Industry Update

Latest developments in stem cell research and regenerative medicine

Latest developments in the field of stem cell research and regenerative medicine compiled from publicly available information and press releases from nonacademic institutions from 1st January 2009 to 28th February 2009.

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Going Global

■ Brazil

Brazil decided to establish a national center for stem-cell research and distribute stem cells around the country for research purposes, inching the debate on human embryonic stem cell (hESC) use in the country to a close. The National Laboratory of Embryonic Stem Cells (Lance), which will open in July, will be split between the Federal University of Rio de Janeiro (www.ufrj.br) and the University of São Paulo (www.usp.br). The announcement came as researchers from UFRJ announced that they were able to produce induced pluripotent stem cells (iPSCs). It is hoped that Lance will be able to provide both iPSCs and hESCs to around 70 public and private laboratories in Brazil, and provide training for scientists to work in the field. Lance will receive around US\$1.7 million from the Brazilian Innovation Agency, the Brazilian Development Bank and the Ministry of Health, a sign that the government is keen to invest in ESCs.

■ Israel/Russia

An Israeli boy with a rare, fatal genetic disease ataxia telangiectasia was treated three times, at ages 9, 10 and 12, with intracerebellar and intrathecal injection of human fetal neural stem cells at an undisclosed location in Russia. He was

diagnosed with a multifocal glioneuronal neoplasm 4 years after the first treatment. Molecular and cytogenetic studies showed that the tumor was of nonhost origin, suggesting it was derived from the transplanted neural stem cells. Microsatellite and HLA analysis demonstrated that the tumor is derived from at least two donors [1]. This is the first report of a human brain tumor complicating neural stem cell therapy. Further work is urgently needed to assess the safety of these therapies.

■ United Arab Emirates

The United Arab Emirates's Ministry of Health became officially the only entity in the Arab world to be part of the International Bone Marrow Transplant Registry (www.cibmtr.org). The Health Minister Humaid Al Qutami and Richard Crotty, Mayor of Orange County Government, representing the USA Transfusion Services – Florida's Blood Centers, signed a Memorandum of Understanding stating that both parties will partner in exchange of scientists in order to advance bone marrow and blood collection, storage, transplantation and/or transfusion in United Arab Emirates. Bone marrow and transfusion services will be available at the Sharjah blood transfusion and research center, which now consists of 38 employees.

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Business Development

■ Collaborations, partnerships & alliances

Banking agreement: Bio-Matrix, NeoCells & AdultCells

Bio-Matrix Scientific Group, based in San Diego, CA, USA (www.BMSN.us), the publicly held maker of disposable instruments for plastic surgeons, is branching into the stem-cell storage business. The company signed its first contract with publicly held NeoCells (www.neocells.com) and AdultCells (www.adultcells.com), both owned by parent company ViviCells of Evanston, IL, USA (www.vivicells.com), to bank adult stem cells in its newly built 15,000 sq. ft. (1400 m²) facility located in Mira Mesa, CA, USA. The cells, derived from umbilical cord blood and peripheral blood, will be kept frozen in the company's liquid nitrogen storage vats for possible future use by patients seeking treatment.

Banking agreement: ProtoKinetix & Zymes

In order to commercialize a unique new product line, ProtoKinetix Inc. from Vancouver, BC, Canada (www.protokinetix.com) formed a Joint Venture with Zymes LLC from Hasbrouck Heights, NJ, USA (www.zymes.com). The Joint Venture is founded on the unique combination of the molecular structures, bioactivities and health care attributes of ProtoKinetix' AAGP™ and Zymes' co-enzyme Q10. Although both AAGP and co-enzyme Q10 are potent antioxidants, the combination of AAGP and co-enzyme Q10 provided significantly greater protection from the harmful oxidative stresses than could otherwise be attained from either molecule acting individually. In addition to skin care, other targeted applications include stem-cell preservation and survival, ischemia, reperfusion injury and wound healing.

Collaboration agreement: AstraZeneca & Cellartis

AstraZeneca, a global pharmaceutical company with a corporate office in London, UK (www.astrazeneca.com), and Cellartis, headquartered in Göteborg, Sweden (www.cellartis.com), have signed an extension to their ongoing collaboration to develop improved safety screening systems based on hESC-derived liver and heart muscle cells. Terms of the collaboration were not disclosed. This represents an extension of a 2-year agreement signed by the companies in July 2006 to explore whether cells taken from established hESC lines could be differentiated into functioning human hepatocytes and myocytes.

Collaboration agreement: Mesoblast & Parkway Group Healthcare

Mesoblast Ltd. from Melbourne, Australia (www.mesoblast.com) has signed a collaborative program with Singapore's Parkway Group Healthcare (www.parkwayhealth.com) to facilitate commercialization of a cartilage stem cell product aimed at preventing or treating osteoarthritis of the knee, both in the elderly and following knee reconstruction surgery. The agreement with Parkway Group Healthcare, which operates 15 private hospitals in Asia, involves a single-injection safety and efficacy trial at Parkway's Clinical Centre of Excellence in Singapore.

Collaboration agreement: Pluristem & Pharmicell

Pluristem Therapeutics Inc. from Haifa, Israel (www.pluristem.com), a bio-therapeutics company dedicated to the commercialization of unrelated donor-patient (allogeneic) cell-therapy products for a variety of disorders, has signed a collaboration agreement with Pharmicell Europe GmbH from Berlin, Germany (www.pharmicell.eu). Under the agreement, upon

approval of Pluristem's Investigational New Drug (IND) and Investigational Medicinal Product (IMP) Dossier submissions in the USA and Europe, Pharmicell will distribute PLX-PAD, Pluristem's placental-derived adherent stromal cell product, to various clinical sites in Germany, in connection with Pluristem's upcoming clinical trials for the indication of critical limb ischemia.

Commercialization agreement: BioTime & Reproductive Genetic Institute

Embryome Sciences, Inc. (www.embryome.com), a wholly-owned subsidiary of BioTime, Inc. from Alameda, CA, USA (www.biotimeinc.com), has entered into an agreement with Reproductive Genetics Institute of Chicago, IL, USA (RGI; www.reproductivegenetics.com), which is granting Embryome Sciences rights to market new hESC lines selected by Embryome Sciences from 294 hESC lines derived by RGI. Embryome Sciences will initially select ten RGI hESC lines, and may add additional cell lines at its option. RGI is a fertility center that screens embryos for genetic disorders, such as cystic fibrosis and muscular dystrophy, prior to implantation. The RGI hESC lines include both normal and cell lines identified as carrying a host of inherited genetic disease genes that Embryome Sciences plans to sell as research products to universities and pharmaceutical companies.

Commercialization agreement: VistaGen & Capsant

VistaGen from South San Francisco, CA, USA (www.vistagen.com) and Capsant from Southampton, UK (www.capsant.com) have signed a strategic commercialization agreement, under which the companies will combine stem-cell biology and 3D cell-culture platforms, respectively, to advance their mutual business

interests worldwide. The new collaboration will focus on late-stage development and commercialization of a new generation of human stem cell-based biological tools enabling clinical trials in a Test Tube™ platform, designed to generate predictive information that results in reduced probability of clinical trial drug failures, especially failures associated with heart and liver toxicity. Capsant's OrganDot 3D™ technology is a novel high-throughput, *in vitro* drug testing platform using stem cell-based 3D 'micro-organ' tissue cultures. Together, the companies are planning to provide reproducible functional human cells that grow as 3D 'micro-organs' in a commercially scalable platform with integrated analytical capabilities. This new agreement advances a common goal of providing new-generation drug-development tools that are a vital necessity to improve drug research and development productivity in the pharmaceutical industry. Capsant was created in 2003 as a University of Southampton spinoff company. Its current major shareholder base is led by the IP Group plc, London, UK.

Licensing agreement: Cellartis & WARF

Cellartis AB, headquartered in Göteborg, Sweden (www.cellartis.com), and the Wisconsin Alumni Research Foundation (WARF), from Madison, WI, USA (www.warf.org), have signed a license for hESC patents that enables Cellartis to commercialize undifferentiated hESC products in the USA. With the addition of the new lines, the WiCell Research Institute National Stem Cell Bank, Madison, WI, USA (www.wicell.org) now has received all 21 cell lines from the six providers listed on the NIH federal registry.

Partnership agreement: Cytori & GE Healthcare

Cytori Therapeutics from San Diego, CA, USA (www.cytoritx.com) formed a partnership with GE Healthcare headquartered in Chalfont St Giles, UK (www.gehealthcare.com) to commercialize Cytori's Celution® System in select European countries. The Celution System is a European-approved, state-of-the-art cell-processing device, which extracts and

makes a dose of a patient's own adipose tissue-derived adult stem and regenerative cells available at the point-of-care. As part of the new relationship, GE Healthcare will commercialize the Celution System in the areas of cosmetic and reconstructive surgery, translational medicine and stem-cell banking in combination with Cytori's StemSource® Cell Bank product. The partnership provides GE Healthcare with exclusive commercialization rights for 18 months in the UK, France, Germany, Norway, Finland, Denmark, Sweden, Austria and Switzerland for the cosmetic and reconstructive surgery market, translational medicine and stem-cell banking. The same terms apply in Belgium, The Netherlands and Luxembourg for translational medicine and stem-cell banking. GE Healthcare was granted a 2-year right of first refusal to sales and distribution rights in the USA and all remaining European countries.

■ Launching new products & services

Biocomposites:

Expanding to Chinese market

Biocomposites, a privately held developer, manufacturer and distributor of synthetic tissue regeneration products from Staffordshire, UK (www.biocomposites.com), announced that the company's unique bone graft substitute geneX® has been cleared for use by the State Food and Drug Administration (SFDA) of the People's Republic of China. geneX is a synthetic bone graft material with a unique biphasic composition manufactured through a proprietary process that confers the product with a reproducible negative surface charge. This property stimulates bone cell activity, accelerating bone formation and fusion by harnessing key proteins and directing osteoblast adhesion and proliferation for rapid osteogenesis. geneX is fully resorbable and is completely replaced by bone.

Invitrogen:

Xenogeneic-free media for hESC culture

Invitrogen, a division of Life Technologies Corporation from Carlsbad, CA USA (www.invitrogen.com), has launched KNOCKOUT™ SR XenoFree Media

for use in the culture of hESCs. Until now, most of the research carried out on hESCs has used mouse-derived feeder cells and culture media containing components derived from bovine sources. Ideally, hESCs research and its translation to therapy would be performed without the use of components from other species, which prevents the contamination of hESC lines with nonhuman proteins that can raise regulatory questions as stem-cell research moves into clinical applications. KNOCKOUT™ SR XenoFree Media is based on KNOCKOUT SR, the gold standard reagent for the maintenance of undifferentiated hESCs while maintaining pluripotency, with more than 200 publications documenting its use. All components are either human derived or synthetic, resulting in a completely xenogeneic-free formulation.

Promega

Promega Corporation from Madison, WI, USA (www.promega.com) launched StemElite™ ID System, which is designed to improve and simplify human cell line authentication in research applications. Understanding that cross-contamination between cell lines is a frequent cause of scientific misrepresentation, StemElite ID is designed to give scientists confidence in the integrity of their results. The StemElite ID System incorporates the same short tandem repeat analysis technology as Promega PowerPlex® products used for genetic identity by forensics laboratories worldwide. StemElite ID includes ten human loci for human cell line authentication and a sensitive marker that specifically detects contamination with the mouse DNA. For stem-cell researchers using mouse 'feeder' cells this is the most sensitive method for detection of mouse contamination in their human stem cell samples. The kit provides all reagents required for co-amplification and three color detection of DNA fragments in a single tube and it is able to detect contamination with mouse DNA (the system incorporates a sensitive mouse marker).

StemCell Technologies:

New sales & distribution office in Singapore

StemCell Technologies Inc., a privately owned biotechnology company based in Vancouver, BC, Canada (www.stemcell.com), opened a new sales and distribution office in Singapore, effective 12 January, 2009. StemCell Singapore PTE Ltd is offering direct service to Singapore customers who will also have immediate access to inhouse scientists and StemCell technical support. Until now StemCell's distribution partner for a number of years for this region was Gene-Ethics (Asia) PTE Ltd from Singapore (www.gene-ethics-asia.com).

The Automation Partnership: Automated stem cell picker

At the Stem Cells World Congress held 20–22 January, 2009, in Palm Springs, CA, USA, The Automation Partnership, a supplier of innovative automation for life science from Cambridge, UK (www.automationpartnership.com), announced CellCelector™, the world's first commercial automated stem cell picker. CellCelector consists of an inverted Olympus microscope, robotic arm and liquid handling station, all fully integrated to powerful image acquisition and analysis software. This walk-away system allows researchers to set parameters for cell or colony types they want (including size, proximity to other colonies or roundness). The picking tool on the robotic arm gently picks and dispenses cells into a microplate well in just 30 s. CellCelector benefits researchers by reducing the time and effort required for this challenging task and assuring the cells chosen are of consistent quality. CellCelector can fit into any standard laminar flow hood, thus ensuring cells remain contamination free. The system can also be fitted with an autoclave compatible metal tool for scraping adherent cells or a disposable glass capillary for picking single cells, both of which are designed with features for maintaining cell integrity and viability. CellCelector,

developed by cell biology automation specialists Aviso from Jena, Germany (www.aviso-gmbh.de) is being successfully used at universities and institutes across Europe with murine and human stem cells. The system is now also available in the USA and Canada, exclusively from The Automation Partnership.

■ Advancements

Advanced Cell Technology

Advanced Cell Technology, Inc. from Worcester, MA, USA (www.advancedcell.com), reported that it is feasible to differentiate hESC into functional human vasculature [2]. The research shows for the first time that human progenitor cells – known as hemangioblasts – have the potential for both endothelial cell and vascular smooth muscle cell lineage differentiation. This dual potentiality is critical for the effective treatment of human vascular disease, especially the repair and formation of mature and larger-size (non-capillary) vessels. Although endothelial cells play an essential role in vasculogenesis and angiogenesis and form capillary vasculatures, they alone cannot complete the process of vessel growth and development. Vascular smooth muscle cells play a critical role in the support of the vascular network by stabilizing nascent endothelial vessels during vascular development and blood vessel growth. This outer layer of cells also protects the fragile channels from rupture and helps control blood flow. Until now, there was no evidence that blast cells (hemangioblasts) or their equivalents generated from hESCs possessed the ability to differentiate into smooth muscle cells. The cells differentiated into both endothelial and vascular smooth muscle cells, and incorporated into blood vessels after injection into ischemic tissues, indicating that these cells are functional both *in vitro* and *in vivo*.

In another study, scientists from Advanced Cell Technology and their collaborators reported that human oocytes have the capacity to extensively reprogram adult human cells [3]. The research demonstrates that although human-to-human clones (human clones) and human-to-animal clones (hybrids) appear similar, the pattern of reprogramming of the

donor human cell is dramatically different. In contrast to the human–animal hybrids, the gene-expression pattern of the human clones was highly similar to normal human embryos. Previous studies have confirmed the ability of animal eggs to support interspecies cell division to the embryo stage, and in a few closely related bovid species, successful development to term. However, there are clear differences in compatibility. Distantly related animal combinations generally arrest at the cleavage stage, although there have been reports of blastocyst formation. Several groups have successfully used eggs to clone closely related species (e.g., Advanced Cell Technology cloned two endangered species – the guar and banteng – using cow eggs). Rabbit eggs have also been used to generate embryos using cells from cats and panda, among others. However, there is no evidence that patient-specific stem cells can be generated using animal eggs, which is consistent with studies that indicate that eggs support nuclear remodeling, but not reprogramming of discordant animal combinations. Studies using cow and rabbit eggs clearly suggest that DNA methylation/demethylation of the donor DNA occurs in a species-specific way, and that the eggs might lack the ability to demethylate repetitive sequences from other species. While cleavage division relies on maternal factors in the egg, further development requires activation of the embryonic genome to ensure correct progression of the cell cycle. The new results suggest that while bovine and rabbit eggs are capable of supporting limited cell division, specific reprogramming towards the normal human embryonic state does not occur.

StemLifeLine

StemLifeLine Inc. from San Carlos, CA, USA (www.stemlifeline.com) reported an improved method for efficient derivation of hESC lines from blastomeres of biopsied embryos [4]. The protocol substituted feeder cells of mouse origin with human foreskin fibroblasts (HFF), limited serum exposure of cells to formation of the initial outgrowth and increased derivation efficacy from 12.5% (one hESC line out of 13 biopsies) to 50% (three out of six

biopsies) by using early population doubling HFF. In addition, it eliminated a need for embryo-blastomere co-culture, thus reducing complexity of the culture and enabling continued development of the

biopsied embryo under optimal conditions. All derived lines maintained normal karyotype and expressed totipotent phenotype including the ability to differentiate into trophoblast and all three germ layers.

Clinical Trials

■ Cytori

Cytori, from San Diego, CA, USA (www.cytoritx.com), announced that the first patient was enrolled in an investigator-initiated safety and feasibility study using adipose-derived stem and regenerative cells to treat stress urinary incontinence. Stress urinary incontinence can have a significant impact on a patient's quality of life, resulting in involuntary release of urine due to a weakened urethral sphincter. The condition is more common in women and often develops following childbirth or menopause. It is estimated that approximately 9 million people in Japan, and more than 13 million women in the USA, are affected by stress urinary incontinence. The ten-patient study is being conducted independently by Nagoya University Hospital in Japan (www.med.nagoya-u.ac.jp/index2.html). Cytori's Celution® 800 System is being used to process and extract the patients' own adipose tissue-derived stem and regenerative cells at the time of surgery. As part of the study, stem cells were injected intramuscularly into the sphincter as well as in combination with a measured volume of the patient's own fat tissue to create a bulking agent to support the urethra. The study will evaluate safety and functional end points including intraurethral pressure and leak point pressure, as well as subjective assessments of patient and physician satisfaction. Current treatments include use of collagen as a bulking agent to provide pressure against and support the urethra.

■ Gamida Cell–Teva

Gamida Cell (www.gamida-cell.com)–Teva (www.tevapharm.com) Joint Venture from Jerusalem and Children

Hospital Orange County (CHOC) from Orange County, CA, USA (www.choc.org) announced that the CHOC Children's Cancer Institute has joined a select group of cancer centers in Europe, the USA and Israel actively enrolling patients for the ExCell study. CHOC Children's is the only pediatric site for the study on the US West Coast. The pivotal registration study is testing the safety and efficacy of StemEx®, a potential alternative transplantation option for adolescents and adults with leukemia, lymphoma and other high-risk hematological malignancies, who are unable to find an adequately matched, bone marrow donor. StemEx is being developed by a Joint Venture equally owned and managed by Jerusalem-based biomedical company Gamida Cell and Teva Pharmaceutical Industries. StemEx is a graft of expanded stem/progenitor cells, derived from a single unit of umbilical cord blood and transplanted in combination with nonexpanded cells from the same unit. While umbilical cord blood has been used mainly for the treatment of small children, the increase in stem/progenitor cells through StemEx boosts the therapeutic potential of this treatment for adolescents and adults. The preliminary results of the Phase I/II study of StemEx presented at the 2004 Annual Meeting of the American Society of Hematology in San Diego, CA, USA, encouraged the further investigation of the safety and efficacy of StemEx in the ExCell study. The ExCell study is recruiting patients between 12 and 55 years of age, with leukemia or lymphoma, who meet all of the eligibility criteria of the study. All of

the enrolled patients will receive StemEx. Visit www.stemexstudy.com for a full list of study sites and investigators.

■ Living Cell Technologies

Living Cell Technologies Ltd, based in Sydney, Australia, and Auckland, New Zealand (www.lctglobal.com), reported positive interim results from the long-term follow-up of insulin-dependent diabetes patients in the Phase I/IIa clinical trial of its lead product, DIABECCELL®, encapsulated porcine insulin-producing cell product designed for the treatment of Type 1 diabetes without the use of immunosuppressive drugs. A total of seven patients have received DIABECCELL implants; five of them have had two low dose (5000 islet equivalents [IEQs] per kg bodyweight) implants at least 6 months apart. There have been no remarkable adverse events attributed to double implants. Two patients have received a single higher dose (10,000 IEQs/kg) with no safety concerns to date. The results of 36–68 weeks follow-up from the first four patients showed that good blood glucose control was maintained as reflected by a reduction in glycated hemoglobin (% HbA1c) levels from a mean of 8% pre-enrollment to 6.8% at the time of clinical review. The American Diabetes Association target for good blood glucose control is HbA1c below 7% and the normal is less than 6.2%. This control was attained despite a reduction of 10–38% in the required daily insulin dose. Although it is too early to assess the second implant for the fifth patient, the patient's HbA1c improved markedly from 9.8% before enrollment to 7.2% accompanied with a small reduction in the average daily insulin

dose of 6%. From two patients that have received the higher dose of DIABECCELL, patient six declined further follow-up at 20 weeks after the first implant when there was no change in glucose control or insulin dose. Patient seven has been followed up for only 4 weeks at which time HbA1c improved dramatically from 8.3 to 4.8% and daily insulin requirement dropped by 60%. The next three patients in Russia are scheduled to receive the 10,000 IEQ/kg dose by April 2009 before testing the top dose of 15,000 IEQs/kg.

■ Osiris

Osiris Therapeutics, Inc. from Columbia, MD, USA (www.osiris.com) announced the final 2-year results for the company's trial evaluating Prochymal® for the treatment of acute myocardial infarction. Prochymal is a proprietary formulation of adult stem cells designed to provide therapeutic benefit by controlling inflammation, promoting tissue regeneration and preventing scar formation. This double-blind, placebo-controlled study of 53 patients, which evaluated safety and preliminary efficacy, found heart attack patients receiving the intravenous therapy had lower rates of adverse events and significantly improved heart function. Throughout the 2-year trial Prochymal demonstrated a favorable safety profile. In this fully immunocompetent patient population, there were no

signs of adverse immune response or infusion toxicities. The adverse events rate experienced by Prochymal patients was lower than that of placebo patients (eight vs 11 per patient). Notably in the class of chest pain events, there were fewer cases in the Prochymal group than placebo (24.2 vs 63.2%; $p = 0.008$). No serious adverse events were attributed to Prochymal, and all-cause hospitalizations trended lower in the Prochymal group (38.2%) as compared with the placebo group (47.4%). Data from this trial strongly suggest that Prochymal is able to attenuate adverse effects typically associated with acute myocardial infarction by blocking scar formation and pathological remodeling. This was evident in cardiac performance measures of both electrical and mechanical function of the heart. During the trial patients receiving Prochymal experienced fewer arrhythmias. This effect was maintained for the duration of the study, with 47.4% of placebo patients experiencing cardiac arrhythmia compared with only 11.8% of Prochymal patients ($p = 0.006$). Ventricular arrhythmias are associated with tissue damage and scar formation in the heart resulting from infarction and can be a sign of poorer prognosis. Along with the conduction effects seen, the mechanical performance data collected from MRI data on a subset of patients showed functional recovery that was maintained through the 2-year study. Left-ventricular ejection fraction (LVEF), which reflects the fraction of blood pumped out of a ventricle with each heart beat, is a common

measurement of overall heart function and typically declines after a heart attack. Patients with significantly compromised LVEF progress to heart failure. MRI data from over 2 years demonstrate that there was a statistically significant improvement in LVEF over baseline, 6.6-point increase in the Prochymal group relative to a 3.9-point improvement in the placebo group. For patients with more severe myocardial infarction, defined as a baseline LVEF of 45% or less, even greater effects were observed. The Prochymal group showed a significant 9.5-point improvement over baseline 2 years post-treatment ($p < 0.05$). This compares favorably with the 3.1-point increase observed for the placebo group.

Prochymal is a preparation of mesenchymal stem cells specially formulated for intravenous infusion. The stem cells are obtained from the bone marrow of healthy adult donors. Prochymal is currently being evaluated in Phase III trials for steroid refractory graft-versus-host disease (GvHD), acute GvHD and Crohn's disease. Prochymal has been granted Fast Track status by the US FDA for all three of these indications. Prochymal also obtained Orphan Drug status from the FDA and the European Medicines Agency for GvHD. Prochymal is being studied in Phase II trials for the treatment of chronic obstructive pulmonary disease, Type 1 diabetes and acute myocardial infarction. In addition, the Department of Defense recently awarded Osiris a contract to develop Prochymal as a treatment for acute radiation syndrome.

Regulations, Approvals & Acquisitions

■ Patent issues

Cytori

Cytori, from San Diego, CA, USA (www.cytortix.com), received US Patent No. 7,473,420 (the '420 patent), which protects important formulations of the Celution System output. This builds upon US Patent No. 7,390,484, issued to Cytori

in June 2008, which covers the Celution System technology for the bedside processing of stem and regenerative cells derived from adipose tissue. Specifically, the patent covers methods to combine the Celution System output with additives such as scaffolds, matrices or other agents to increase therapeutic effect, optimize or localize cell

delivery, enhance specific cell properties or promote cell differentiation. The additives covered by the claims include, but are not limited to, the following: an agent that promotes cell differentiation, including growth factors, cytokines, proteins, pharmaceuticals, among others; demineralized bone, tissue or tissue fragment; biological

or artificial scaffold; and immunosuppressive compound, antibiotic or antimicrobial agent. Delivery of the stem and regenerative cells may be improved by combining the cells with a scaffold or matrix; for example, demineralized bone may be combined with the Celution output to support orthopedic repair. The Celution output may also be combined with drugs, proteins, genes or other agents to enhance cell properties or to induce the cells to differentiate. For example, combining adipose-derived stem and regenerative cells with hyaluronic acid has been shown preclinically to repair damaged spinal disc. The company's intellectual property portfolio related to the devices, methods and uses of adipose-derived stem and regenerative cells includes nine USA and international patents and over 120 applications pending worldwide.

Cytori also received a Notice of Allowance from the US PTO on a patent application which broadens protection of the company's Celution System. The more than 40 allowed claims create several new layers of protection related to the automated processing of stem and regenerative cells from adipose tissue. Key claims include the following: alternative Celution System configurations beyond those covered in the company's core device patent (US Patent No. 7,390,484); expanded protection for cosmetic and reconstructive surgery applications; and technology within the device that optimizes intravascular delivery of adipose-derived stem and regenerative cells. Alternative configurations could accelerate processing times, be tailored to smaller volume procedures or include a desktop version. These claims also expand the array of Celution output compositions protected by Cytori's intellectual property, so that combinations of cells, matrices and other additives specific to the needs of reconstructive and cosmetic surgery, as well as other applications are protected more broadly. The claims related to intravascular delivery are potentially applicable to a variety of clinical applications for the Celution System output, which include cardiovascular disease, liver disease and renal failure.

Neuralstem

Neuralstem Inc. from Rockville, MD, USA (www.neuralstem.com) has received Official Notice of Allowance for its

patent application, number 10/047,352, for Stable Neural Stem Cell Lines from the US Patent and Trademark Office (US PTO) (www.uspto.gov). This patent covers the technology to immortalize any human neural stem cell, rendering its growth robust and long-lasting. The newly patented method uses cMyc-ER, a recombinant fusion of two proteins normally present in cells, c-Myc, a human cell-cycle regulating protein, and ER, a human protein that is activated by estrogen. By transiently increasing the concentration of the c-Myc protein, a cell inserted with cMyc-ER is able to overcome the natural aging process inherent in the cell without losing any of its stem cell properties. The immortalized human neural stem cell can be grown for over 60 cell-doublings and is clonally expandable.

Vitro Diagnostics

Vitro Diagnostics, Inc. from Golden, CO, USA (www.vitrodiag.com) has received notification from the US PTO (www.uspto.gov) indicating the successful examination of its patent application entitled, 'Generation and Differentiation of Adult Stem Cells' (App No 11/256,673). The US PTO issued an Official Notice of Allowance indicating the allowance of certain claims and the impending issuance of a new US patent.

■ Green light

BioE

BioE, Inc., from St. Paul, MN, USA (www.bioe.com) received 510(k) clearance from the FDA for its PrepaCyte®-CB Processing System for umbilical cord blood. PrepaCyte-CB provides public and private cord blood banks with a simple and cost-effective method for processing umbilical cord blood to obtain potentially therapeutic cells – total nucleated cells and CD34⁺ hematopoietic stem/progenitor cells – for eventual transplantation in humans. Available immediately, PrepaCyte-CB is the only cord blood processing system of its kind cleared by the FDA.

Geron

Geron Corporation from Menlo Park, CA, USA (www.geron.com) received a clearance of the company's IND application from the FDA for the clinical trial of

GRNOPC1 in patients with acute spinal cord injury. The clearance enables Geron to move forward with the world's first study of a hESC-based therapy in man. Geron plans to initiate a Phase I multicenter trial that is designed to establish the safety of GRNOPC1 in patients with 'complete' American Spinal Injury Association (ASIA) grade A subacute thoracic spinal cord injuries. Patients eligible for the Phase I trial must have documented evidence of functionally complete spinal cord injury with a neurological level of T3 to T10 spinal segments and agree to have GRNOPC1 injected into the lesion sites between 7 and 14 days after injury. Geron has selected up to seven US medical centers as candidates to participate in this study and in planned protocol extensions. The sites will be identified as they come online and are ready to enroll subjects into the study. Although the primary end point of the trial is safety, the protocol includes secondary end points to assess efficacy, such as improved neuromuscular control or sensation in the trunk or lower extremities. Once safety in this patient population has been established and the FDA reviews clinical data in conjunction with additional data from ongoing animal studies, Geron plans to seek FDA approval to extend the study to increase the dose of GRNOPC1, enroll subjects with complete cervical injuries and expand the trial to include patients with severe incomplete (ASIA grade B or C) injuries to enable access to the therapy for as broad a population of severe spinal cord-injured patients as is medically appropriate.

GRNOPC1, Geron's lead hESC-based therapeutic candidate, contains hESC-derived oligodendrocyte progenitor cells that have demonstrated remyelinating and nerve growth-stimulating properties leading to restoration of function in animal models of acute spinal cord injury. Geron submitted evidence of the safety, tolerability and efficacy of GRNOPC1 to the FDA in a 21,000-page IND application that described 24 separate animal studies requiring the production of more than 5 billion GRNOPC1 cells. Included

in the safety package were studies that showed no evidence of teratoma formation 12 months after injection of clinical grade GRNOPC1 into the injured spinal cord of rats and mice. Other studies documented the absence of significant migration of the injected cells outside the spinal cord, allodynia induction (increased neuropathic pain due to the injected cells), systemic toxicity or increased mortality in animals receiving GRNOPC1. *In vitro* studies have shown that GRNOPC1 is minimally recognized by the human immune system. GRNOPC1 is not recognized *in vitro* by allogeneic sera, natural killer cells or T cells [5]. These immune-privileged characteristics of the hESC-derived cells allow a clinical trial design that incorporates a limited course of low-dose immunosuppression and provide the rationale for an off-the-shelf, allogeneic cell therapy.

Also included in the IND application were published studies supporting the utility of GRNOPC1 for the treatment of spinal cord injury. Those studies showed that administration of GRNOPC1 significantly improved locomotor activity and kinematic scores of animals with spinal cord injuries when injected 7 days after the injury [6]. Histological examination of the injured spinal cords treated with GRNOPC1 showed improved axon survival and extensive remyelination surrounding the rat axons. These effects of GRNOPC1 were present 9 months after a single injection of cells. In these 9-month studies, the cells were shown to migrate and fill the lesion cavity, with bundles of myelinated axons crossing the injury site.

GRNOPC1 is produced using current good manufacturing practices (cGMP) in Geron's manufacturing facilities. Geron's GRNOPC1 production process and clean-room suites have been inspected and licensed by the state of California. The cells are derived from the H1 hESC line, which was created before 9 August, 2001. Studies using this line qualify for US federal research funding, although no federal funding was received for the development of the product or to support the clinical trial. Geron's H1 hESC master

cell bank is fully qualified for human use and was shown to be karyotypically normal and free of measurable contaminants of human or animal origin. Production of GRNOPC1 from undifferentiated hESCs in the master cell bank uses qualified reagents and a standardized protocol developed at Geron over the past 3 years. Each manufacturing run of GRNOPC1 is subjected to standardized quality control testing to ensure viability, sterility and appropriate cellular composition before release for clinical use. GRNOPC1 product that has passed all such specifications and has been released is available for the approved clinical trial. The current production scale can supply product needs through pivotal clinical trials. The existing master cell bank could potentially supply sufficient starting material for GRNOPC1 to commercially supply the US acute spinal cord injury market for more than 20 years.

The production and commercialization of GRNOPC1 is protected by a portfolio of patent rights owned by or exclusively licensed to Geron. Patent rights owned by Geron protect key technologies developed at Geron for the scalable manufacturing of hESCs, as well as the production of neural cells by differentiation of hESCs. The fundamental patents covering hESCs are exclusively licensed to Geron from the WARF for the production of neural cells, cardiomyocytes and pancreatic islets for therapeutic applications. US PTO (www.uspto.gov) in a re-examination proceeding recently confirmed the validity of these patents. Geron funded the original research at the University of Wisconsin-Madison that led to the first isolation of hESCs. The production of oligodendrocytes from hESCs is covered by patent rights exclusively licensed to Geron from the University of California. These patent rights cover technology developed in research collaboration between Geron and University of California scientists.

Mesoblast

Mesoblast Ltd., from Melbourne, Australia (www.mesoblast.com), had received Australian institutional ethics approval to begin the first human trial of adult stem cell treatment for prevention of knee osteoarthritis after an acute traumatic knee

injury. The randomized, placebo-controlled, Phase II clinical trial design will evaluate whether Mesoblast's allogeneic, or 'off-the-shelf', adult stem cell product, RepliCart™, can slow or prevent the development of knee osteoarthritis after reconstruction of a ruptured anterior cruciate ligament. The trial will enroll 24 patients aged between 18 and 40 years old who have undergone recent ligament surgical reconstruction within 6 months of a traumatic knee injury. Patients will be randomized to receive either one of two doses of RepliCart injected into the knee joint together with hyaluronan, or hyaluronan alone. The trial's primary end point will be safety of the stem-cell therapy at 12 months, and its secondary end point prevention of cartilage loss and knee osteoarthritis during this period.

Pluristem

Pluristem Inc. from Haifa, Israel (www.pluristem.com) has received approval, in the form of a declaration, from Bridgent, UK-based Biotec Services International Ltd. (www.biotec-trial-supplies.com) stating that Pluristem's IMP, PLX-PAD, is manufactured in accordance with standards of GMP, equivalent to those applied in the EU. Biotec Services is an international clinical trials supply company providing release services of the IMP into the European Union by a Qualified Person. Upon IND and IMPD clearance by the regulatory authorities, Pluristem plans to initiate clinical trials in Europe and in the USA, administering PLX-PAD to patients afflicted with critical limb ischemia, which has not responded to traditional medical or surgical interventions.

ReNeuron

ReNeuron from Guildford, Surrey, UK (www.reneuron.com) has received a green light from British regulators to proceed with a clinical trial of its stem-cell therapy for stroke damage. In the trial, which will take place in Glasgow, 12 stroke victims will be divided into three groups and treated with fetal stem cells in 6–24 months following a stroke. Doses will start at around 2 million brain cells and grow to 20 million. The stem cells being tested are extracted from the fetuses of aborted babies, which had sparked controversy in the UK. ReNeuron

was the first stem-cell company to go public in the UK in 2000, it then went private in 2003 before relisting in 2005.

Vitrolife

Vitrolife from Göteborg, Sweden (www.vitrolife.com) has received approval from the FDA, so-called 510(k) clearance, for its new needle for the collection of human oocytes, Swemed Sense™ (patent pending), which reduces the risk of tissue damage and pain. The needle has previously received European approval, a so-called CE mark. The Swemed Sense oocyte aspiration needle is designed with a thinner front part and tip so as to minimize tissue damage, bleeding and pain. However, the rear part of the needle has a larger diameter. This makes the needle stable and therefore does not reduce the handling ability. Furthermore, the procedure is just as fast as when a thicker needle is used, which is not the case with uniformly thin needles. A patent application has been submitted for Swemed Sense in all major markets.

■ Pending

Neuralstem

Neuralstem Inc. from Rockville, MD, USA (www.neuralstem.com) announced that its spinal cord stem-cell trial to treat amyotrophic lateral sclerosis (Lou Gehrig's disease) is on clinical hold and that the FDA has provided the company with specific comments, questions and recommendations for modifications to its protocol.

Pluristem

Pluristem Inc. from Haifa, Israel (www.pluristem.com), has filed an IND application with the FDA to begin clinical trials with its placental-derived stromal cell product, termed PLX-PAD, for the treatment of critical limb ischemia. Following clearance of the IND application by the FDA, Pluristem will initiate Phase I dose-ranging clinical trials during the first half of 2009 at two clinical sites in the USA. As in the clinical trials with PLX-PAD that are to begin in Europe, the patients in the US trials will be considered 'late stage' and defined as patients whose limb ischemia has not responded to traditional medical or surgical interventions and are facing amputation.

■ FDA

The FDA announced the availability of a draft document entitled 'Guidance for Industry: Current Good Tissue Practice (cGTP) and Additional Requirements for Manufacturers of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps)' dated January 2009. The draft guidance document provides establishments that manufacture HCT/Ps with recommendations for complying with cGTP requirements. The draft guidance can be obtained at either www.fda.gov/cber/guidelines.htm or www.regulations.gov.

■ Capital market & finances

Garnet

Garnet Biotherapeutics, from Malvern, PA, USA (www.garnebiot.com), a clinical stage regenerative medicine company targeting the acceleration of healing and reduced scarring associated with surgical procedures and other dermatologic conditions, won a \$10.4 million financing round. Garnet Biotherapeutics will use the financing to support research and Phase II clinical trials for its proprietary human adult bone marrow-derived cells, along with manufacturing and development. Garnet Biotherapeutics has identified a distinct population of bone marrow-derived cells capable of reducing inflammation and promoting healing. In addition, the company has developed proprietary scalable cell expansion technology that can generate a large number of patient doses from a single source. The company is initially developing its cell-based therapy for cosmetic and dermatologic applications where accelerated healing and reduced scarring are desirable. Cosmetic applications, such as liposuction, breast augmentation, abdominoplasty and facelifts, represent an addressable market opportunity of more than US\$1 billion worldwide. Garnet Biotherapeutics believes that the cell-based therapy may also be applicable in burns, autoimmune disorders such as psoriasis, and in other conditions where inflammation or scar formation plays an important role in disease pathology. Investors include Safeguard Scientifics, Alliance Technology Ventures and SCP Vitalife Partners.

Geron

Geron Corporation, from Menlo Park, CA, USA (www.geron.com), announced the pricing of an underwritten public offering of 7.25 million shares of its common stock. On 12 February, 2009, the last reported sales price of Geron common stock on the Nasdaq Global Market was \$7.77. UBS Investment Bank has been acting as sole book-running manager and Lazard Capital Markets LLC was acting as co-manager for this offering. Geron intended to use the net proceeds from the offering for research and development, clinical trials and general corporate purposes. The offering closed on 18 February, 2009, with a stock price of \$5.74. The price continued to drop to only \$3.79 on 6 March 2009. Market analysts believe that the likely reason behind the dive in Geron's stock price was the publication of a medical paper detailing the case of a boy with a fatal brain disease who was treated with an experimental concoction of fetal neural stem cells [1].

Histogen

Privately held SkinMedica of Carlsbad, CA, USA (www.skinmedica.com), has filed a patent infringement lawsuit against new start-up Histogen, San Diego, CA, USA (www.histogeninc.com) and Gail Naughton, its founder and CEO. The lawsuit, which was filed in federal court in San Diego, alleges that Histogen and its affiliated Histogen Aesthetics business infringe on two SkinMedica patents related to proprietary conditioned cell media technology. SkinMedica complaint asks for unspecified relief for the "misappropriation of SkinMedica trade secrets and confidential information by Histogen." A patent infringement lawsuit against Histogen prompted a group of angel investors to withdraw their planned US\$2.4 million investment in Histogen at the end of January and Histogen was forced to layoff all 36 of its employees immediately. The ensuing funding crisis has been playing out behind the scenes at Histogen, even while Naughton reported promising findings on the start-up's experimental hair regrowth treatment at the 4th Annual Stem Cell

Summit in New York in February 2009. The company says that patients using the ReGenica treatment showed increased, thicker hair growth after 12 weeks of treatment, with no adverse reactions. ReGenica is an injectable liquid product made by culturing cells from newborns and collecting growth factors and other molecules that the cells secrete.

Intercytex

The regenerative medicine developer Intercytex, which in addition to its head office in Cambridge, UK, has a GMP clinical production facility with research and development laboratories in Manchester, UK, and additional laboratories located in Boston, USA (www.intercytex.com), axed half of its 76 staff at the end of January at facilities in the UK and Boston, providing enough money to fund operations through 2009. The cuts came after Intercytex failed to raise £9 million (US\$12.7 million) from investors. Its stock price plunged 30% on the news. Furthermore, approximately a month later, Intercytex announced that the Phase III study of Cyzact® for the treatment of venous leg ulcers has failed to meet its primary end point. The primary end point was demonstration of a statistically significant ($p < 0.05$) increase in complete wound closure at up to 12 weeks compared with four-layer compression bandaging alone. The 396-patient Phase III trial was conducted in the UK, USA and Canada. The three-arm study involved all patients receiving four-layer compression bandaging (the current standard of care for venous leg ulcers) with either Cyzact ($n = 196$), vehicle (a fibrin disc with no cells, $n = 100$) or standard of care alone ($n = 100$). The primary end point of the study was the incidence of complete wound closure at up to 12 weeks for the

Cyzact arm of the study versus the standard of care arm. No statistically significant difference was seen between any of the groups. Data from the secondary end points of the trial have yet to be collated. Apart from completing the data analysis, no further work on Cyzact is planned in any indication.

Pluristem

Pluristem Therapeutics Inc. from Haifa, Israel (www.pluristem.com) a bio-therapeutics company dedicated to the commercialization of nonpersonalized (allogeneic) cell therapy products for a variety of degenerative, ischemic and autoimmune indications, on 29 January, 2009, entered into definitive agreements to sell restricted common stock and warrants for aggregate gross proceeds of US\$1,200,000. Pursuant to the agreement, the company sold 1,034,000 restricted shares of common stock at a price of \$1.16 per share and 5-year warrants for purchase of an additional 1,034,000 shares of common stock at an exercise price of US\$1.90 per share. Several days later, on 5 February 2009, Pluristem received a Nasdaq Staff Notification of Deficiency and Compliance Letter indicating that the company had violated the shareholder approval rules under Nasdaq Marketplace Rule 4350(i)(1)(D)(ii) (the 'Rule') in connection with its sale during November and December of 2008 of units consisting of an aggregate of 1,662,500 common shares and warrants to acquire 1,662,500 common shares and an option to purchase additional units consisting of 886,667 shares and warrants to acquire 886,667 shares. The company regained compliance after the investors in such issuances agreed that no warrants or options to purchase additional units consisting of shares and warrants would be exercised until 6 months after the original agreement dates. As a result, the Nasdaq Staff determined that the company has regained compliance with the Rule and that this matter is now closed.

ZenBio

ZenBio, Inc. from Research Triangle Park, NC, USA (www.zenbio.com) has been awarded a Phase II SBIR grant to commercialize primary human peritoneal mesothelial cells. The US\$1.88 million award from the NIH will fund continued optimization and commercial development of this unique tool for cancer, obesity and Type 2 diabetes research. Mesothelial cells are specialized epithelial cells that line the peritoneal, pleural and pericardial cavities (as well as internal organs), providing a frictionless barrier that facilitates movement of organs and tissues. The grant will allow ZenBio to make well-characterized mesothelial cells available to the wider research community.

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