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Research Shows Potential of Stem Cells in Treating Brain Diseases

Neural stem cells reduce symptoms of Parkinson's, help recovery after stroke in animal models

WASHINGTON, DC — Scientists today reported significant advances in the search for stem cell-based treatments for degenerative brain disorders, such as Huntington's and Parkinson's diseases. Other studies used stem cells to promote brain repair after a stroke and to understand how Alzheimer's and Parkinson's diseases spread across the brain, providing insight into new potential treatments. The findings were presented at Neuroscience 2014, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

Neurodegenerative disorders have a large and increasing impact on today's society. Alzheimer's disease affects 44.3 million people worldwide and is expected to increase to 115.4 million people by 2050. An estimated 7 million to 10 million people around the globe are living with Parkinson's disease. And while it's difficult to determine the number of people with Huntington's disease worldwide, in Western countries, it is estimated to affect about five to seven people per 100,000.

Today's new findings show that:

- Stem cells made from patients have been used to identify the mechanism through which Alzheimer's and Parkinson's diseases can spread across the brain, shedding light on a potential treatment approach (Martin Hallbeck, PhD, MD, 133.22, see attached summary).
- Neural stem cells derived from unfertilized human eggs reduce symptoms of Parkinson's disease when they are transplanted into the brains of animal models (Ruslan Semechkin, PhD, 137.01, see attached summary).
- Scientists have been able to reduce the presence of a defective protein responsible for Huntington's disease, without affecting the levels of its normal protein counterpart, according to studies in stem cells derived from Huntington's disease patients (Philip Gregory, PhD, 769.12, see attached summary).
- Stem cells can better promote repair of brain tissue after a stroke when they are injected into the damaged area as part of a precise mix of supporting cells (S. Thomas Carmichael, MD, PhD, 771.05, see attached summary).

Another recent finding discussed shows that:

- Neural stem cells unique to the motor system transplanted into a mouse model of Machado-Joseph disease alleviate its motor deficits and limit its destruction of cells in the brain's cerebellum, the area responsible for regulating muscle movement (Luis Pereira de Almeida, PhD, 770.02, see attached summary).

"Researchers are using stem cells both as a tool to better understand diseases that attack the brain and as a potential therapy for such diseases," said moderator Jane Roskams, PhD, of the University of British Columbia, Canada, an expert in the regeneration of damaged cells of the nervous system. "The work presented here today builds on the belief that science can someday end the devastation caused by neurodegenerative diseases."

This research was supported by funding from a variety of private and philanthropic organizations. Find out more about stem cell research at *BrainFacts.org*.

Related Neuroscience 2014 Presentation:
Presidential Special Lecture: Stem Cells in the Brain: Glial Identity and Niches
Tuesday, Nov. 18, 5:15–6:25 p.m., Hall D, WCC

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Abstract 133.22 Summary

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Stem Cell Culture System Helps Show How Alzheimer's and Parkinson's Diseases Spread Across the Brain

Findings may point to new direction for treatment research

Working with stem cells in the laboratory, scientists are discovering how Alzheimer's and Parkinson's diseases are spread from neuron to neuron across the brain, prompting widespread destruction of brain cells. The findings were presented today at Neuroscience 2014, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

"Toxic proteins accumulate and are packaged within one neuron, and that package is released into the gap between neurons where it can be taken up by another neuron," explained lead author Martin Hallbeck, PhD, of Linkoping University in Sweden. "We can now use this new knowledge, together with new model systems, to search for drugs that can block the progression of neurodegenerative diseases."

Today, only symptomatic treatments are available for Alzheimer's and Parkinson's diseases. In both diseases, research indicates that bundles of toxic proteins — called oligomers — damage cells and act as seeds for the formation of additional bundles, spreading the damage across the brain and causing progressive degeneration of brain function. Recent studies suggest that oligomers are transferred from one neuron to the next, but it was unclear how this transfer takes place.

To solve this puzzle, Hallbeck and his colleagues used stem cells obtained from healthy adult human cells. They induced these cells to develop into neurons that could be grown and studied in the laboratory. The researchers then fluorescently tagged the two oligomer types involved in Alzheimer's and Parkinson's diseases. Tracking the fluorescent oligomers in the cell culture, they discovered that the oligomers are packaged in tiny vesicles, called exosomes, which are released into the gap between neurons. Once there, the exosome and its toxic protein passenger are taken up by another neuron, spreading disease.

The researchers also found that transfer between neurons could be prevented by using a small molecule that inhibits the uptake process — a finding the points to a potential new class of drugs that could be developed to block the continuous cognitive deterioration of neurodegenerative diseases.

Research was supported with funds from The Swedish Alzheimer's Foundation, The Swedish Research Council, Swedish Brain Power, The Swedish Dementia Foundation, and the Hans-Gabriel and Alice Trolle-Wachtmeister Foundation for Medical Research.

Scientific Presentation: Sunday, Nov. 16, 9–10 a.m., Halls A-C

133.22, Mechanisms for neuron-to-neuron transmission of neurodegenerative proteins
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TECHNICAL ABSTRACT: Neurodegenerative (ND) disorders such as Alzheimer's disease and Parkinson's disease have a large and increasing impact on today's society. There is an emerging understanding of intracellular oligomeric protein aggregates as drivers of these diseases. Synaptic transfer of such aggregates from neuron-to-neuron in a prion like fashion have been shown and could, together with seeding capabilities, explain the progression of these diseases between anatomically associated regions. However, the cellular mechanisms of this spread have not been known, nor whether it is possible to interfere with the transfer. We have developed a neuronal 3D co-culture cell model to study the synaptic transfer of fluorescently labeled ND proteins (Nath et al. J. Neurosci 2012). Using this model we have now studied the cellular mechanisms of neuron-to-neuron transfer of fluorescently tagged oligomeric beta-amyloid (oAB) and oligomeric alpha-synuclein (oaSyn). We show that, in addition to neuronally differentiated human SH-SY5Y cells, neuronally differentiated human induced

pluripotent stem cells (iPSC) also can be used to study transfer in our cell model. Both oAB and oaSyn were shown to transfer between synaptically connected cells. Confocal microscopy of cells with fluorescent labeled oAB or oaSyn oligomers and co-labelled with immunocytochemistry for exosomal proteins (e.g. Flotilin-1) showed that both types of aggregates were present in intracellular exosomes. Exosomes containing the fluorescent labeled oligomers were isolated from concentrated media and fed to new cells, which internalized the oligomers. This cellular uptake of oligomer containing exosomes could be inhibited using Dynasore (Sigma-Aldrich), an inhibitor of dynamin dependent endocytosis. In conclusion this study shows the feasibility of neuronal differentiated iPSC's as a model for neuron-to-neuron transmission of oligomers implicated in neurodegeneration; that oligomers of neurodegenerative proteins are secreted at synapses in exosomes; and that ND oligomers can be transferred from neuron-to-neuron in a dynamin dependent way via exosomes. These results have important implications for understanding the propagation of neurodegenerative diseases.

Abstract 137.01 Summary

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Neural Stem Cells Reduce Symptoms of Parkinson's Disease in Animal Studies

Implanting stem cells derived from unfertilized human eggs appears safe, effective in animal models

The transplantation of neural stem cells derived from unfertilized human eggs into the brains of animal models of Parkinson's disease has been shown to preserve dopamine levels and cells and reduce disease symptoms six months after cell transplantation, according to findings presented today at Neuroscience 2014, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

"Cell-based therapies hold great promise in the treatment of Parkinson's disease, for which there is currently no cure," said lead author Ruslan Semechkin of the International Stem Cell Corp. in Carlsbad, Calif. "We believe these human neural stem cells provide a valuable new approach, and the results presented here bring the development of a disease-modifying treatment for Parkinson's a step closer to reality."

An estimated 7 million to 10 million people worldwide are living with Parkinson's disease, marked by symptoms including tremors, muscular rigidity, and slow, imprecise movement. Parkinson's lowers levels of the neurotransmitter dopamine and causes the degeneration of a group of structures at the base of the brain involved with the coordination of movement. Current treatments manage the symptoms, but they do not stop disease progression and many produce unwanted side effects.

The researchers reported results from three preclinical studies evaluating the safety and efficacy of implanting neural stem cells derived from unfertilized human eggs in both nonhuman primates and rodents. Neural stem cells have the ability to differentiate into all the neural cells (neurons, astrocytes, and oligodendrocytes) that constitute the nervous system. The researchers worked with a type of stem cells called human parthenogenetic neural stem cells, which, unlike embryonic stem cells, do not require the destruction of embryos. These cells can be expanded indefinitely in the laboratory, providing the large cell numbers needed to treat Parkinson's patients.

Results from one of the nonhuman primate studies showed that six months after cell transplantation, Parkinson's symptoms were reduced and many healthy behaviors had returned to close to normal levels. Rodent studies indicated that the stem cells are safe, even at very high doses, with no signs of abnormal tissue, tumors, or stem cells spreading to other parts of the animal. In addition to behavioral improvements, higher levels of dopamine were detected in the treatment groups when compared with the control groups that did not receive transplanted cells.

These results will form part of an Investigational New Drug Application with the Food and Drug Administration supporting the use of these cells for the treatment of Parkinson's disease.

Research was supported with funds from the International Stem Cell Corp.

Scientific Presentation: Sunday, Nov. 16, 8–9 a.m., Halls A-C

137.01, Preclinical development of a neural stem cell based therapy for Parkinson's disease
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TECHNICAL ABSTRACT: Cell-based therapies hold great promise in the treatment of Parkinson's disease (PD). Long-term symptomatic relief has been observed in some PD patients after implantation of fetal neural grafts, but their source is limited and ethically controversial. Human parthenogenetic stem cells (hpSCs) offer a more practical alternative because they can be expanded indefinitely *in vitro* and are derived from unfertilized eggs, avoiding the destruction of a

potentially viable human embryo. We have previously reported that intracranial administration of hpSC-derived neural stem cells (hpNSCs) promote host repair of the nigrostriatal system and ameliorate symptoms in preclinical models of PD. We observed higher dopamine levels in the striatum and higher number of TH positive dopaminergic neurons in the substantia nigra of animals transplanted with hpNSCs, without signs of ectopic tissue, tumors, or biodistribution to other organs. Here we present IND-enabling data of three preclinical studies testing the safety and therapeutic potential of hpNSCs. Master and working cell banks of hpNSCs were manufactured under cGMP conditions and used in a pharmacology and toxicology study in non-human primates (NHP); a dose escalating efficacy study in PD rats; and a tumorigenicity and biodistribution study in athymic nude rats. In the NHP study, Parkinson scores and healthy behavior of MPTP-lesioned African green monkeys with moderate to severe Parkinsonism were recorded before and every month after transplantation. Interim results of this study show a reduction of Parkinson scores and an increase in healthy behavior six months after cell transplantation. Bilateral administration of hpNSCs into the caudate nucleus, putamen and substantia nigra appears to be safe with no adverse events such as dyskinesia or dystonia. We report interim results from the rodent studies that indicate hpNSCs are safe and effective in treating PD and bring the development of this cell therapy closer to the clinic.

Abstract 769.12 Summary

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Novel Therapeutic Approach Improves Huntington's Disease Symptoms in Animal Model

Stem cell culture used to investigate potential new treatment; findings point to new class of drugs

Working with both stem cell cultures and animal models, researchers have succeeded in reducing the production of a defective protein responsible for Huntington's disease, and they did so while leaving levels of the protein's normal counterpart unchanged — a critical goal in the search for effective treatment for this progressive, fatal disease. The findings were presented today at Neuroscience 2014, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

Huntington's disease is a degenerative brain disorder that results from the ongoing destruction of nerve cells in the brain. Afflicted individuals lose their ability to walk, talk, think, and reason. Previously, research in animal models has shown that reducing the levels of the defective Htt protein can prevent, or even reverse, Huntington's disease. However, most methods used previously to lower Htt levels decreased both the protein's "good" and "bad" forms.

"Our approach hones in on the defective form of Htt, reducing disease symptoms and laying the groundwork for the development of a new class of drugs to treat Huntington's," said senior author Philip Gregory, PhD, senior vice president of research and chief scientific officer of Sangamo BioSciences Inc. in Richmond, Calif.

The study reports a novel therapeutic approach based on an engineered version of a class of natural proteins called zinc finger protein transcription factors, or ZFP TFs, that control the expression of specific genes. The researchers custom designed a ZFP TF to recognize the defective version of the gene that serves as a template for the dangerous form of the Htt protein. Because most cells in the human body produce Htt, the researchers were able to test the engineered ZFP TF in skin stem cells (fibroblast cells) derived from Huntington's patients. In these cell cultures, the ZFP TFs lowered the production of mutant Htt by more than 90 percent, while leaving levels of the normal protein unaffected. Importantly, mutant Htt was also reduced in neurons that originated from Huntington's patient cell cultures, and this reduction reversed several disease-related neuron defects, including their susceptibility to cell death. And in mouse models of Huntington's disease, ZFP TFs significantly reduced the production of mutant Htt, reversing disease-related motor defects.

Research was supported with funds from CHDI and Shire International GmbH, Sangamo's partner in the development of this therapeutic.

Scientific Presentation: Wednesday, Nov. 19, 1–4:15 p.m., Room 147B

769.12, Engineered zinc finger transcriptional repressors selectively inhibit mutant huntingtin expression and reverse disease phenotypes in Huntington's disease patient-derived neurons and in rodent models

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TECHNICAL ABSTRACT: Huntington's disease (HD) is a fatal autosomal dominant neurodegenerative disease caused by CAG-trinucleotide repeat expansion in exon 1 of the Huntingtin (Htt) gene. Repeat lengths of 35 or fewer CAGs are not linked to pathophysiology, whereas 40 or more CAGs invariably lead to HD, which most severely affects the basal ganglia and cerebral cortex and is characterized by a progressively worsening chorea, cognitive and psychiatric dysfunctions. Based on results from rodent studies that show that reduction of Htt levels lead to phenotypic improvement, multiple Htt-lowering strategies, such as RNAi and antisense oligonucleotides, are being pursued as potential therapies for HD. However, most of these methods either simultaneously down-regulate mutant Htt as well as wild type Htt, which may play critical roles in various cellular processes, or target mutant Htt based on single nucleotide polymorphisms (SNPs) that are only applicable to various subpopulations of patients. Engineered zinc-finger protein transcription factors (ZFP TFs) can be designed to bind virtually any DNA sequence and regulate gene expression. By designing ZFPs to specifically recognize the CAG expansion, we sought to develop a therapeutic

strategy that can selectively target the mutant Htt and be applied to the majority of the patient population. We showed that ZFPs can be engineered to minimally regulate the normal Htt alleles (CAG15-21) while driving approximately 90 percent repression of mutant Htt alleles (CAG40-69) in multiple fibroblasts lines derived from HD patients. Such allele-specific repression of mutant Htt can also be achieved in neurons differentiated from HD patient stem cells. Moreover, ZFP repressors of mutant Htt reversed multiple phenotypes exhibited by HD neurons, including reduced energetics and susceptibility to cell death upon growth factor withdrawal. To test their *in vivo* efficacy, ZFPs were delivered using adeno-associated virus (AAV) vectors to the striatum of HD model mice. In R6/2 mice, ZFPs led to significantly reduced levels of mutant Htt mRNA without affecting normal Htt levels; they also increased expression levels of medium spiny neuron markers, suggesting ZFP-mediated protection of those cells. Significant improvements in motor defects, such as clasping, were also observed in ZFP-treated R6/2 mice. In Q175 mice, ZFPs not only prevented mutant Htt aggregation when delivered at two months of age, but also led to significant clearance of existing Htt aggregates when delivered at six months of age. Together, these results support the further development of allele-specific ZFP repressors as a therapy for HD.

Abstract 771.05 Summary

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Bioengineered Cell Gel Enables Stem Cells to Boost Recovery After Stroke

Animal study shows improved survival and maturation of stem cells, integration into brain tissue

Researchers have created a unique mix of cells and supportive molecules that, when injected into the site of stroke in animal models, improves recovery and survival. The mixture forms a nurturing environment in which stem cells can develop into full-fledged nerve cells, according to findings presented today at Neuroscience 2014, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

"Stem cells can do more for stroke recovery when they are delivered within a supportive environment," said senior author Tom Carmichael of the University of California, Los Angeles. "Our approach promoted the survival of transplanted stem cells at the very site of the stroke, which enabled their development into mature brain cells and their integration with other brain tissue."

Stroke is the leading cause of adult disability, and there is no medical therapy that consistently promotes brain repair and recovery after stroke. Neural stem cells have moved into clinical trials as a potential remedy for stroke-related brain damage, but their effectiveness has been limited by poor survival of the transplanted cells.

To overcome this limitation, the researchers targeted the actual stroke cavity so that the stem cell transplant would not disrupt normal tissue, and they used a naturally occurring molecular matrix found in the brain, called hyaluronic acid, or HA. HA is important in brain development and is found close to areas of the brain containing stem cells. The researchers combined HA with a mix of growth factors that support stem cell survival and differentiation, as well as molecules that signal a normal brain environment. Stem cells were encapsulated in this matrix, which assembles itself into a gel once injected into the brain.

Researchers tested the HA gel in mice. One group of mice received the gel a week after an induced stroke. A second group received only stem cells after stroke. Survival was high in first group, and poor in the second. When engulfed in the HA gel, the stem cells not only survived in large numbers, but also differentiated into mature brain cells and gradually integrated into the host brain tissue. The HA gel promoted growth of new blood vessels into the graft, a critical requirement for rebuilding brain tissue. Furthermore, scientists report that the HA gel can be visualized on routine magnetic resonance imaging scanning, achieving an important goal of tracking a brain recovery over time.

Research was supported with funds from the California Institute of Regenerative Medicine.

Scientific Presentation: Wednesday, Nov. 19, 1–4:15 p.m., Room 152B

771.05, Biopolymer hydrogels promote neural precursor cell survival as a transplanted matrix in stroke
*****P. MOSHAYEDI[1], A. R. BERG[1], J. P. LAM[2], L. NIH[2], W. LOWRY[3], T. SEGURA[2], **S. T. CARMICHAEL[1]**; [1]Dept. of Neurol., [2]Dept. of Chem. and Biochem., [3]Dept. of Mol. Cell and Developmental Biol., Univ. of California Los Angeles, Los Angeles, CA

TECHNICAL ABSTRACT: Stroke is a leading cause of disability in the world. Neural stem or progenitor cell transplantation in stroke has been shown to induce repair and recovery. However, cell transplantation in stroke is limited by poor transplant survival. Here we have employed self-polymerizing hyaluronan (HA)-based gels to encapsulate neural precursor cells (NPCs) upon transplantation into a cortical model of brain infarction. Hydrogel parameters such as elasticity, cross-linking characteristics and incorporation of growth factors or motifs for proteins commonly found in precursor cell-extracellular matrix interactions are crucial in tissue reconstruction. Examining a range of elasticities revealed that injecting a hydrogel of 350 Pa, which is close in elasticity to that of the brain, will promote the best replacement of infarcted tissue but imposes no stress on surrounding brain, as measured by gliosis and inflammation. By using different crosslinkers, HA gels can be rendered sensitive or resistant to matrix metalloproteinases (MMPs), enzymes that are active in stroke and might interact with the HA matrix. This study revealed that MMP-sensitive HA gels promote angiogenesis by 9-folds; possibly because MMP-secreting endothelial cells are able to digest their way up in the gel matrix. We further enriched HA gels by incorporating motifs of extracellular matrix (ECM) proteins and growth factors. To extend these capabilities to cell transplantation approaches, human NPCs derived from induced-pluripotent stem (iPS) cells were encapsulated into HA gels with varying

concentrations of RGD, YIGSR and IKVAV motifs (from fibronectin and laminin molecules) as well as BMP4 and BDNF. Subsequently the optimized combination that promoted the highest cell survival *in vitro* was discovered. This finding was confirmed when the optimized gel induced the highest proliferation of encapsulated cells *in vivo*. In addition, the same combination of HA gel promoted the highest rate of neuronal differentiation in encapsulated cells, as well as angiogenesis and integration with astrocytic backbone of the host tissue. We then showed that encapsulation in optimized HA gel improves survival of cells upon transplantation into cortical infarcts of drug-induced immunosuppressed wild-type mice. These preliminary results show HA-based biopolymers are a viable method of NPC delivery to infarcted brain, and they can also promote cells' survival once they are supplemented with an optimized combination of growth factors and motifs of proteins present in the neural precursor cell niche. Future studies will now focus on discovering any advantage for HA-based hydrogels for the improvement of motor deficits after cortical stroke.

Speaker Summary (770.02)

Speaker: Luis Pereira de Almeida, PhD
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Transplantation of Neural Stem Cells Improves Motor Coordination, Neuropathology in Mouse Model of Machado-Joseph Disease

Nanosymposium: Repeat Diseases From SBMA to Motor Neuron Disease
Wednesday, Nov. 19, 1–3 p.m., Room 146C

Our research indicates that transplantation of neural stem cells made from the part of the brain most affected in Machado-Joseph disease alleviates motor and neuropathological impairments in its mouse model and therefore may provide a therapy for this inherited ataxia.

Machado-Joseph disease/spinocerebellar ataxia type 3 is a genetic neurodegenerative disease causing enormous suffering without effective treatment. The disorder is caused by over-repetition of the trinucleotide cytosine-adenine-guanine (CAG) that translates into an overlong polyglutamine tract within the protein ataxin-3, which becomes aggregation-prone and toxic. Machado-Joseph disease patients exhibit severe impairments in gait and coordination of voluntary movement, and in articulation and swallowing. These impairments are associated with multiple neuropathological changes including mutant ataxin-3 aggregation in the patient's brain. The condition is marked neuronal loss and atrophy of major neurons in the cerebellum, brainstem, and striatum.

Despite now having a greater understanding of its pathology, there is still no therapy able to modify the progression of the disease. Extensive neurodegeneration in symptomatic patients suggests an effective treatment of symptomatic Machado-Joseph disease patients may require cell replacement.

Neural stem cells (NSC) are self-renewing, multipotent cells with the ability to differentiate into all the neural cells (neurons, astrocytes, and oligodendrocytes) that constitute the nervous system. In this work, we investigated for the first time whether transplantation of nonmutant mouse cerebellar neural stem cells (cNSC) into the cerebellum of adult Machado-Joseph disease transgenic mice would alleviate motor coordination and neuropathological defects.

We found that upon transplantation into the cerebellum of adult Machado-Joseph disease mice, cNSC differentiated into neurons, astrocytes, and oligodendrocytes. Importantly, cNSC transplantation mediated a significant and robust alleviation of the motor behavior impairments in rotarod, foot-print and beam-walking tests as compared to control MJD mice injected with HBSS (2.1; 5.3; 1.7 and 2.9 fold improvement, respectively). This improvement correlated with a reduction of Machado-Joseph disease associated neuropathology, namely reduction of Purkinje cells loss, reduction of cellular layers shrinkage, and reduction of mutant ataxin-3 aggregates. Additionally, a significant reduction of cerebellar neuroinflammation markers and an increase of neurotrophic factors levels were observed, indicating that transplantation of cNSC also triggered important neuroprotective effects.

Thus, cNSC have the potential to be used as a cell replacement and neuroprotective approach for Machado-Joseph disease therapy. Our next step is to evaluate whether neural stem cells generated by induction of patient fibroblasts can mediate similar benefits, opening the way for transplantation of neural stem cells generated from a patient's own cells.

Research was supported with funds from the National Ataxia Foundation, the Richard Chin and Lily Lock Machado-Joseph Research Fund, the European Regional Development Fund, Competitive Factors Operational Program – COMPETE, and the Portuguese Foundation for Science and Technology.