143(3):224-230

12. Terstegge, S and Brüstle, O. (2009) *Automated method for embryonic stem cell culture* In: *Emerging technology platforms for stem cells* (Lakshmipathy, U., Chesnut, J, Thyagarajan, B, eds.) Wiley, New Jersey, pp. 275-281

13. Conti, L and Cattaneo, E. *Neural stem cell systems: physyological players or in vitro entities?* Nat Rev Neurosci. 2010 Mar;11(3):176-87

14. Gorba, T et al. *Prospects for neural stem cell therapy of Alzheimer's Disease* In: *Stem Cells & Regenerative Medicine: From Molecular Embryology to Tissue Engineering* (Appasani, K and Appasani, R eds.) Springer, ISBN: 978-160761-859-1, October 2010

15. Fulton, N et al. *Derivation of adherent, non-immortalized neural stem cells from different regions of the human fetal human brain as automated and scalable platform for cellular assays*. Neurochemistry International; Special Issue: Stem Cells for 21st Century Neuroscience. Invited article, Draft manuscript, (Sep 2010)

16. Diaferia, G et al. *Systematic chromosomal analysis of cultured mouse neural stem cell lines.* Stem Cells and Development. Submitted (Aug 2010)

17. Marco Onorati, M et al. *Preservation of positional identity in fetal-derived Neural Stem (NS) cells from different mouse central nervous system compartments.* Submitted Revised.

18. Paina, S et al. *Wnt5a is a transcriptional target of Dlx homeogenes and promotes differentiation of interneuron progenitors in vitro and in vivo.* Submitted Revised.

Support for patents

1. Neural Tumor Stem Cells and Methods of Use Thereof. Inventors: Peter Dirks, Austin Smith, Ian Clarke, Steven Pollard, (some now **Partner 5**) Owners: University of Edinburgh, Hospital for Sick Children (Toronto) PCT/CA2008/001741
2. Neural Stem Cells. Inventors: Luciano Conti, Steven Pollard, Austin Smith (some now **Partners 5** and **6**). Owners: University of Edinburgh (licensed to **Partner 1**). PCT/GB2005/002289
3. Automated Culture of Stem Cells. Inventors: Hazel Thomson, Julie Kerby (Partner 1) Owners: **Partner 1**. PCT/GB2008/000813
4. Scalable process for culturing undifferentiated stem cells in suspension. Inventors: Stefanie Terstegge and Oliver Brüstle (**Partners 4** and **7**). Owners: Partner 7. WO/2006/027229

Patents under preparation

1. Defined protocols for oligodendrocyte differentiation from human neural stem cells (**Partner 5**)

Publicisable Exploitable Results

SD1

The main achievement of this project is the transfer of knowledge from science base to application. The work here described moves strongly from the traditional academic focus on understanding stem cells to a comprehensive commercial focus. The close collaboration between industry and academy, the standardization of all processes, the use of efficient tracking systems and the study of the administrative/legal aspects of stem cell line distribution, created a successful workflow to be applied to the management of a stem cell bank and to the distribution of highly validated cells. The integration of a biorepository such as Partner 1's in the development of new strategies, which have a strong potential in regenerative medicine, helped the sharing of valuable information and materials generated by this novel project and it will place Europe at the forefront of a commercial stem cell application.

=== REPORT ENDS ===