

REGENERATIVE MEDICINE: A NEW FRONTIER FOR THERAPEUTIC INTERVENTION

HIGHLIGHTS FROM THE SOCIETY FOR MEDICINES RESEARCH SYMPOSIUM HELD ON MARCH 11, 2010 AT GRANTA PARK, CAMBRIDGE, UK

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SUMMARY

Regenerative medicine is one of science's new horizons, holding the promise of groundbreaking new therapies to repair, replace or regenerate disease-damaged cells, tissues or organs. It is a multidisciplinary field of research that is acquiring increasing importance in drug discovery and development portfolios, but the realization of this promise depends on overcoming a number of significant challenges. On March 11, 2010, the Society for Medicines Research held a 1-day meeting entitled "Regenerative Medicine: A New Frontier for Therapeutic Intervention". This symposium, organized by Wendy Alderton, Richard Davenport and Paul Fish, brought together a panel of international

speakers across the breadth of research and development in the field of regenerative medicine to present and discuss the advances that are currently being made. A number of case studies on important new approaches to regenerative medicine were presented by both academic and industry leaders in the field, and the challenges of conducting clinical trials in this area were discussed.

FUNDAMENTAL BIOLOGY OF STEM CELLS

Professor Austin Smith (Wellcome Trust Centre for Stem Cell Research, Cambridge) described his research interests, centered on the biology of embryonic stem cells (ESCs). ESCs (Fig. 1), which are derived directly from the pluripotent cells of the early mammalian embryo, can be propagated and manipulated in vitro, while retaining the ability to generate every cell type of the organism. Neural stem cells (NSCs) can be manipulated in a similar way in vitro, but are restricted to generating cell types associated with the central nervous system.

A variety of extrinsic and intrinsic regulators of the pluripotent state have been identified by comparing cultured ESCs with the resident pluripotent cells in the mammalian embryo. Tool compounds can be used to probe some of these regulators, although the selectivity of any tool compound must be fully characterized (1, 2). The 2i (inhibitor) system using an MEK and GSK-3 inhibitor was described, which can be used to block the progression of ESC development and hold the cells in the ground state, leading to the postulate that naïve pluripotency is the basal cellular state. If this is true, it should be possible to use 2i to make ESCs from any mouse or rat embryo, and experiments have been performed to confirm this. Unfortunately, human ESCs do not obey the 2i rule, and it is postulated that this may be because they have two phases of pluripotency.

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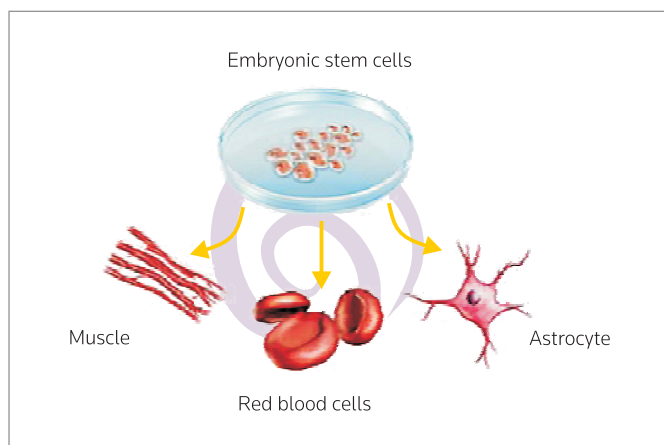


Figure 1. Embryonic stem cells retain the ability to generate every cell type of the organism.

RETINAL PIGMENTED EPITHELIAL CELL THERAPY FOR THE TREATMENT OF AGE-RELATED MACULAR DEGENERATION

Professor Peter Coffey (Institute of Ophthalmology, University College London) described the latest research efforts in using human embryonic stem cells (HESCs) as a potentially unlimited source of progenitor cells for use in the repair of retinal diseases, such as age-related macular degeneration (AMD). This is a condition that cannot be resolved by glasses, and occurs when damage to the retinal pigment epithelium (RPE) means nutrients are unable to reach the photoreceptors, leading to impaired vision. There is no real cure, although multiple eye surgery procedures can yield some positive results. The goal was to use new RPE cells, which can be derived from undifferentiated HESCs, to provide a source of new RPE to replace the damaged area, with the aim of going from a cell therapy to the clinic in 5 years. In the first instance, the new RPE was successfully grown on a polyester base with transplantation performed on a pig during a 2-h operation. Improved vision was noted within 1 day (compared to the 6 weeks with more traditional eye surgery). No immune response was noted, and only a little local inflammation around the surgical area. The approach is currently being optimized with respect to improving both the yield and reproducibility of RPE cells, with the aim of performing a retinal tears phase II trial in 2011.

GE HEALTHCARE STEM CELL RESEARCH

Dr. Stephen Minger (R&D Technologies, GE Healthcare) described the belief within the GE life sciences business that it should align itself with the expanding field of stem cell research. As a consequence, it has formed an alliance with Geron Corporation in the field of chronic degenerative diseases, with GE Healthcare bringing its expertise in developing emerging technologies. The alliance's long-term goal is to develop an infrastructure around manufacturing large quantities of stem cells which would be required for any novel treatments. In addition, GE Healthcare is currently focusing on two areas to assist drug discovery. Firstly, the identification of novel cardiomyocyte and hepatocyte assays using cultured stem cells to help screen out the toxic effects of new drugs. This approach is currently being assessed by the pharmaceutical industry, with the cardiomy-

ocyte assay already being validated with known cardiotoxic drugs. The second is the development of a high-content analysis automated microscope, which enables researchers to look at cell differentiation (key for stem cell research). These cells can be viewed with and without potential new drugs, with a screening capacity of up to 20,000 compounds per day.

SMALL-MOLECULE APPROACHES FOR MODULATION OF STEM CELL ACTIVITY

Dr. Mark Bunnage (Pfizer Regenerative Medicine - Chemistry, Sandwich, U.K.) gave a presentation which illustrated the use of small molecules in directing stem cell fate (3). This point was illustrated with several examples, including small molecules that directed HESCs or induced pluripotent stem cells (iPS) towards pancreatic β -cells (e.g., Fig. 2, **1** and **2**), and small molecules that could replace some of the four transcription factors (Oct-3/4, SOX-2, Klf4, c-Myc) in taking committed cells and returning them to an induced pluripotent state (e.g., **3** and **4**).

The exploration of stem cell biology with small-molecule probes needs to be performed with chemical tools that have been fully characterized, and this point was illustrated with dorsomorphin (**5**). Dorsomorphin is reported to be a potent and selective inhibitor of

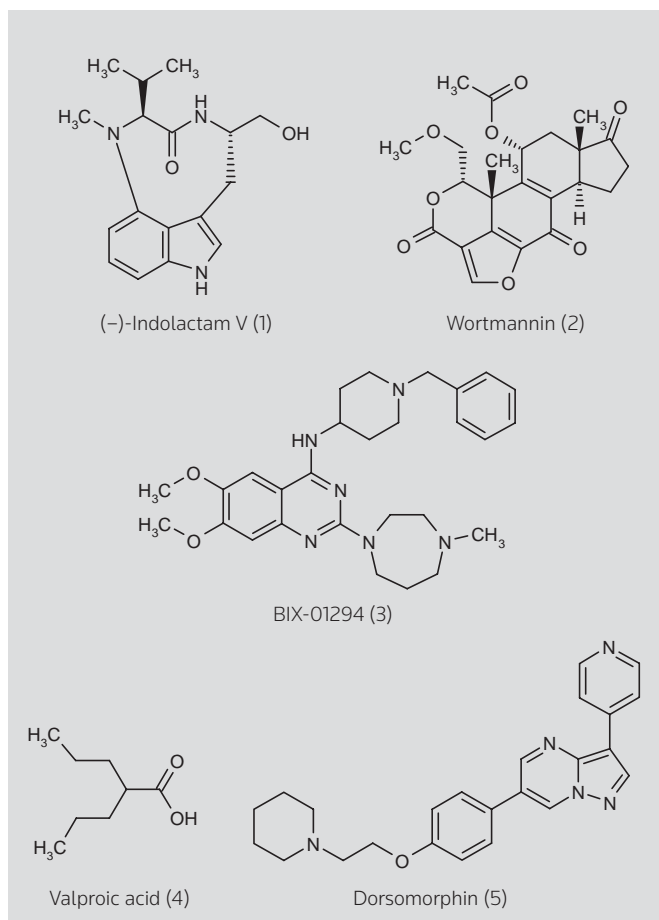


Figure 2. Small-molecule tools.

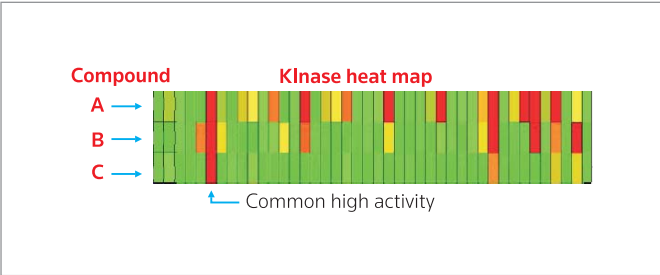


Figure 3. Illustration of the use of Pfizer's chemogenomics library.

AMP kinase, although in-house screening at Pfizer showed many other activities, making it difficult to align dorsomorphin's effects with a particular primary pharmacology.

Pfizer has created a chemogenomic library, which consists of 3,155 compounds aligned against 726 target mechanisms classified by gene family. There are up to five orthogonal compounds per target, giving a high confidence in mechanistic association if all chemogenomic tools for a given target are active. The Pfizer chemogenomic library was used to identify a new mechanism, modulation of which could promote differentiation of pancreatic endodermal progenitor cells towards β -cells (Fig. 3) (Note that compounds **A-C**, the primary mechanism and the β -cell markers were not disclosed).

PERSPECTIVE ON THE CHALLENGES OF CONDUCTING CLINICAL TRIALS IN REGENERATIVE MEDICINE

Dr. Paul Kemp (Intercytex [now Regenerative Medicine Assets], U.K.) gave a very informative and open presentation on Intercytex's experience in the clinical development of Cyzact® (ICX-PRO) as a cell therapy for the treatment of hard-to-heal dermal ulcers. The active wound dressing of Cyzact® is composed of allogeneic human dermal fibroblasts in a human fibrin matrix. The clinical challenges included: the absence of a unified view of the global regulatory bodies and how Cyzact® was classified (device or biologic); unlike previous products, the FDA required a cellular-free control arm (fibrin only); the commitment of Intercytex to physicians to have product continually available; and the introduction of a four-layer compression bandage as a new standard of care.

Intercytex's phase III clinical trial with Cyzact® centered at 62 sites across 3 countries and 400 patients. Patient inclusion criteria were venous leg ulcers of at least 3 months' duration, nonresponsive to conventional therapy, and roll-in of patients on four-layer compression bandaging with healing of < 30% in 1 month. The endpoints of the trial were either rate and incidence of closure (MHRA) or complete healing at 12 weeks (FDA). However, Cyzact® plus four-layer

bandage failed to show clinical significance when compared to four-layer bandage plus fibrin matrix or four-layer bandage alone (Table I). Subsequent reanalysis of the data did show a benefit for Cyzact® plus four-layer bandage over four-layer bandage alone if the healing cut off was reduced from 30% to 20% or 10%.

Intercytex's portfolio post-2008 has seen the successful divestiture of a number of products (e.g., cell line SHEF-1), and in 2010 the company now has a smaller, more focused portfolio. An example is Vavelta® (ICX-RHY) as a treatment for epidermolysis bullosa (dermal blistering through minor contact) or severe wound burn contractures. Case histories were presented that supported the use of Vavelta® for these conditions. Vavelta® is a suspension of human dermal fibroblasts (HDFs) in cell storage medium. When injected into the skin, HDFs are believed to lay down collagen within the dermis, which restructures and repairs the extracellular matrix.

THE MAKING OF CHONDROCELECT®: CLINICAL AND REGULATORY SUCCESSES AND CHALLENGES

Francois Meurgey (Tigenix, Belgium) described the development of ChondroCelect®, an autologous, cell-based medicinal product for cartilage regeneration, which is the first product to receive EU Marketing Authorisation under the advanced therapy medicinal product (ATMP) framework. Currently, approximately 2 million cases of articular cartilage defects of the knee are diagnosed worldwide, and the spontaneous healing capacity of cartilage is limited. ChondroCelect® was approved in October 2009 for the treatment of single symptomatic cartilage defects of the femoral condyle of the knee, and to date approximately 500 patients have been treated. The treatment bridges the gap between symptomatic relief and joint replacement. The product is comprised of characterized chondrocytes, which are harvested from the patient during diagnostic knee arthroscopy, expanded from ~200,000 to 12 million cells, and then reimplanted under open knee surgery using a collagen membrane to cover the defect. The speaker discussed the development challenges for ChondroCelect®, which included the changing regulatory framework landscape, since no guidelines were in place when trials started in 2001, and ensuring stringent product release criteria and consistent manufacturing for a complex and inherently variable cell-based product. Quality control criteria for identity, purity, potency and sterility were developed, and protocols for all aspects of the process, from surgeon training to enzymatic digestion, the expansion process and shipping were necessary. Also, a clinical score for cartilage was developed and validated during the clinical trials. The concept was assessed in a goat joint injury model, whereby goat chondrocytes were implanted into a cartilage defect, and good basal and lateral integration and surface repair were observed after 10 weeks. Control implantation of fibroblasts and dedifferentiated chondrocytes did not lead to repair.

Table I. Cyzact® clinical trial data.

Year of informed consent	Cyzact® + four-layer bandage	Four-layer bandage	Four-layer bandage + fibrin matrix
2005/2006	26% (22/85)	14% (6/42)	18% (7/40)
2007	24% (18/75)	37% (15/41)	29% (12/42)
2008	44% (16/36)	41% (7/17)	39% (7/18)

The pivotal clinical trial of ChondroCelect® compared chondrocyte implantation with microfracture, a surgical procedure in patients with grade III-IV symptomatic cartilage defects of the femoral condyle (4). The primary endpoint was structural repair assessed at 12 months by histology, with the secondary endpoint being referred to 36-month data on clinical benefit using the knee injury and osteoarthritis score (KOOS). Characterized chondrocyte implantation resulted in significantly better structural repair ($P = 0.003$) and clinical outcome (KOOS score of 26.08 vs. 17.09; $P = 0.048$). No major safety concerns were highlighted, and adverse events were as expected after open knee surgery procedures. Since therapy prevents patients developing osteoarthritis 10 years earlier than those without treatment, reimbursement is complex, and Mr. Meurgey discussed this issue and emphasized the need for the “value story” to be an integral part of the development plan.

ONTARIL®: AN AUTOLOGOUS ADIPOSE TISSUE TREATMENT

Dr. Eduardo Bravo (Cellerix, Spain) described the development of Ontaril® (Cx-401), a medicine based on expanded mesenchymal adult stem cells (eASCs) obtained from adipose tissue. Adipose tissue is not only readily accessible by liposuction, but is also rich in stem cells, containing 100-1,000 times more stem cells than in other tissues commonly used. Ontaril® was awarded orphan drug status in July 2005, and is currently in clinical trials for the treatment of complex perianal fistulas of cryptoglandular origin and for fistulas associated with Crohn's disease. A perianal fistula is an abnormal communication between the perianal space and the outer surface of the skin. Fistulas are considered complex when treatment is associated with a high risk of causing loss of anal continence, the fistulous track covers more than 30% of the external sphincter, several tracks are present and the fistula is recurrent or the patient suffers from incontinence, local irritation or Crohn's disease. The condition is debilitating, painful and gives rise to a compromised quality of life. It is estimated that there are 70,000 patients in Europe. Treatment of complex fistulas has a low success rate, is costly and is associated with a risk of fecal incontinence.

A phase I study with Ontaril® demonstrated the safety of the product, and was the first clinical study to be carried out with stem cells derived from adipose tissue. A subsequent phase II study in 50 patients confirmed the safety and efficacy of Ontaril®, demonstrating that it had the capacity to activate the body's own physiological repair mechanisms through its ability to interact with affected tissue. The primary objective of this study was to clinically and objectively assess complete closure and healing of the fistula. When treated with Ontaril® 71% of the fistulas closed in a severe patient population (80% had previously had surgery), and after 1 year, 80% of the fistulas remained closed. This was compared to the use of fibrin glue, after which only 16% of fistulas were closed. Two phase III studies in patients with and without Crohn's disease are currently underway comparing treatment with Ontaril®, Ontaril® and fibrin glue, and fibrin glue alone. However, interim results have revealed that fibrin glue alone had closed 40% of fistulas, raising doubts as to whether the efficacy of Ontaril® can be successfully demonstrated in

this study. Ontaril® is licensed to Axcan Corporation for rights in the North American market.

Dr. Bravo also described Cellerix's strategy to progress from the development of a local autologous product (Ontaril®) to systemic allogeneic products for the treatment of inflammatory and autoimmune diseases. Cx-601 is an allogeneic eASC in a phase I/IIA trial for perianal fistulas in Crohn's patients, and uses the same manufacturing and control processes as for Ontaril®. Cx-601 is also being tested for the treatment of rectovaginal fistulae. Cx-611 consists of allogeneic eASCs for systemic administration, for which encouraging preclinical results were obtained in a mouse model of collagen-induced arthritis. Treatment with eASCs significantly reduces swelling in joints, inhibits Th1 cytokine induction and elevates the Th2 response. Clinical trials of Cx-611 are expected to be initiated in 2011.

CONCLUSIONS

In recent years, significant progress has been made in bringing stem cell products forward into clinical trials and on to the market. Due to the novel nature of these products and a changing and heterogeneous regulatory environment, difficulties in conducting clinical trials have been experienced. Biotechnology companies have been the first to navigate and influence the regulatory agencies, building up a wealth of knowledge and experience. Larger pharmaceutical companies are also now investing significantly in bringing cell therapies to the market to treat important diseases and conditions. Of the products currently on the market or approaching the market, a variety of issues have emerged. In the case of autologous products, the product expansion process and cost of shipping represent factors that need to be taken into account, while for allogeneic products the challenges lie more with product yield and reproducibility.

In the area of small molecule manipulation of stem cells, the worldwide research community is investing time and effort in dissecting the targets and pathways important to the maintenance and developmental process of stem cells. There is particular interest in the identification of small molecules that can replace the four transcription factors (Oct-3/4, SOX-2, c-Myc, Klf4) in the induction of iPS (5). Ultimately, it will be of great interest to see if small molecules can work in harmony to replace all the transcription factors in reprogramming to induce iPS. Furthermore, small molecules that can efficiently promote stem cells or iPS to differentiate along a particular cellular lineage are likely to have considerable utility in the development of superior cell-based therapies or pharmacotherapies. To aid this activity, the small-molecule probes need to be fully characterized, such that their primary and secondary pharmacological activities are understood, i.e., their biochemical fingerprints. The use of such tools, or ideally a combination of orthogonal tools, would give higher confidence in mechanistic association.

DISCLOSURES

Dr. Wendy Alderton is a consultant at CB1 Bio, Ltd. Dr. Richard Davenport is a group leader in the Medicinal Chemistry department at Takeda Cambridge. Dr. Paul Fish is the exploratory chemistry leader for the Regenerative Medicine unit at Pfizer, UK. The SMR would like to thank Pfizer Regenerative Medicine for hosting this

meeting. The SMR Committee organizes conferences on behalf of the Society for Medicines Research four times a year. These 1-day conferences are multidisciplinary in nature and focus on various aspects of medicines research. Details of forthcoming meetings can be found at <http://www.smr.org.uk> or by e-mail to secretariat@smr.org.uk.

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