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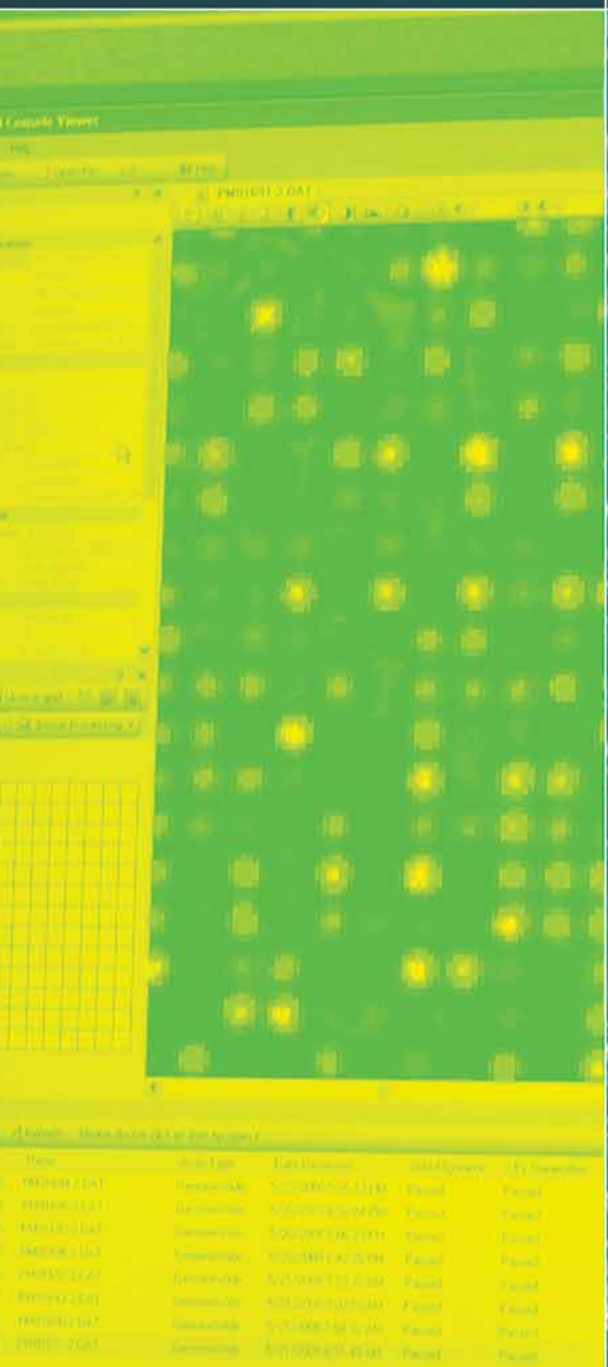

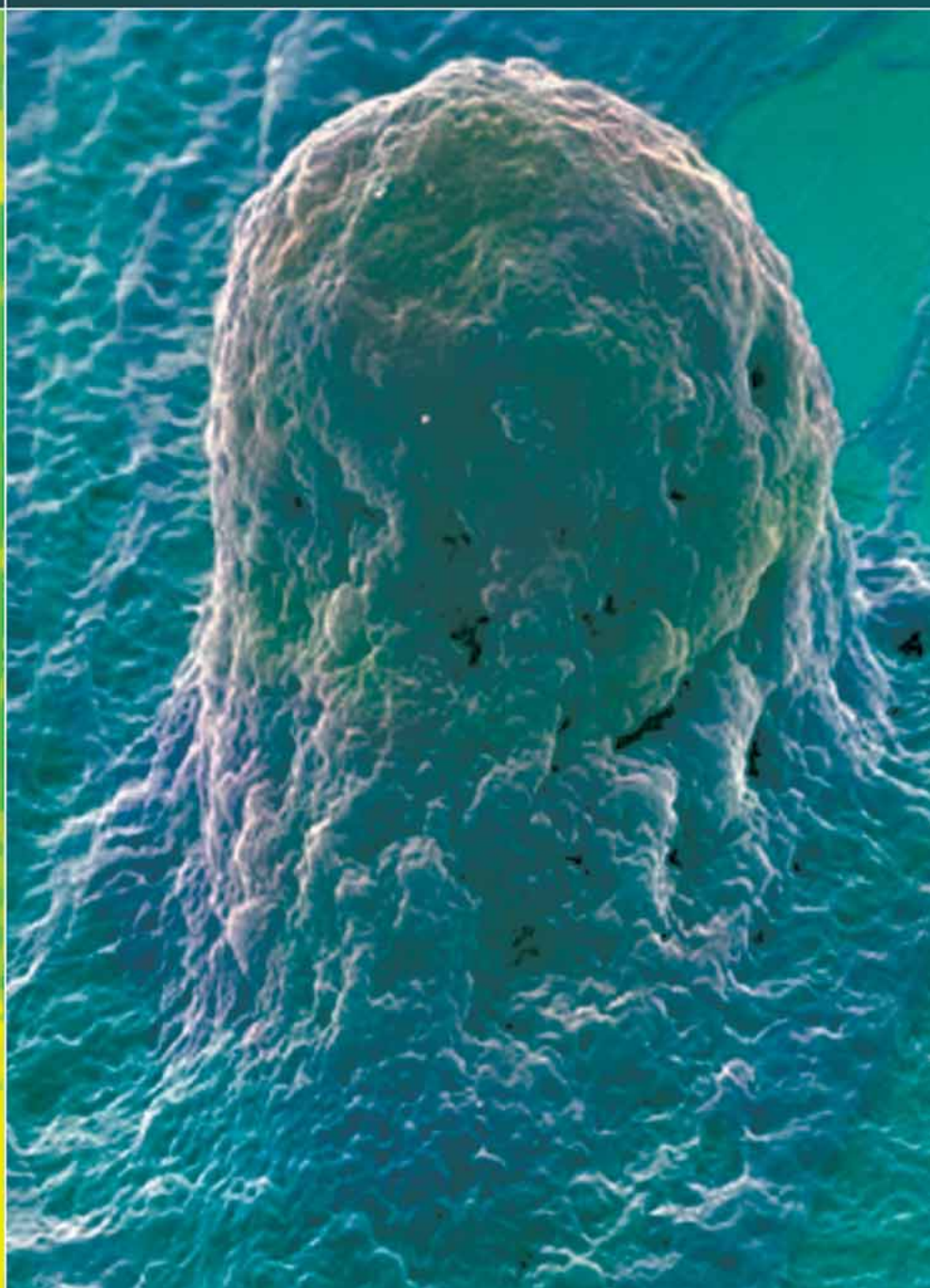

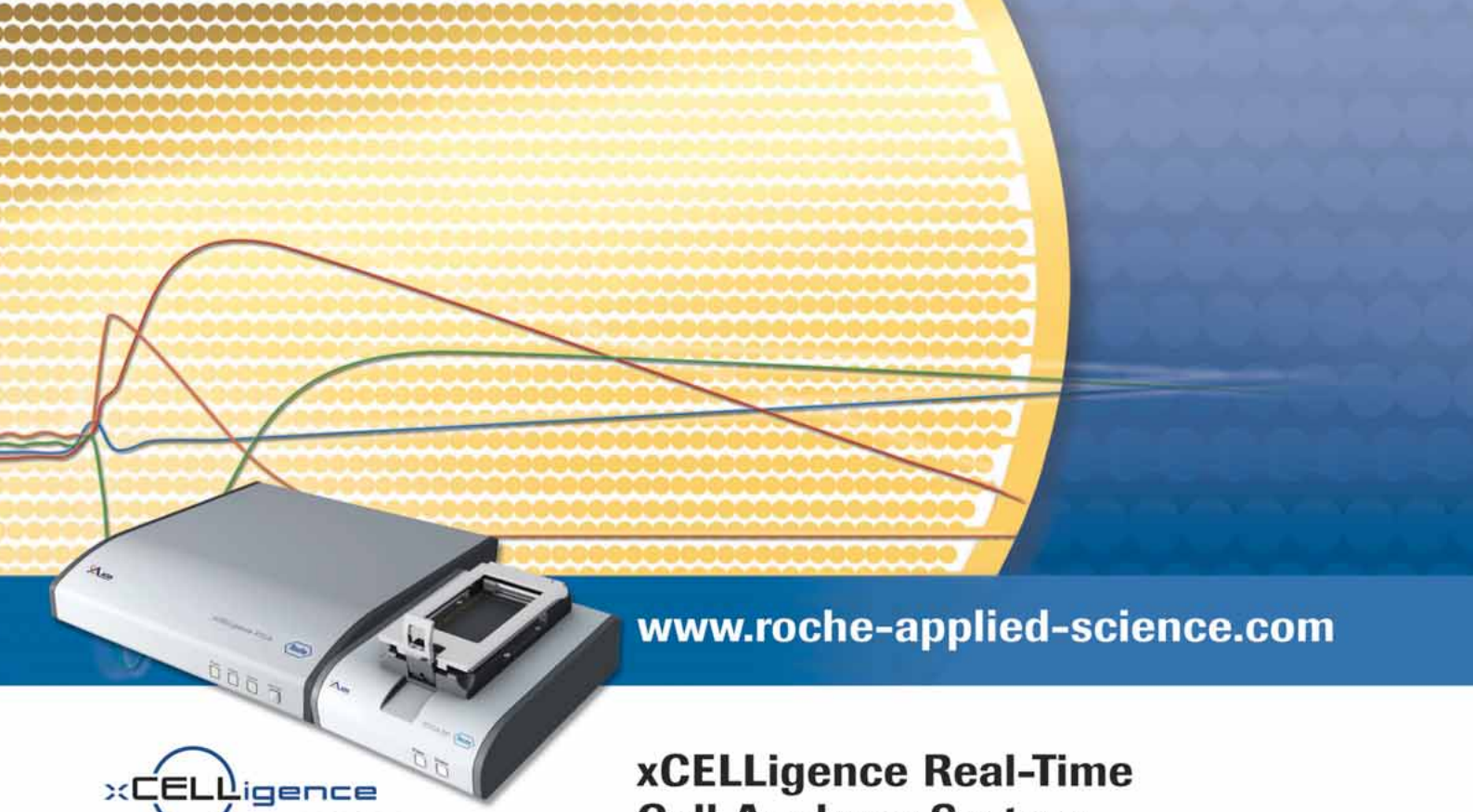

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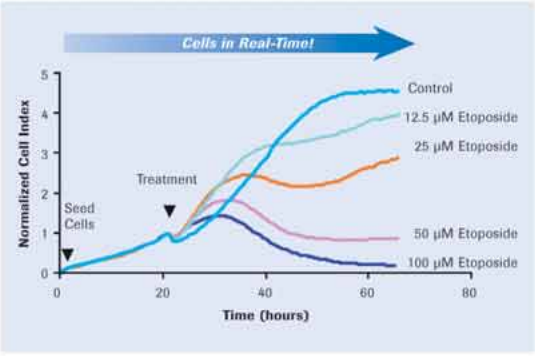

Figure 1: Real-time monitoring of cytotoxicity through DNA damage. Etoposide is a DNA damaging agent which induces apoptosis in high concentrations, while at lower concentrations it leads to S-Phase and/or G2 arrest.

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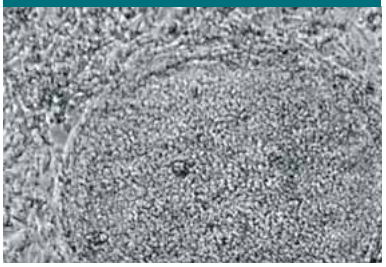

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MAGAZINE

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Stem Cells and Regenerative Medicines – Gathering Pace in 2008

The next phase of stem cell market is commencing. While ongoing tasks have to be finished and technologies to be improved, new challenges are awaiting. It's not anymore industry just following academic research.

TEXT | HUGH ILYINE, GUEST EDITOR

Over the last twelve months a number of significant funding initiatives have emerged, including the activation of California's Institute for Regenerative Medicine (CIRM) and the new European Union Innovative Medicines Initiative (IMI) in April this year. Exciting new technologies such as iPS cells have been confirmed by a number of research groups, and progress continues to be made towards novel stem cell based regenerative medicines. At the same time, improved tools and technologies to grow and differentiate stem cells under GMP conditions, with new serum free or animal component free media and new stem cell based assays for drug discovery and toxicology are emerging. Automated stem cell production has arrived. New stem cell banks have now been established in the US and the UK, to provide libraries of stem cell lines for research purposes. Development and refinement of guidelines and regulations for stem cell based therapies is underway in Europe and America. The International Stem Cell Initiative, which brought together the world's leading embryonic stem cell researchers to improve characterisation of a wide range of human embryonic cell lines, has moved on in its second phase to evaluate the cell line responses to a range of cell culture media, including longer term genetic changes. Drawing on the expertise of seventeen labs in eleven countries, it is a truly international collaboration, and indicative of the growing momentum now seen in this field.

INFO AUTHOR, GUEST EDITOR

Hugh Ilyine (1952) works as life sciences consultant, following eight years with Stem Cell Sciences plc from 2000-2008, where he acted as a director and Chief Operating Officer of the company. Prior to 2000, Hugh Ilyine spent seventeen years with the Rhône-Poulenc group in various international and managing director roles in France, Indonesia and Australia. He is a member of the Advisory Board of the Scottish Stem Cell Network, and an industry representative on the UK National Stem Cell Network.

Funding, … although in limited amounts

New national bodies such as the UK National Stem Cell Network have been established, dedicated to providing effective co-ordination between governments, funding agencies, researchers and industry. Stem cell conferences and symposia occur every month at the moment across Europe, the US and Asia, demonstrating the market for strong demand for knowledge. Despite the considerable challenges of finding adequate funding for early stage biotechnology companies, and stem cell companies in particular, funding has however been achieved if in limited amounts. Stem cell companies are active in developing products for the research tools and platform technology segments, as well as for cell based therapeutic. Others are combining both areas via hybrid business models, with this depending on their sources of IP and competencies. Media and reagent companies have commenced in-licensing of products emerging from academic and stem cell companies. Although the level of investment made in stem cells by the pharmaceutical industry has been small to date, signs are that significant increases in R&D spending in are now on the point of arriving, as discovery technologies are progressively validated their scaleability, cost and superior functional performance.

Turning science into tangible values

In this issue we have attempted to cover some of the elements of change across this very dynamic and broad field of endeavour, featuring an interview with Alan Trounson, CIRM's new President, and his views on the evolving landscape. A detailed review of cancer stem cells by Joanne Mountford and Tessa Holyoake of Glasgow University, with their application potential in drug discovery, and some of the early success and challenges facing this emerging field of research combines with Zhan Feng Cui's coverage on identifying the needs to grow stem cells at industrial scale without which exciting discoveries won't reach more than just a few patients, continue to remain in academic labs, or be exploited outside Europe and the US. Then, there are rather practical issues of sourcing human tissue which Morag McFar-

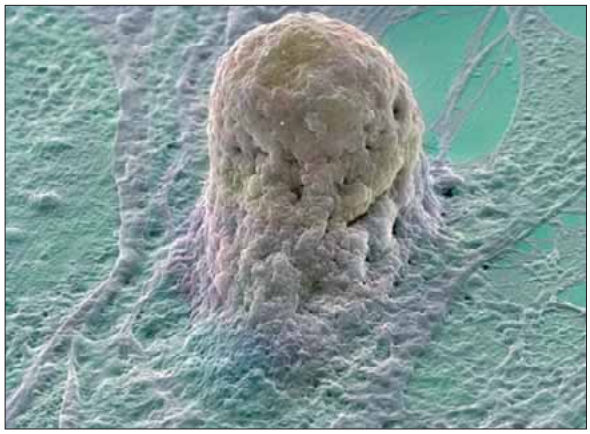
lane discusses, the technical task of improving cell culture media Mark Gerber and Dan Allison describe, as well as conforming to regulatory requirements which Steve Gibson explores. While William Lensch offers a stimulating glimpse into a very promising future of stem cell research, namely induced pluripotent stem cells, Michael Stierwald and Susana de Azevedo Wäsch provide a down to earth overview on bone replacement materials, which clearly shows that innovation has to build on prior art. At issue is turning science into tangible values both returns to attract further investments and benefits for the patient. Other contributions in this magazine like the report on pharma industry's views of stem cell technologies, Harald Stallforth's experience with a corporate investment in a regenerative medicine start-up, or Jonathan Knowles reflecting on personalised medicine, are vivid reminders that any professional discussion about stem cells should always strive to better inter-relate with ongoing other developments in science, business, and society.

New funding models needed

All in all, it's an exciting time to be engaged in the stem cell and regenerative medicine field. However, managing new discoveries all the way through to final products and applications remains challenging if not perilous for biotechnology companies, engaged as they are in co-ordinating IP, product development, regulatory conformity and approval, manufacturing and clinical trials. As investment is generally required with long-term time-frames, this translates to governments being essential to the process, both in the traditional financial support for academic research, as well as assisting development of stem cell technologies into the marketplace.

New funding models are under active consideration and implementation at this time, particularly to address the so called 'valley of death' faced by early biotech companies as they develop new products, and accelerate their progress to market. New funding models are particularly important to overcome the general lack of appetite by venture capital and the pharmaceutical industry to invest in the stem cell field. A recent exception to this has been Novocell, successfully raising US$25 million in July 2007 for the company's human embryonic stem cell R&D programme for diabetes. The funding was lead by the Johnson and Johnson Development Corporation, J&J's venture capital subsidiary. The 'gap' between the 'technology push' coming from the biotechnology companies, and the 'market pull' of users remains an issue, with the general complaint that the industry over-promises and under-delivers. Converting biological discovery at the bench to reproducible technology is never easy however. The new Innovative Medicine Initiative (IMI) project with its 2 billion euro budget committment, and its unique public-private funding model is of larger interest. Under IMI the European Commission will contribute 1 million euros, and a further €1bn contribution will be made from European Federation of Pharmaceutical Industries and Associations (EFPIA) companies. Calls have opened for eighteen initial projects with a total budget of €295m. Significant and equivalent expenditure is underway and planned by CIRM in the US designed to accelerate the pace of discovery and delivery into the clinic of stem cell therapies. The recently started UK Public Private Partnership known as SC4SM (Stem Cells for Safer Medicines), albeit on a much smaller funding level, has nonetheless brought together the UK government and national industry funding of research to deliver pre-competitive applications of stem cells in new toxicology assays. In Scotland, the Intermediate Technology Institute (ITI) for Life Sciences has invested for a number of years in the development of stem cell technologies, using government funding to accelerate academic/industry interactions and development of new products and services. Currently, other European governments like those in France or Spain are emulating this example.

Annie Cavanagh and Dave McCarthy

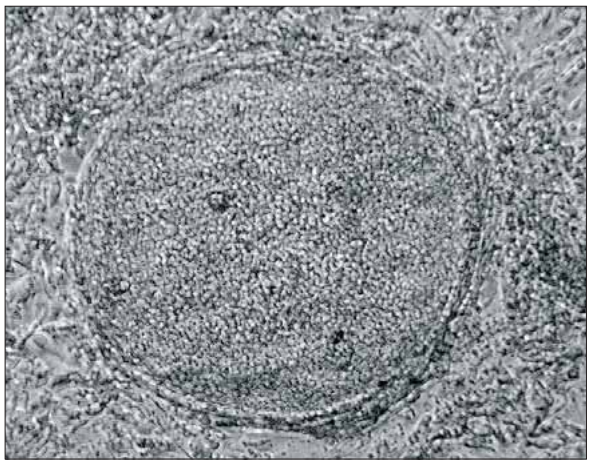


Human embryonic stem cell (gold) on a layer of supporting cells (fibroblasts), were grown by Jessica Cooke in Stephen Minger's labs at King's College London.

Varying business models

Many stem cell companies have focussed on developing non embryonic stem cells as the way to clinical applications, with a variety of foetal and adult sources. Blood and transplantation services are expanding their business. In parallel, tissue banks and cord blood banks, concomitant with refined logistics (supply chains, cryo-storage facilities) and direct to consumer services have been established in many countries, especially in Italy, to preserve this rich source of stem cells for current and future therapeutic options. By

In Hyun Park



Colony of human iPS cells (see also page 14).

contrast, far fewer companies are working with embryonic stem cells, indeed the typical business model is the academic operating unit providing colleagues-and-friends services.

Autologous stem cell therapies offer shorter time-frames through to clinical applications, as well as fewer safety issues, but have higher individual patient costs. Tengion's autologous cell based neo-bladder using a 3-D matrix has successfully demonstrated to date urinary bladder regeneration six months after implantation, along with functionality, in pre-clinical studies.

Adult stem cell therapies, such as those being developed by Osiris Therapeutics with their adult mesenchymal stem cells for Graft versus Host Disease (GVHD) and Crohn's disease, have with Phase I and Phase II trial success, moved on to enrolment for Phase III clinical studies, and represent exciting treatments options when the proven.

iPS cells: «a small step for stem cell research, but…»

Human embryonic stem cell therapies, while less advanced than adult stem cell therapies, continue to offer the prospects for broad scale applications across a range of illnesses or conditions such as spinal cord injury. Geron Corp remains a world leader in this segment, despite the FDA putting the company's IND submission for its cell therapy for spinal cord injury being placed on clinical hold.

A broad review of the emerging technologies would not be complete without some regard to 'Induced pluripotent cells', commonly abbreviated to iPS cells, and currently 'the hottest cells in town'. These cells are made through the integration of a small number of DNA transcription factors into adult somatic cells, and represent an exciting new development in the stem cell field. The iPS technology provides a novel reprogramming methodology to obtain pluripotent cells, an alternative to using embryonic stem cells or somatic cell nuclear transfer. Being able to take skin biopsies from patients and easily convert them into pluripotent cells opens up the possibilities of disease modelling, regenerative medicine, and their applications to drug discovery.

iPS cells were first discovered by Shinya Yamanaka and co-workers of Kyoto University, when they published in Cell in August 2006. Using retroviruses to transfect mouse fibroblasts with four stem cell associated genes, Oct-3/4, SOX2, c-Myc, and Klf4, they produced pluripotent cells. Yamanaka followed up by demonstrating iPS cells could be produced without the use of the gene c-Myc, but this early news was received in a relatively low key manner. However, in June 2007 with the simultaneous publications of Yamanaka, Jaenisch and Hochedlinger on iPS cells, confirmed the technology and its ability to produce pluripotent cells. Its appeal in avoiding using human embryos or undertaking somatic cell nuclear transfer was also quickly understood by interest groups outside the scientific community.

In November 2007, two groups, Shinya Yamanaka and also James Thompson at the University of Wisconsin-Madison created international excitement in their respective publications, covering reprogramming of adult human cells to a pluripotent state. In December 2007, George Daley of Harvard Medical School in Boston and his colleagues demonstrated that iPS cells could be generated from a wide variety of adult cells. Many stem cell research labs have now commenced work on the cells, given the tremendous opportunities to learn more about these cells and their properties, including how similar these cells are to human embryonic stem cells. Work to date indicates these cells as having similar morphology, cell surface markers, growth rates, differentiation and teratoma formation. Researchers agree the best way to proceed at the moment, however, is to continue with research into both human embryonic and iPS cells.

Thinking 'pharma' and 'chemical'

While these developments relate to medical applications other profitable stem cell businesses outside the therapeutic field are generally overlooked such as toxicology testing in pharmaceutical development. There may be a clear advantage for companies in Europe, near to the chemical manufacturers. Europe not only has the world's largest chemical industry, but also a REACH regulation that requires tox testing of chemicals. This regulation promises to be a gold-mine for those stem cell and tissue engineering companies able to think not 'pharma' but 'chemical', meaning small margins and large bulk volumes or high margins but short product development cycles in the case of fine-chemical manufacturers.

The beginning of the next phase

It looks like the coming twelve months will be an exciting year for the stem cell field, and one of real progress and increased momentum. Already, European in contrast to US pharmaceutical companies are voicing their own individual positions in use of stem cell technologies. They are setting the pace for others to follow behind. The beginning of the next phase that London-based financial analyst Navid Malik prognosed in BioWorld *EUROPE's* last year stem cell edition appears to have commenced. □

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Cancer stem cells – lessons from leukaemia

Whilst not new, the cancer stem cell hypothesis is gathering support as primitive disease-sustaining cells are being identified in an increasing range of tumours. Worryingly, it appears that these primitive cells may be comparatively resistant to existing anti-cancer treatments. It is anticipated new drugs targeted specifically to cancer stem cells will improve future treatments of a range of cancers.

JOANNE MOUNTFORD, TESSA HOLYOAKE | TEXT

The cancer stem cell hypothesis proposes that tumours originate from and are sustained by a small sub-population of stem cells (SC). These cancer stem cells (CSC) have the capacity to self-renew (Fig. 1) and also to differentiate into the heterogenous cells that form the bulk of a tumour. In contrast to previous dogma it also proposes that only the CSC population, and not the majority of the tumour cells, have the ability to propagate the disease. In this way it is thought that cancer development may mimic normal tissue formation and maintenance by the activity of endogenous SC.

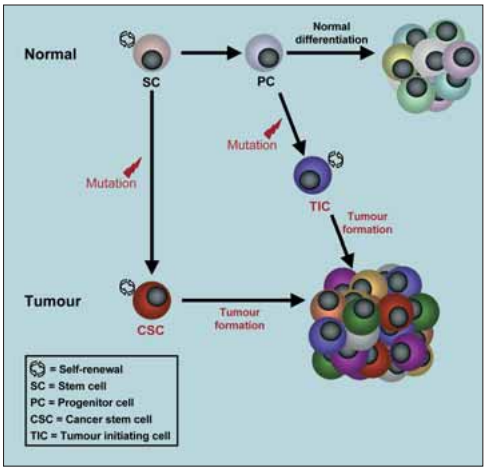


Figure 2: Models for Cancer Stem Cell (CSC) and Tumour formation.

The origin of CSC remains a matter of some debate. In one model it is thought that a normal SC becomes mutated and transforms into a CSC. An alternative mechanism is that a more differentiated progenitor cell, which has lost the capacity to self-renew, becomes mutated and re-acquires stem cell characteristics including self-renewal. These transformed progenitors are also referred to as tumour initiating cells (TIC) as they may not meet all the criteria of stem cells (Fig. 2).

However, it is becoming increasingly clear that in most tumours there is a population of cells with SC properties. Although originally described in leukaemia, CSC have now been characterised from melanoma; prostate, breast and colon tumours; neuroblastoma, medulloblastoma and glioblastoma. These have been reviewed in (Lobo et al., 2007). CSC are being sought in most tumours now, but investigations of haematological tumours lead the way for a number of reasons. Firstly, samples of malignant tissue are relatively easily obtained from bone marrow or circulating blood, unlike many solid tumours. Secondly, the haemopoietic hierarchical development system has been studied and characterized in depth for over 4 decades, including haemopoietic stem cells(HSC). ,Although postulated for many years, the first formal demonstration of a CSC population was made from acute myeloid leukaemia (AML) in 1997 by Bonnet & Dick (Bonnet et al., 1997).

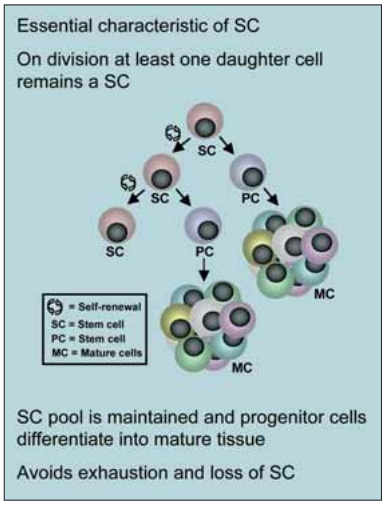


Figure 1: Self reneval

Towards Cancer Therapies and Targeted Drugs for CSC's

The existence of a CSC population which sustains tumours raises a number of issues when considering therapies, particularly with respect to targeted drugs. To eradicate a cancer and not just control the tumour, it will be essen-

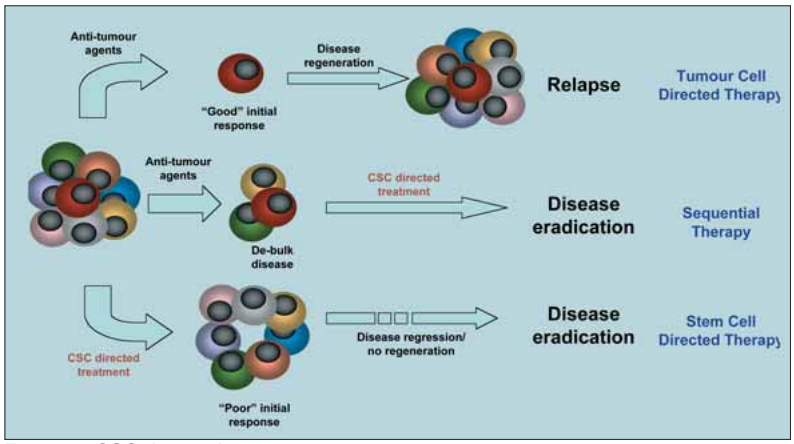


Figure 3: CSC directed treatment

tial to ensure that the CSC population is destroyed. Sequential or combination treatment with separate drugs to target the bulk tumour population as well as the CSC population may be required (Fig. 3). Studies show CSC are considerably more resistant to radiotherapy, chemotherapeutic agents and even molecularly targeted small molecules, representing new challenges in discovery to find find selective toxic compounds to CSC that do not kill 'normal' stem cells or non-tumour cells. Academic as well as industry researchers have now started down this path.

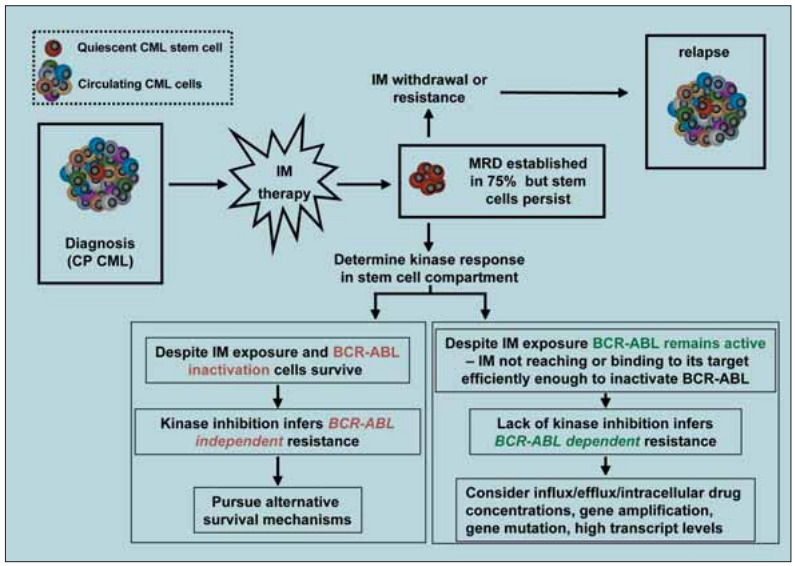
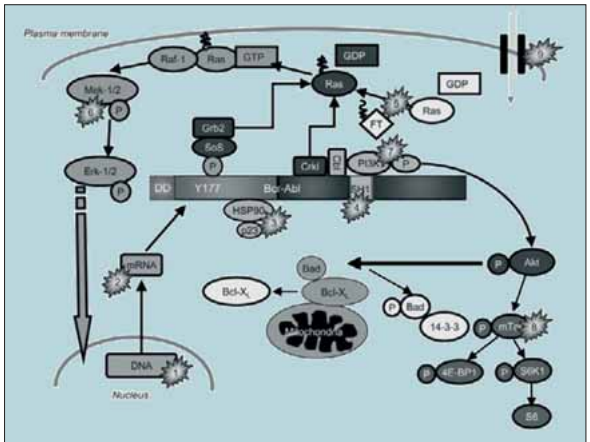


Figure 4: Combining IM with other drugs.

Our work at the University of Glasgow's Division of Cancer Sciences and Molecular Pathology has focussed on chronic myeloid leukaemia (CML), a cancer where too many myeloid cells (one of the main types of white blood cells) are produced. Incompletely maturing cells fill up the bone marrow, preventing it from making other blood cells properly, with risk of infection and chronic illness following. CML results from a single genetic mutation, a translocation of chromosomes 9 and 22 forming the Philadelphia chromosome, and the resulting oncogenic fusion protein Bcr-Abl. Bcr-Abl is a constitutively active tyrosine kinase which causes activation of multiple anti-apoptotic and pro-proliferative signals. The reliance of CML cells upon Bcr-Abl has been exploited through the development of imatinib mesylate (IM), a tyrosine kinase inhibitor (TKI). However, a significant amount of work demonstrates that CML SC are not eradicated by IM, and indeed, IM has a cytostatic effect on these cells, resulting in their relative accumulation. Additionally, both CML SC and more mature cells can develop resistance to IM via protein mutation, reducingdrug



	Pathway / molecular target	Example of Inhibitor
1	Bcr-Abl point mutations	**Nilotinib, Dasatinib**
2	mRNA	siRNA vs anti-apoptotic genes
3	HSP-90 antagonists	**17-AAG**
4	Tyrosine kinase inhibitors	Adaphostin
5a	Farnesyl transferase inhibitors	**Lonafarnib**, R115777, **BMS-214662, BMS-225975**
5b	Farnesyl / geranylgeranyl transferase	Zoledronate
6	Mek (MAPK or ERK kinase) inhibitors	PD098059, PD184352, **U0126**
7	Phosphatidyl inositol 3-kinase inhibitors	**LY294002**
8	mTor inhibitor	Rapamycin
9	Active transporters	Range of agents

Figure 5: Possible signalling pathways that may be targeted to complement TKI activity in CML SC.

INFO AUTHORS

Author

Joanne C. Mountford (1969) studied Biological Science (1991: BSc. Hons Microbiology) and obtained her PhD in Immunology from the University of Birmingham (UK) in 1995. She did post-doctoral research at IGBMC, Strasbourg (FR), in the Department of Pharmacology Universities of Oxford (UK) and Medical School Birmingham, and later at St. Jude Children's Research Hospital, Memphis TN (US) until 2002. Since then she works as Lecturer in Stem Cell Technology at Glasgow University's Faculty of Medicine.

Author

Tessa L. Holyoake (1963) qualified in Medicine and Surgery (1985: MB, ChB) at the University of Glasgow, MRCP (U.K.) in 1989, MRC Path. (U.K.) 1995 and was awarded her PhD in Haematology at the University of Glasgow (UK) in 1996. During that time and later she worked at several hospitals and clinical research laboratories in Scotland as well as Terry Fox Laboratories, Vancouver (CA). In August 2001 she joined University of Glasgow's Royal Infirmary as Reader in Academic Transfusion Medicine and Honorary Consultant in Haematology/Bone Marrow Transplantation. In 2004 she was nominated Professor in Experimental Haematology and is currently Head of Section of Experimental Haematology and Haemopoietic Stem Cells at Gartnavel's Paul O'Gorman Leukaemia Research Centre. She has collaborated in several clinical trials with major pharmaceutical companies among them Chugai, BMS and Novartis and serves as Advisor to commercial and non-commercial clinical trials on an international scale.

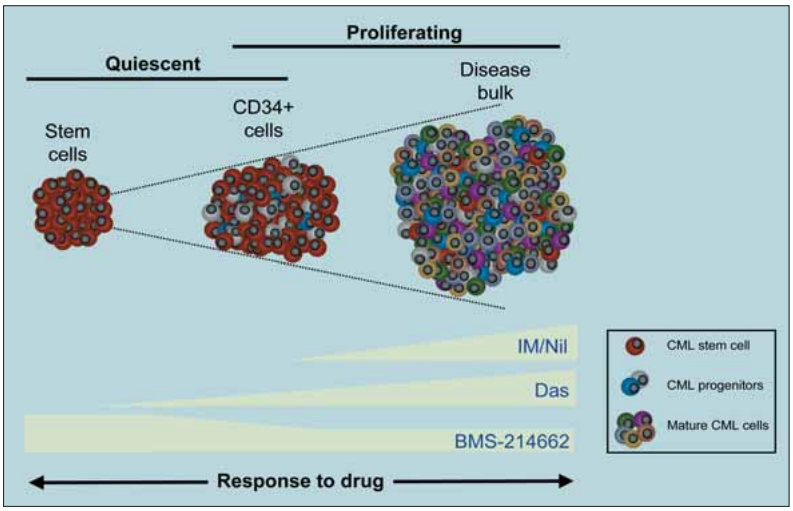


Figure 6

binding, , decreases IM cellular uptake and increases IM removal. As well,over-expression of the Bcr-Abl protein results in cell proliferation of the CSC, the opposite to what is desired in a treatment. While new generation TKIs have been developed to overcome these issues and increase efficacy, many of the same problems persist. Nilotinib inhibits most mutant forms of Bcr-Abl but is relatively ineffective against CML SC. Dasatinib, a dual src-kinase/abl inhibitor although much more potent and with a greater effect against cycling CML SC or progenitor cells (PC). still fails to induce apoptosis of primitive CD34+/ CD38- non-cycling CSC isolated from chronic phase CML samples. (Jorgensen et al., 2007).

It should be noted that while in conventional anti-cancer treatment a cytostatic response is a desirable effect, inducing quiescence/cell cycle arrest of CSC is unwelcome, as it results in the persistence of a reservoir of diseased cells, which can accumulate additional mutations, develop further resistance mechanisms, and hence fuel re-emergence of the disease.

Attempts in our labs to date to increase the efficacy of Tyrosine Kinase Inhibitors against CML SC by combining IM with other drugs (Fig. 4) have, with the exception of the farnesyl transferase inhibitor (FTI) lonafarnib, not had positive effects. Indeed, most of them exacerbated the cytostatic effect. Attempts to break the quiescence inducing effects, including use of granulocyte colony stimulating factor (G-CSF) have not met with success. Inhibition of Bcr-Abl by TKI appears insufficient to kill CML SC. Further understanding of the CML SC survival mechanisms is worthy of further research.

Based on the slight effect of lonafarnib we looked further at additional FTIs and discovered that BMS-214662 had a profound effect on CML CSC. Although cytotoxic FTI has been reported to preferentially kill non-proliferating cells, we found that in *in vitro* assays BMS-214662 causes apoptosis of both dividing and quiescent CML cells, including the most primitive CSC. In *in vitro* long term stem cell colony assays BMS-214662 was found to cause almost complete elimination of colony forming capacity and, more importantly, overcame the cytostatic effect of either IM or dastinib (Copland et al., 2008). As the number of compounds tested on CSC increases, we expect new candidate compounds will emerge to control cancers. Interestingly, an analogue compound (BMS-214975) with almost identical FTI activity does not kill CML cells, indicating BMS-214662 action is via an unknown mechanism, with an off target effect (Fig. 6).

Conclusion

The discovery of cancer stem cells in tumours, and the importance of selectively killing these cells to improve therapeutic success has opened up a new and important research area in drug discovery. Further basic discovery in conjunction with industry partners will be important in testing candidate compounds, such as has been demonstrated by the CSC/ CML cell *in vitro* assays. Similar efforts are going on in the neural cancer stem cells.

A major challenge in developing drugs that selectively target CSC is when sharing many fundamental properties with normal SC (Fig. 7), specificity of targeting will be perhaps more difficult, but critical to successful development.

Better understanding of the biology of cancer stem cells remains crucial, as well as their normal counterparts. In conjunction with new culture systems and assays to study cancer stem cells, an important and exciting new field of discovery is now emerging. □

New targets for SC directed drugs?
Notch pathway
Wnt/b-catenin pathway
NfkB pathway
Hedgehog (Hh/Shh) signalling
ROS resistance
Efflux transporters
Hypoxic adaption
Glucose transport
Fatty acid synthesis
SC-niche interactions

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Bone Replacement Materials

An overview of bone replacement materials particularly for dental applications is provided. Critical factors of bone replacement materials are discussed as well as the latest regulatory requirements for such products.

TEXT | MICHAEL STIERWALD, SUSANA DE AZEVEDO WÄSCH

Bone is besides blood one of the most common materials to be transplanted. The need to replace damaged or diseased bone tissue is constantly increasing, mainly due to the demographic trends towards an aging population. In the dental field, functional and aesthetic viewpoints getting more and more important. Patients needing tooth replacement do often not have adequate bone to provide sufficient stability for a dental implant. Measures for bone regeneration are necessary in about 40% of all implantation procedures.

The understanding and knowledge of bone tissue and bone regeneration phenomena is a fundamental prerequisite for the development of new bone substitute materials and regeneration techniques. Bone and bone tissue fulfill several crucial functions, for example as calcium reservoir of the body. Bone is continously remodelled in response to changes in mechanical forces. Osteoblasts are the bone-forming cells and therefore important during bone tissue regeneration, whereas osteoclasts resorb old bone or bone that is not under any load. A number of physicochemical variables influences bone repair. For instance healing is inhibited by movements. The highest proportion of bone is mineral such as carbonat ions, magnesium, sodium, hydrogenophosphate ions and trace elements, the rest is water and matrix. Bone tissue represents the largest proportion of the body's connective tissue mass.

A plethora of methods and compositions is used to repair or regenerate bone tissue *in vivo*.

Bone substitute materials comprise natural or synthetic polymers, ceramics, composites, natural or processed bone, biological materials such as growth factors etc. In general, bone graft material should be osteogenic, i.e. it should have the ability to create new bone. The material should support the attachment of osteogenic precursor cells (osteoconductive) and additionally be osteoinductive, which constitutes the ability to induce undifferentiated stem cells or osteoprogenitor cells to differentiate into osteoblasts.

In general bone replacement material tries to mimick the chemistry and microstructure of human bone. Certain parameters such as crystallinity, solubility, particle size, porosity, pore structure and pore size of the material are of paramount importance and may greatly influence bone compatibility and bone integration. An inappropriate combination of those parameters leads to failure of bone repair. Excessively large pore size and high porosity of the material can lead to excessive resorption rates that often causes an inflammatory response. Small pore size and low porosity of the material will lead to low resorption rates causing encapsulation of matrix particles. It is generally believed that reabsorption should occur within several months and the ideal dissolution rate would be almost identical to the bone growth rate. Different pore sizes are required for cell in-growth and proliferation, vascularization or cell adhesion processes. A three-dimensional coexistent network of interconnected pore sizes may lead to optimal results. Autologous bone graft and allograft materials, bone replacement material of natural and synthetic origins are the main categories of bone replacement materials.

1. Autograft

Autologous bone is harvested from a first anatomical site and reimplanted into a second anatomical location. Autograft represents the gold stan-

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dard for bone grafting. It is the most reliable method to repair bone defects. However, it has some serious drawbacks including prolonged operative and rehabilitation time and problems that may occur at the second surgical site where the graft is implanted. Formation of scars, significant blood loss, increased risk of infection, additional short-term and long-term pain at the site of harvesting as well as donor-site morbidity are the inherent risks of this technique. Autologous bone is only available in limited quantities.

2. Allograft

Allograft is bone graft between individuals of the same species, i.e. form another patient. It is believed that allografts have a lower osteogenic capacity than autografts and therefore the rate of new bone formation might be lower. Even though there is little probability, allografts bear the risk of disease transmission, bone resorption, larger immunogenic response up to rejection of the material. Allografts may require the administration of immunosuppressant drugs to the patient. Prior to implantation, allograft bone material is duly processed with chemical treatments. The need to remove immunogenic cellular materials may conflict with the biomechanical properties of the graft material. Allograft bone material is available only in limited quantities and the variable bone quality and physical characteristics of the donor may influence the use of such materials. Demineralized bone particles or demineralized bone fibres are variants of allograft bone materials.

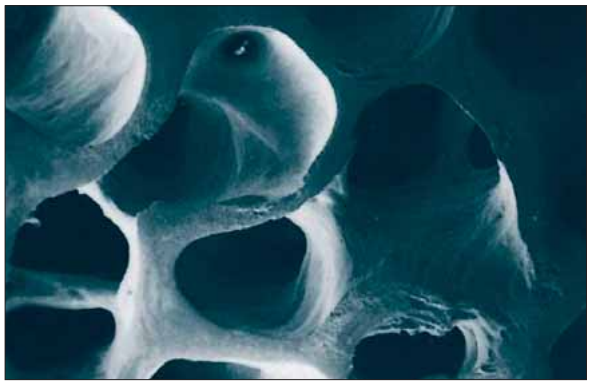
3. Bone replacement material of natural origin

The use of bone replacement materials of natural origin including xenograft bone has gained increasing acceptance since techniques for removal of potentially pathogenic organisms have become more reliable. However, some bone replacement materials of natural origin are very well established and exist for more than twenty years. The main advantage of bone replacement materials of natural origin is the high similarity to the microporosity of the spongy structure of the human bone. The preserverd porous architecture and fine-crystalline structure of the natural bone serves bone cells as guide rail. Bone replacement materials of natural origin are mainly extracted from corals or bones of bovine or equine origin. The extraction procedures often comprise substantial cleaning steps with different kinds of solvents. The resorption rates of bone replacement materials of natural orgin are rather slow. In contrast to other bone replacement materials, certain bone replacement materials of natural origin are described extensively in the literature over many years. Together with synthetic materials bone replacement materials of natural origin are available in almost unlimited quantities.

4. Synthetic materials

Synthetic bone substitutes represent a further alternative of bone replacement products. Typical synthetic bone materials include calcium phosphate compounds (hydroxyapatite, tricalcium phosphate etc.), bioglass, calcium carbonate and the like, used alone or as mixtures. Among the synthetic materials calcium sufate hemihydrates was one of the first materials investigated. In general synthetic bone materials may be prepared cheaply in any quantity. There is no risk of disease transmission. Synthetic bone replacement materials represent a rather heterogenous group. The biocompatibility among synthetic bone replacement materials may differ tremendously. Resorption and dissolution rates, cell toxicity effects and immunogenic reactions are the most important distinctive features.

In the last years there has been a focus on calcium phosphates as hydroxyapatite and tricalcium phos-



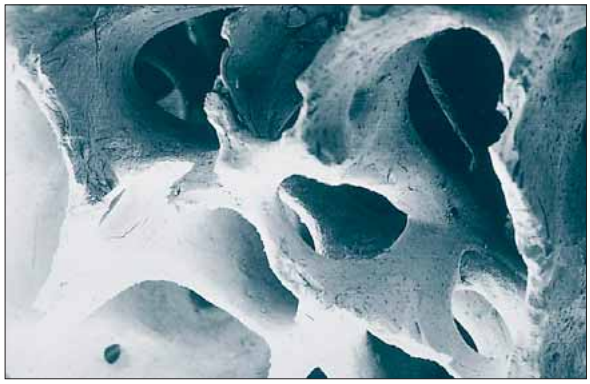


TABLE 1 Bone substitutes currently available on the market

	Manufacture / Distribution	Origin	EU	US
GEM21S	Osteohealth	Synthetic beta-TCP & rhPDGF	-	PMA
InductOs	-	Collagen Sponge (ACS) & rhBMP2	-	PMA
BIOGEN®	BIOTECK Italy	Natural: equine bone	CE0373	-
Biogran®	BIOMET 3i USA /ORTHOVITA	Synthetic (Glas mix Silicagel Ca, Ph)	CE 0483	
BioResorb® / BioResorb® Macro Pore	Oraltronics Dental Implant Technology GmbH	Synthetic beta-TCP	CE0297	510(k)
Biosite®	Vebas Milano	HA + Collagen		-
BONITmatrix®	DOT GmbH	Synthetic	CE	-
CERASORB®	Curasan AG	Synthetic beta -TCP	CE 0483	510(k)
Ceros®	Synthes	Synthetic beta -TCP	-	-
Collos®	Ossacur	Natural: Bovine collagen	CE1275	
easy-graft	Degradables solutions	Synthetic	CE	-
Emdogain®			CE 0086	
Fisiograft®	Ghimas S.p.A.	Synthetic: PGA & PLA	CE	-
FRIOS ® ALGIPORE®	Dentsply - Friadent 2003	Natural: marine algae	CE 0123	510(k)
Geistlich Bio-Oss®	Geistlich Pharma AG CH	Natural: bovine bone	CE0123	510(k)
Geistlich Bio-Oss® Collagen	Geistlich Pharma AG CH	Natural: bovine bone & porcine	CE0123	510(k)
Nanobone®	ARTOSS GmbH	Synthetic	CE0482	-
NuOss®	ACE	Natural: bovine	-	
Ossaplast®	Ossacur	Synthetic TCP	CE1275	510(K)
OsSatura®	Isotis	Synthetic HA/TCP		510(k)
OsteoGraf® N	Dentsply International	Natural: Bovine and Synthetic (HA)		510(k)
Ostim®	Aap biomaterials GmbH&Co.KG/ Heraeus Kulzer, Inc.	Synthetic: HA	CE	510(k)
Pepgen P-15TM	DENTSPLY Friadent Ceramed	Natural:bovine (P15) and Synthetic (HA)	CE	
PerioGlas®	Novabone Products	Synthetic: Glas (Si, Ka, Na, Ph)	CE	510(k)
Puros®	Zimmer	Natural: allograft	-	-
RootReplica	Degradable solutions AG	Synthetic	CE	-
Strauman® Bone Ceramic	Biora AB/ Straumann GmbH	Synthetic	CE 0510	
TARGOBONE®	Ossacur AG	Natural: Bovine collagen with antibiotic	CE	-
Tutogen®	Tutogen	Natural: Bovine	CE 1275	

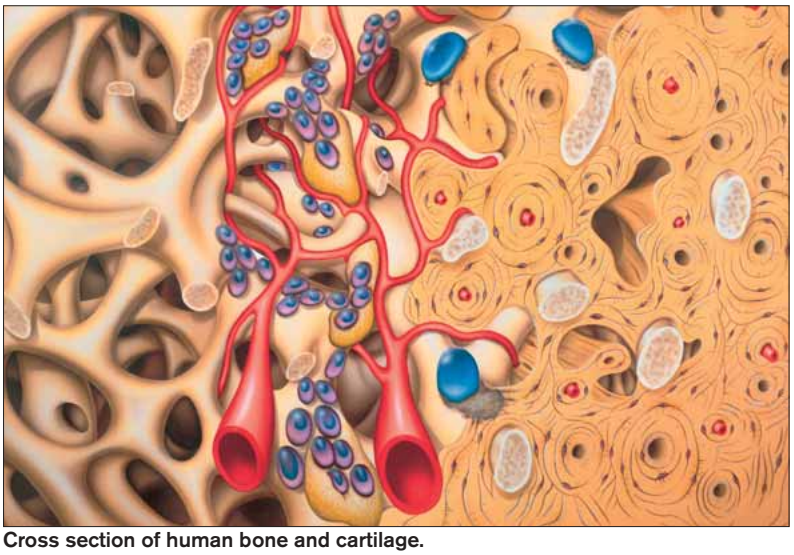
phate. Calcium phosphate itself has a relatively slow resorption rate, from several months up to several years. Hydroxyapatite is crystallographically and chemically similar to inorganic bone substance and biocompatible with bone. It has a very low solubility in a living body and complete replacement is practically impossible. Tricalciumphosphate degrades much faster than hydroxyapaptite. Tricalcium phosphate occurs in two polymorphisms, as beta-phase (hexagonal crystal structure) and alpha-phase. The beta-phase is used more often. There are lots of different methods of producing a porous beta-Tricalcium phosphate material. One method is the mixing of tricalciumphosphate-powder with a pore forming agent that decomposes at high temperature into gaseous decomposition products without leaving any solid residue.

Another example of synthetic bone replacement material is calcium sulfate, mainly used as gypsum or as plaster of paris mixed with water or saline to form a hardening paste. It has a dissolution rate between 25 and 60 days. Further examples of synthetic bone replacement materials comprise magnesium substituted calcium phosphate, carbonate hydroxyapatites containing magnesium, crystalline calcium phosphate embedded in a silica xerogel matrix, apatite/collagen composite fibers, compositions of several different ceramic particles in combination with cements and different types of polymers etc.

Besides the discovery of new materials, the technological trends of bone replacement materials directs to combinations of carriers with biologically active materials, such as stem cells, proteins, growth factors etc. Combinations of certain bone replacement materials with stem cells or growth factors are already on the market. In the future one major issue will be the development of drug release or delivery systems and better handling possibilities of the materials.

Regulatory requirements

Currently most bone substitutes that are available on the worldwide marked are regulated as medical devices having a natural or synthetic origin (see Table 1). Regulatory requirement for synthetic bone substitutes are most manageable since they are comparable with other inactive medical device implants. More demanding for a regulator are bone substitutes made of natural material, in particular made from animal sources e.g. porcine or bovine origin. Emphasis is put on safety issues that are particular related to the possibility of disease transmission (e.g. Viral and TSE/BSE and to avoid immunological reactions due to the presence of risky proteins). Consequently



Cross section of human bone and cartilage.

most companies use animal sources from which diseases are known and well observed due to the fact that these animals are processed for comestible purposes.

To fulfil the regulatory requirements for animal origin sources, a company has to comply with a variety of directives and standards and be continuously up-to-date with evolution and presence of animal diseases world wide. An extended safety review of the entire process focusing on animal source, safety assurance processing steps in the manufacture, and post marked surveillance are the basis of confidence for patients, medical practitioner, authorities and not lastly for the employees of the own company.

The request for faster and more differentiated ossification is often addressed by combination products like bone substitutes together with medicinal products. These medicinal products are often recombinant proteins, like growth factors, that somehow activate and hopefully accelerate the healing process. Device/drug combination products can theoretically be regulated as medical device or as medicinal product depending on the primary mode of action of the product. Therefore, it is crucial at the very beginning of the development of these kinds of products to consider the later regulatory process. The correct selection of design input criteria and endpoints during the preclinical and the subsequent clinical studies is fundamental for fast targeted regulatory process avoiding unexpected setbacks.

Furthermore, classification and evaluation of device/drug combination products might differentiate by FDA and EMEA. Combination products that are successfully available for several years on the US market are not yet on the European marked due to the delay that resulted from the different regulatory classification process. These companies have sometimes to perform additional pre-clinical studies, in the worst case to make further clinical studies and finally to rewrite the whole registration documentation. Fortunately, EMEA has presented in the last two years an easy process for small companies to get scientific or regulatory advice for these borderline products. It is advisable to benefit from this kind of EMEA consultation.

To provide bone substitutes combined with living human cells for perfect integration and optimal healing in patients, are one of the challenges of the future for manufactures. These products are currently developed by several manufacturers in most cases together with academic partners. The regulation process in Europe is specific for each individual type of combination products («case by case» is the EMEA wording) and this regulation process might require the cooperation between the notified body (for the device part) and the EMEA for human cells or the human cell based product. In December 2008 the guideline for human cell based medicinal products will be effective and certainly apply to several combination products of this kind. The technical evolution, from the non-living matrix, via the activated scaffold to the living bone substitute, correlates with increasing regulatory requirements that have to be considered by manufacturers. This evolution is an exciting field for ambitious regulatory employees since they can be involved in the development of a new regulatory area.

In the last years great improvements have been achieved with newly developed bone grafting materials. Finally it is the clinical requirement combined with the surgeon's preference that determines the market potential of each product. □

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- Directive 2003/32/EC regards medical devices manufactured utilizing tissues of animal origin.
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Challenges to stem cell bioprocessing

How to grow a lot of stem cells for therapeutic and biotechnological applications? How do we know what we get are the desired stem cells? How to store stem cells for future use?

TEXT | ZHANFENG CUI

Stem cells offer unprecedented potential for the treatment of many diseases and disorders such as Parkinson's disease, bone cell degeneration, Duchenne's muscular dystrophy, heart disease, and vision and hearing loss, as well as for tissue and organ regeneration [1]. Stem cells may be derived from the embryo, foetus, umbilical cord blood, or adult tissues. More recently it was discovered that differentiated cells, such as skin cells, can be reprogrammed to reverse the differentiated cells back to stem cells [2]. Stem cells are also the ideal cell source for engineered tissue development [3]. This is because most tissues consist of more than one cell type and the co-culture of differentiated cells to form complex tissues remains challenging. On the other hand, engineered tissues composed of multiple cell types may, in principle, be easier to construct by seeding stem cells and controlling their microenvironments at different locations during culture. A further emerging application of stem cells is for drug efficacy and chemical toxicology testing. The investigation of drug effect on proliferation and differentiation of human stem cells may provide more reliable data than simple monolayer culture of cell lines and be established as an alternative to animal testing [4].

For all the important potential applications of stem cells, either in regenerative medicine or for drug-testing, bioprocessing of stem cells is challenging. For example, how to culture stem cells efficiently, economically, reproducibly and on a large scale? Even for autologous stem cell therapy, i.e. using patient's own stem cells for therapy, a single treatment would typically need 100 million to 1 billion cells. A biopsy from bone marrow, for example, would yield around 1000-10,000 cells. Hence for a practical clinical application, the stem cell number must be increased by at least 4-5 orders of magnitude. Allogenic therapies or industrial tissue engineering will require even more stem cells of the same type; thus expansion of stem cells becomes increasingly critical. In addition the stem cells are prone to embark on differentiation pathways, which should be avoided during the expansion protocols.

Current way to grow stem cells in a cell biology lab is to use cell culture flasks or plates to passage the cell. This is a labour intensive inefficient method which heavily depends on operators and has high risk of contamination. Efficient expansion of stem cells needs novel processes using bioreactors. Many types of bioreactors, similar to those established in the biopharmaceutical industry may be used potentially to scale-up the stem cell culture. However to design and operate bioreactors at the scale relevant to clinical and industrial applications, the desired culture conditions, including cell culture medium compositions, oxygen, three dimensional support, etc, need to be determined and optimised. Unfortunately these remain largely unknown for many types of human stem cells. Multiple parallel perfused microbioreactors, which maintain stable and well controlled cell culture environment [5], can be useful to identify the most suitable stem cell culture conditions.

The second challenge to stem cell bioprocessing is the monitoring of stem cells and their functions. The commonly used methods to characterise stem cells depend on cell surface markers, which are not definite and can not be used to monitor stem cell growth in bioreactors. The method to ascertain the 'stem-ness' of the obtained cells is to use various differentiation assays, i.e. to check whether the cells can be differentiated into various cell lineages. This method takes days or even weeks and obviously is not suitable for process

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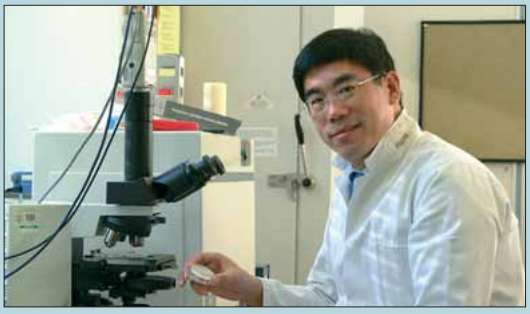

Professor Zhanfeng Cui obtained a BSc in Chemical Engineering from Inner Mongolia Polytechnic University, Beijing in 1982 and his Ph.D. in Chemical Engineering at Dalian University of Technology (CN) and held academic positions in Edinburgh University, UK (1991-94) and Oxford University UK (1994-2000) prior to the current post. Since 2000 he has been Donald Pollock Professor of Chemical Engineering Director of the Oxford Centre for Tissue Engineering and Bioprocessing (OCTEB), part of the recently established Oxford Institute of Biomedical Engineering (IBME). His research interests cover two distinctive but related areas: (i) tissue engineering and stem cell technologies, and (ii) bioseparation and bioprocessing.

monitoring and control. There is an urgent need for non-invasive non-destructive rapid techniques for monitoring stem cell status and functions.

One technique, particularly useful for stem cell differentiation monitoring, and for establishing and calibrating other assays, is the multi-photon microscopy (MPM) with spectral imaging analysis [6]. The technique uses near femtosecond infrared laser to excite fluorescent molecules either existing within the cellular system (auto-fluorescence) or the introduced molecular probes and obtain three dimensional images by layer-by-layer scanning. Compared to confocal microscopy, which uses UV laser, multiphoton microscopy can achieve deeper penetration and more importantly does not cause DNA damage hence enabling in situ long term observation once the laser power is optimised. The obtained 3D images can show stem cell co-location, cell-cell interaction, and 3D distribution in scaffolds. For example Fig 1 shows a 3D image of green fluorescence labelled human bone marrow mesenchymal stem cells (hMSC) cultured in Matrigel™ (the 3D video can be viewed at www.oxford-tissue-engineering.org). The emitted fluorescence signal, apart from visualisation, can be linked to specific cell functions. Many molecular probes, i.e. fluorescent chemical indicators on cell functions such as viability and cell membrane integrity, mitochondrial membrane potential, intracellular pH, calcium, oxygen, and metabolites, or stem cell functions are available for this type of monitoring. Fig 2 shows the differentiation of hMSC towards neuron-like cells assayed by Calcein AM-Peopidium Iodide probes.

Another bioprocessing challenge is how to freeze stem cells for long term storage. Cryopreservation is the process of choice for cell supply logistics and to ensure the off-the-shelf availability. The survival rate of human embryonic stem cells is typically less than 10% after cryopreservation. The most common cryoprotecting agent, DMSO, may cause gene mutation and its effect on stem cell functions is not clear. For clinical applications, animal product free and well defined cryoprotecting agents should be used, but there are no effective alternatives yet. Further requirement is the precise control of heat and mass transfer rate during each step of cryopreservation – addition of cryoprotecting agents, freezing, thawing and removal of cryoprotecting agents, as each step imposes additional stress to stem cells which may damage stem cells and their functions.

To speed up the translation from stem cell science to clinical and commercial applications, bioprocessing issues such as those exemplified above must be addressed. It should be pointed out that many fundamental scientific questions on stem cell biology need to be answered first. The combination of skills of bioprocess engineers and stem cell biologists and working with clinicians are essential to realise the promised potential of stem cells both clinically and commercially. □

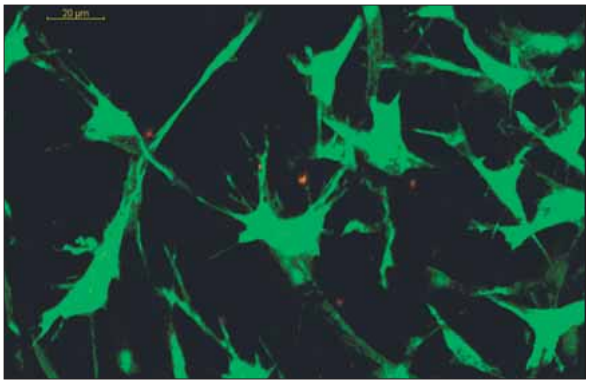

Fig 1 GFP-labelled human bone marrow stem cells culture in 3D Matrigel™

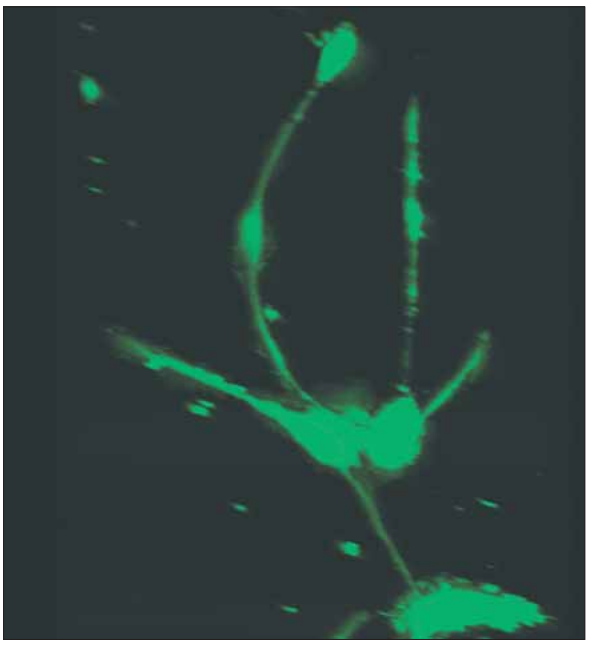

Fig 2 Human MSC differentiation towards neuron-like cells (Calcein AM-Peopidium Iodide assay)

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iPS Cells: The Needle of the Compass

The field of regenerative medicine is building steam due to recent breakthroughs in stem cell research. Newly generated induced pluripotent stem (iPS) cells point the way to tomorrow's medical advances whether used to screen for novel compounds or to facilitate the refinement of cells for use as therapy.

TEXT | M. WILLIAM LENSCH

Regenerative medicine is a phrase that sounds good, but how real is it? It's difficult to think of another area of biomedical research bearing as much promise and perhaps as many challenges. A large part of both is due to stem cells and their biological capacity to divide in a way that preserves functionally identical daughter cells (termed self-renewal) and also to differentiate into mature cells throughout the life of the organism. These two properties make stem cells the workhorses in regenerative medicine and efforts to refine our understanding of their biology occupy the field today. What's to be gained by forging ahead is enormous and includes a capacity to replace damaged or decayed tissues, discover drugs with key efficacy such as against rare cells, and define new approaches to disease management or even cures.

The hurdles to be cleared before these goals are met are multifaceted (Figure 1). Issues such as cellular delivery to sites of injury and the amelioration of prior scarring are areas in need of refinement or outright development. There are more barriers still such as purifying or generating enough cells to do the job (i.e. to meet the clinical scale needed) and for cells to be of the correct variety, whether considering immune system tolerance, disease state, and/or cellular type. While work using tissue-derived, so-called "adult", stem cells will undoubtedly continue to make important inroads, their rarity, difficulty to culture in the laboratory, and in most cases inaccessibility in living donors unfortunately hampers their success.

What's more, it seems clear that not all tissues in the human body are maintained by adult stem cells. While certain organs such as pancreas and heart had stem or progenitor cells in their earliest stages of growth, their tissue-specific progenitors fail to manifest or preserve stem cell activity later in life. Researchers will continue to seek ways to revive or direct tissue self-repair though other sources of pluripotent stem cells, those with the capacity to make any tissue in the post-natal human body, are subjects of intensive study.

Pluripotent stem cells: ES and iPS

Pluripotency is a biological term that describes the capacity to form cells from all three embryonic germ layers: ectoderm (including skin and nerves), mesoderm (such as muscle, bone, blood, and heart), and endoderm (gut, liver, and lung among others). Just as not all adult stem cells are functionally equivalent, all pluripotent cells are not the same where different types can be grown from fetal primordial germ tissue (so-called embryonic germ or EG cells), germ cell-related tumors such as teratocarcinomas (embryonal carcinoma or EC cells), or from normal, pre-implantation embryos (embryonic stem or ES cells).

Recently added to these are a variety called induced pluripotent stem or iPS cells. First described in mouse (Takahashi and Yamanaka, 2006) iPS cells are very similar to the types of pluripotent cells mentioned above though different from them in at least one major way: iPS are not isolated from a biological source such as a tumor or embryo. Rather, they are engineered, generated by a process of

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cellular reprogramming from a differentiated source such as skin cells via a method that "winds back" their developmental clock. While there are several reasons why iPS cells are a breakthrough of incredible proportions, one of the foremost is that they may represent a method whereby stem cells with a particular genetic makeup might become available, such as those matching a specific patient's immune system.

Why cellular reprogramming works

In a series of fascinating experiments ranging back well over 100 years, individuals such as August Weismann (1880's) and Hans Spemann (1920's and 30's) sought to explain the central question of developmental biology: "How does one cell divide to make two that are different from one another?" The principle that Spemann defined is known as nuclear equivalence and simply states that as cells divide going forward in development, each daughter cell inherits the same genetic information. Cells differ from one another due to restrictive use of subsets of genes to create tissue-specific patterns. King and Briggs (1950's) in the USA and Gurdon (1960's onward) in the UK built upon an understanding of nuclear equivalence to demonstrate amazing feats of cellular gymnastics by cloning new animals from the nuclei of cells taken from later stages of development that were then placed into egg cells whose own nucleus had been discarded (so-called nuclear transfer or NT). Unknown factors in the egg cytoplasm reprogrammed the mature nuclei to an embryonic pattern of gene expression.

Wilmut and colleagues in the UK took advantage of nuclear equivalence (and NT) to create Dolly the sheep from the differentiated nucleus of a mammary epithelial cell. A key observation from the use of NT is that it is possible to reprogram gene expression in a mature cell (such as a mammary epithelial cell) back to an early type such as an ES cell. Working in Japan in 2006, Takahashi and Yamanaka were the first to demonstrate that this could be done directly using defined genes.

Making iPS cells

A brilliant genetic screen permitted Takahashi and Yamanaka to narrow a list of candidate genes to four capable of reprogramming mouse skin fibroblasts to pluripotency: c-Myc, Klf4, Oct4, and Sox2. These genes were delivered to cells in a cocktail of retroviruses and within a few weeks post-infection, reprogrammed a small fraction of skin cells to be very similar to mouse ES cells. The mechanistic details behind how these four genes work are still being investigated though two, Oct4 and Sox2, are key transcription factors; master regulators of other pluripotency-promoting genes. The other pair, c-Myc and Klf4 are known oncogenes that promote cellular survival and enhance a cell's flexibility to shift patterns of gene expression.

In late 2007, three laboratories (including Yamanaka's) reported the derivation of human iPS from skin fibroblasts (Park et al., 2008b; Takahashi et al., 2007; Yu et al., 2007). These reports were hailed by some as breakthroughs as iPS cells require neither eggs/NT nor the destruction of human embryos (though they build upon information gleaned from both of these activities). In August 2008, George Daley's laboratory in the USA published the generation of a cadre of human iPS cell lines representing 10 different genetic diseases including juvenile dia-

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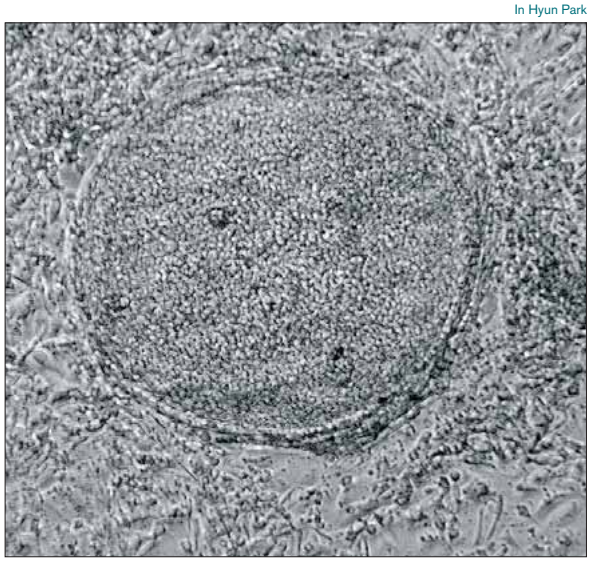

Phase-contrast image of a growing colony of human iPS cells on a background of mouse «feeder» cells.

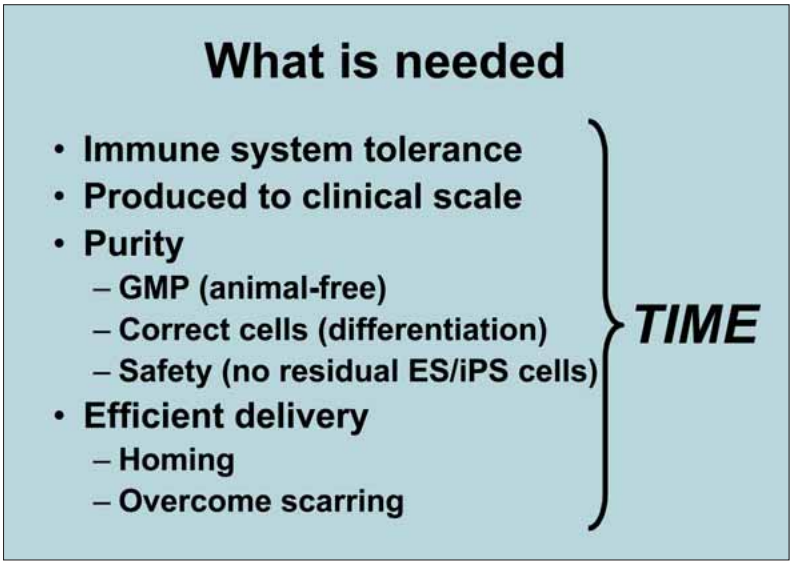

Figure 1: For stem cells to have the best chances for effective medical use, several important hurdles must be overcome including: immune system matching, sufficient numbers to be of benefit, and acceptable purity. Furthermore, delivery issues remain difficult for many organs and tissues and stem cells must be either purified or generated and directed to the cell of interest (when needed) in a manner that permits timely use in the clinical setting.

betes, Parkinson disease, and Down syndrome; all as part of an effort to glean greater mechanistic insights into how these complex conditions developmentally unfold (Park et al., 2008a).

Looking ahead

What is it going to take to use cells as medicine? For one, iPS cells are not ready for clinical use in their current form. While now capable of being established without the use of the cancer-causing c-Myc gene, as of today, the iPS process still requires the use of retrovirally-delivered transgenes to direct reprogramming. Viral transgenesis carries the risk for cancer due to the potential for activating nearby oncogenes or by mutating a gene when the virus inserts its DNA into a cell's genome. Retrovirus-free cellular reprogramming is the next technological wave and efforts are well underway in this area. Chemical modulators or completely transgene-free systems might also be possible where a recent report indicates that the histone deacetylase valproic acid improves reprogramming efficiency (Huangfu et al., 2008).

Beyond this, the greatest hurdle for iPS cells is the same as for other pluripotent cells, namely, learning to efficiently direct them to mature into the cells of interest. Human iPS cells have recently been generated from a patient with ALS and successfully directed to form neural cells (Dimos et al., 2008). While a great deal remains to be learned about how to enforce particular pathways of directed cellular differentiation, as an example, the availability of large amounts of ALS-affected neurons (due to the scalability of iPS and other pluripotent cells in culture) presents a potentially invaluable platform for high-throughput screening of compounds with specific efficacy in ALS; work that would otherwise be incredibly challenging. Such drug screens may well prove to be the first successful uses of pluripotent stem cells. No matter whether they facilitate an indirect advance by mining for new compounds or lead to the next generation of patient-matched cells capable of direct clinical use, human iPS cells are the needle of the compass pointing the way to regenerative medicine. □

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Critical elements in sourcing of human tissue for research in Europe

Most scientists' eyes glaze over when they learn about relevant regulations and guidelines covering the removal, storage, use and disposal of human bodies, organs and tissue for research applications. Key issues to be addressed early on.

MORAG MCFARLANE | TEXT

Most scientists' eyes glaze over when you, as a consultancy, start quoting all the relevant regulations and guidelines covering the removal, storage, use and disposal of human bodies, organs and tissue for research applications. In general terms, there are a number of key issues that must be addressed when sourcing human tissue for research use, whether this is from approved Research Tissue Banks or when establishing a prospective collection. These cover organs unsuitable for transplantation; samples donated specifically for research e.g. blood, other body fluids or small biopsies; post mortem material or so called "surplus tissue" left over from clinical and diagnostic procedures. The challenge is to translate this into a practical level in terms of obtaining the specific human tissues for their research.

Ethical Approval

You need to consider whether ethics approval is required for your project. For example, in the UK there is a requirement for all human tissue research to be approved by a recognised Research Ethics Committee (REC) or for the end user to have a licence from the Human Tissue Authority (HTA) (England, Wales & Northern Ireland). For further information please see the refer to the web links listed below.

Informed Written Consent

Consent is the central focus of legislation covering the donation and use of tissues for research and access to anonymised patient information. All donors must be fully informed in writing about the intent of the research and any potential commercial interests. Obtaining consent should be part of a dialogue to allow time to review the information and raise any questions. Depending on the circumstances, consent can be given by the individual themselves, a person with parental responsibility, by a nominated individual or a qualifying relative. There must also be room for consent to be withdrawn for whatever reason.

Governance and Quality Systems

When sourcing tissues from Research Tissue Banks you must ensure that they are properly licensed and that they have systems to ensure the provision of safe tissue with reliable quality. Their premises, facilities and equipment must be suitable for the storage of human tissue.

Audit Trails to Track Tissues

Systems must be in place to allow tissues to be tracked for health and safety reasons e.g. in case of infection. These records should include details of who gave consent; exactly what this related to including any restrictions on use; processes applied to the tissue e.g. RNA extraction; when and to whom a tissue is transferred (if applicable), e.g. to a third party, and finally when and where disposal is undertaken.

Having said this, one key thing is to ensure that what you are trying to source is actually feasible. Scientists do not always realise that what may work in an in vivo model may not necessarily translate directly into a human situation. For any prospective collection you need to get clinical input as soon as possible, ideally at the project planning stage. □

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Morag McFarlane (1968) obtained 1st Class Honors Degree in Molecular Biology from the University of Glasgow (UK) in 1989 and her PhD at MRC Institute of Virology in 1992, working as post-doctoral research assistant in molecular genetics until 1996. From Scotlab, Glasgow, she moved on to BioReliance, Stirling (UK) and joined in 1999 Scottish Biomedical working until 2007 as Principal Scientist. In October last year she co-founded Tissue Solutions Ltd. where she is Chief Scientific Officer.

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Improved Cell Culture Systems for Regenerative Medicine

While donor source and genetic engineering of donor cells are issues of keen interest, the need for high-quality, clinical grade, regulatory friendly raw materials remains as a critical concern. The article is discussing cell culture media, defined supplements, closed and/or disposable culture systems, and the efforts needed to address these issues.

TEXT | MARK A. GERBER, DANIEL W. ALLISON

The earliest realization of stem cell-based therapy occurred in 1968 with successful completion of the first bone marrow transplants 1,2. Since that time, the landscape of stem cell research and its impact on the treatment options for a number of human diseases has expanded tremendously. This expansion has been accompanied by increasingly complex regulatory issues that must exist to ensure that such treatments are safe as well as effective. Recent technological advances have brought the concept of personalized medicine from possibility to likelihood, and while donor source and genetic engineering of donor cells justifiably emerge as issues of keen interest, the need for high-quality, clinical grade, regulatory friendly raw materials remains as a critical concern. We will briefly discuss the issues in this area, including cell culture media, defined supplements, closed and/or disposable culture systems, and the efforts needed to address these issues.

TABLE 1 Regulatory issues associated with cell culture systems for cell therapies

Component	Issue(s)	Solution(s)
Cells	Biological contaminants	Source tested for various viral agents with a validated bank of clean material
	Reproducible cell sources	Validated culture methods with effective screening for reproducibility
Medium	Biological contaminants	Animal-component free formulations
	Undefined components	Chemically-defined formulations
Vessels	Biological contaminants	Closed systems for culture to limit exposure
	Other contaminants	Disposable systems for one-time use
Extracellular Matrices	Biological contaminants	Synthetic (non-animal) materials
	Reproducibility	Validated methods for manufacture

Stem cell culture medium regulatory concerns

Given that the typical mode of therapy requires the direct infusion of cultured cells into patients, the regulatory issues surrounding cell therapies are often more complicated than those for standard small molecules or biopharmaceuticals. These types of therapeutics have stringent procedures in place for release, such as cleanup steps to remove potential contaminants and strict identification procedures required for release. This represents the problems often associated with cell-based therapies. Use of animal-derived components in the culture systems leads to the potential for the introduction of adventitious agents into patients. Cells exposed to these animal-derived components must be considered potentially contaminated, because cleanup of cells in culture is not really a practical alternative. Reproducibility of cell culture techniques can also be an issue due to the use of undefined raw materials such as serum or plant hydrolysates, which often have unknown effects on the phenotype of the cells. Since there is not often a robust assay to define the potential efficacy of the cells in culture prior to treatment, this can also represent a severe regulatory hurdle. Every component of the cell culture system should be tightly controlled in order to make a regulatory friendly and consequently safe cell therapy.

Media components for clinical grade cell therapeutics

Because of the aforementioned regulatory concerns surrounding stem cell culture media and systems for therapeutic purposes, the push to develop products that are "regulatory friendly" is becoming an industry-wide initiative. As with the bioproduction of recombinant protein therapeutics, where defined components and systems are highly desired, stem cell culture systems specifically geared for therapeutic uses are trending in the same direction. The latter undoubtedly poses a more significant challenge than the former, as the complexity of stem cell culture systems (broad and varied use of growth factors, supplements, and matrices) far exceeds that of traditional biotherapeutic protein production.

Optimization of systems that maintain fidelity (expansion/differentiation potential) and robustness and meet or exceed safety and regulatory concerns remains as a major challenge as cell-based therapies continue to become more widely used and

validated. Many of the products currently available that promote fidelity are far from acceptable with respect to issues of regulatory compliance. Strides are being made to develop products for this niche, however, and the development of media and supplements that are either fully recombinant, animal component/serum free, or chemically defined currently exist for culturing a small number of specific cell types, and are likely on the horizon for several others. Moving forward, these development projects will rely on integrating medium optimization technologies with more elegant strategies to characterize cell populations, such as gene expression profiling, transcriptional networks, and mechanisms that trigger and regulate lineage commitment and differentiation. A more thorough understanding of these key principles relies heavily on basic research, and makes such development feasible and ultimately more efficient.

While specific components of culture media are key in satisfying regulatory requirements, the hardware components of the culture system are nearly of equal importance. Especially in cases where donor tissues and cells are processed to generate therapeutic material for others, careful containment and prevention of contamination is paramount. With developments of better media components and growth-supporting extracellular matrices, disposable vessels that are currently used for other types of large-scale cell culture will likely be adapted to make them suitable for a variety of stem cell applications. A wide range of disposables already exist for other culture systems, making this a seemingly more straightforward challenge for the stem cell therapy industry. As scale-up occurs, however, close attention must be paid to culture fidelity, so thorough validation of such systems will be critical.

Establishing compliant, yet therapeutically useful culture systems for cell-based therapies remains as a significant challenge for modern regenerative medicine. Continued research and development will be needed to improve performance and consistency in cell characterization while maintaining the appropriate environment necessary to produce clinical grade cell therapeutics.

□

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Mark A. Gerber (1976) studied at Missouri State University where earned a B.Sc. in Cell and Molecular Biology (1999). He was awarded a Ph.D. in Biochemistry and Molecular Biology from Saint Louis University School of Medicine for his research on RNA polymerase II transcription elongation factors (2004). His postdoctoral research was conducted at Washington University in St. Louis, studying the signaling cascades involved in meningioma oncogenesis. In 2006, Mark joined the R&D division of Sigma-Aldrich, where today he is a Senior Scientist in Regenerative Medicine. While in industry, Mark's work has focused on improving cell culture systems that have application in a number of fields, primarily biotherapeutics and regenerative medicine. His current efforts are directed toward the development of reagents and systems that can be used in basic stem cell research.

Daniel W. Allison (1972) studied at Quincy University where he earned a B.Sc. in Biological Sciences (1994). He was awarded his Ph.D. in Cell and Structural Biology at the University of Illinois Urbana-Champaign for his work on the cell biology and structure of the mammalian synapse (2000). In the same year he moved on to Sigma-Aldrich in St. Louis, Missouri, where he is today a Principal R&D Scientist in Regenerative Medicine. Since moving to industry, his work has focused on the development of culture systems for a variety of fields, including cells involved in both regenerative medicine and recombinant therapeutic protein expression. Much of this work has focused on the development of culture media for adult stem cells and primary cells. Currently his group is working to develop both better cell culture systems and molecular biology tools to be used in basic stem cell research.

Human Embryonic Stem Cells: Biosafety Challenges

Human derived cells are widely used to manufacture biopharmaceuticals, for example MRC-5 and WI38 cells in the manufacture of vaccines, HEK293 and PER.C6 cells to produce adenoviral genetherapy products, foreskin derived fibroblasts for allogeneic skin transplants, modified cancer cells to develop anti-cancer vaccines. hES cells, although human derived, present a number of additional biosafety issues to be addressed.

TEXT | **STEVE GIBSON**

INFO | **AUTHORS**

Author

Steve Gibson (1954) studied chemistry at Newcastle Polytechnic and also obtained his PhD there in 1983. After a 10 year period with Pharmacia Biotech, St Albans (UK), in technical and sales roles, he joined Q-One Biotech in 1995. After Q-One Biotech, Glasgow (UK) was purchased by BioReliance he was in charge of sales in Asia Pacific until the summer 2006. He is a co-founder of Vitrology and responsible for sales and I.T.

Human derived cells are widely used to manufacture biopharmaceuticals. Some examples are the use of MRC-5 and WI38 cells in the manufacture of vaccines, HEK293 and PER.C6 cells to produce adenoviral genetherapy products, foreskin derived fibroblasts for allogeneic skin transplants, modified cancer cells to develop anti-cancer vaccines, etc. In these examples, each of the cell lines used presented different biosafety profiles which had to be addressed to allow clinical trials to proceed. Subsequently a number of products derived from these human cell lines have now become licensed biopharmaceuticals.

Human Embryonic Stem (hES) cells offer mankind tremendous potential to cure many serious and life-threatening illnesses, by their ability to be converted to other cell types and then used clinically to replace depleted cell populations and restore lost function. hES cells, although human derived, present a number of additional biosafety issues to be addressed before the full therapeutic potential of stem cells can be realised. To understand these, a basic knowledge of how biosafety is addressed by the regulatory bodies, such as the FDA and EMEA, in their different guidelines is helpful.

Biosafety, A Basic Regulatory Guide

Worldwide many regulatory documents are in place to both advise and direct organisations on the issues they need to address to obtain a licence to market their therapeutic product. These are constantly being updated to take account of new technologies, knowledge and experience gained. However specific regulatory guidelines which take account of the issues surrounding the therapeutic use of hES cells and advise organisations on the regulators' concerns, and most importantly potential ways to address these concerns, are not available. An organisation's main option is to extrapolate from existing regulatory documents and address those concerns that they can identify for themselves.

The issues raised by all regulatory documents can generally be ascribed to one of the following three categories:

- Safety. Ensuring the product at its prescribed dose is not a threat to the patient.
- Efficacy. Demonstrating that the product has a therapeutic benefit for the patient.
- Consistency. Demonstrating that the characteristics of the product are consistent from batch to batch, and that there is continuity of supply to meet expected demand.

For early clinical trials, out of the three above categories, it is the safety of the product that regulators have identified as absolutely critical for organisations to address, prior to permission being given for early clinical trials to progress.

For a biotherapeutic, this safety issue can generally be broken down into the seven categories below:

1. **Toxicity**. That the product or any of its breakdown products do not pose a serious risk of poisoning to the patient.
2. **Microbiological safety**. The threat from bacteria, fungi, yeast, mycoplasma and viral contaminants.
3. **Identity**. Demonstrating that the original cell line developed, is identical to cells at different manufacturing stages and no contamination by other cell lines has occurred.

4. **Tumourigenicity**. The potential for the cells to form tumours In vivo. This has important relevance, irrespective of whether whole cells or cellular products (e.g. r-proteins, vaccines) are the therapeutic agent.
5. **Oncogenicity**. If the cells have tumourigenic potential, then it is considered that there is a threat from oncogenic components such as oncogenes (DNA & mRNA) and their protein products. Generally, in such an outcome, the manufacturing process has to be validated as capable of removing such components. In rare cases where this threat has existed, extensive In vivo studies have been performed to demonstrate that this threat is negligible.
6. **Genetic stability**. For a manufacturing process to be robust and deliver consistent product, the cell substrate used should be genetically stable. Of equal or greater importance, cell lines that are not genetically stable have potential to evolve from a non-tumourigenic line to one which is tumourigenic, with all the additional safety concerns this raises.
7. **TSE** – Tissue Spongiform Encephalopathy. The threat that the cell line itself is harbouring the potential to cause new variant Creutzfeldt-Jakob Disease (nvCJD) or contamination with Bovine Spongiform Encephalopathy (BSE) agent by the use of bovine foetal calf serum during production.

hES Biosafety Issues

Mankind has learned how to isolate hES cells from embryos and tissues and cultivate these to increase the quantity available, we have also discovered how to activate some of the pathways that lead to therapeutically useful cell lines, so we are tantalisingly close to having the potential to cure previously incurable diseases. However, compared to other existing human cell derived biotherapeutics, stem cells present us with some very challenging biosafety concerns. If the regulatory guidance given in existing guidelines was interpreted strictly, then the potential benefits of stem cells may be lost, as stem cells would fail the biosafety criteria of those existing guidelines. To understand what these issues are, we need to consider a hypothetical hES stem cell project, i.e. how an organisation might isolate an hES cell line and develop this into a therapeutic cell line for clinical use. The path described is an idealised one, the difficulties of working with stem cells, our current lack of extensive knowledge around their manipulation and differentiation, would probably prevent this path from currently being realised.

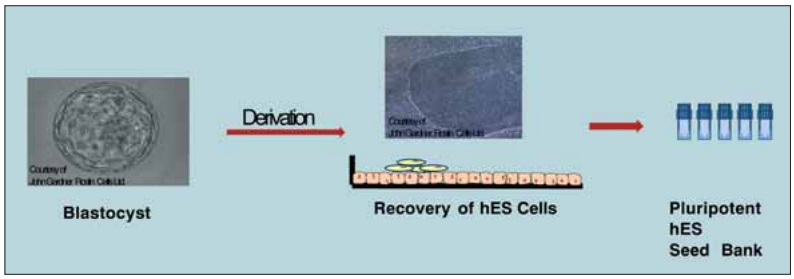

Figure 1: Recovery of Pluripotent hES Cell Line

The inner cell mass from the embryo is isolated and cultured on a monolayer of «feeder cells». hES cells do not reach senescence, so they continue to divide and multiply as long as they receive nutrients and the correct biological signals, while other cell types senesce and die.

Early hES cell lines were recovered on a murine derived feeder cell line. This has potential problems, as all murine cell lines encode endogenous retrovirus sequences within their own genome, and have the potential to express infectious retrovirus. hES cell lines derived by such means will require extensive testing to demonstrate that endogenous murine retrovirus has not crossed the species barrier into the human hES cell line, and become a new human pathogen.

Most commonly today, human fibroblast feeder cell lines are used and with these, endogenous retrovirus is not considered to be a major concern, but the threat from infectious retroviruses such as HIV, HTLV, etc has to be considered. A number of supplier/reagent companies are working on the development of media and technologies that would allow for cell free and bovine serum-free recovery of hES cells, which would be the best solution of all.

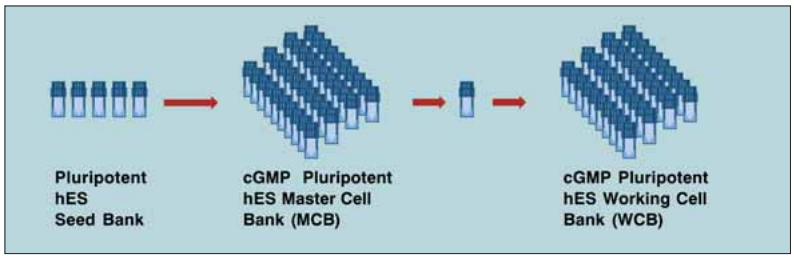

Figure 2: Expansion into Master and Working Cell Banks.

Generally, under cGMP, a manufacturer would create a cell banking system, each cell bank being a homogenous collection of vials each with a similar quantity of cells in cryopreservative. Typically 100 to 200 1ml cryovials each containing 5 x106 to 1 x 107 cells in DMSO/media are created and stored in the vapour phase of liquid nitrogen. If a WCB is required, an

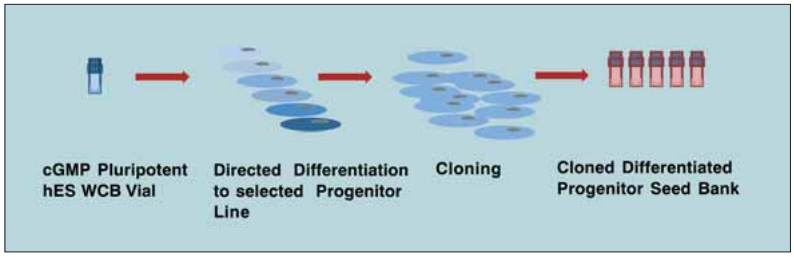

Figure 3: Directed Differentiation of Pluripotent hES Cell Line to Progenitor Line and Cloning

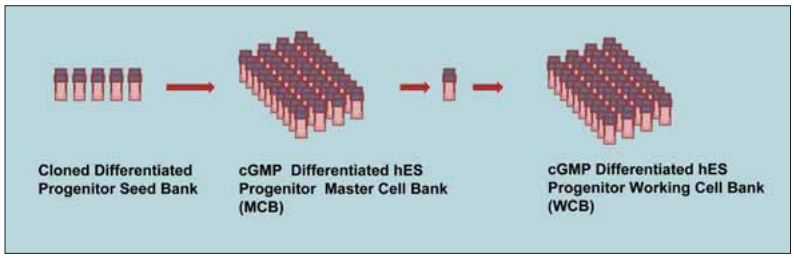

Figure 4: Cloned Cell MCB and WCB Manufacture

MCB vial is recovered and expanded to create a similar quantity of WCB vials. A key concept here is the homogeneity of each vial when compared to any other vial in the bank.

It could be argued that hES lines are already clonal, as they were derived from a single cell originally, however, the concept of clonality in the biotherapeutic area generally implies a level of genetic stability such that cloned daughter cells are all identical and behave in a similar manner. This statement does not hold true for pluripotent stem cells. When a stimuli is applied to cause differentiation down a particular pathway, only a small percentage of the hES cells will arrive at the final desired destination, 5% to 10% perhaps. This raises biosafety concerns, as you not only have to demonstrate the 5% to 10% of cells you want are safe, but equally you have to demonstrate that the remaining 90% to 95% of undesired cells are just as safe.

A potential solution is to clone out a line from the cells that have been down the directed differentiation pathway. This is easy to say, to describe and even to draw, but with our current level of stem cell knowledge and lack of ability to manipulate, it is currently, probably not feasible. Some of the technology barriers being:

– Stem cells do not «enjoy» being cultured in isolation, so expanding from a population of 1 is difficult and potentially impossible with today's technology.
– The differentiated line that has been produced is probably senescent, so it could only undergo a limited number of population doublings, which might not allow for a viable commercial process to be developed.
– Once cloned, we may find that it still lacks genetic stability, and when it is expanded again, it diverges to produce a heterogeneous cell population.

These are difficult barriers to overcome, but we may find from a biosafety perspective that our alternatives are very limited.

Assuming the technology barriers have been overcome to clone the differentiated line, then a MCB would be created, and WCB, if senescence is not an issue. This MCB would be one focus where many of the biosafety concerns would need to be addressed, particularly screening for microbiological safety.

Production of a clinical batch would commence with recovery of a vial from the MCB, or WCB if available, and seeding on to the selected culture technology. If feeder cell lines are being used, probably by this stage cGMP MCB and WCB feeder cell banks will have been created and tested to demonstrate suitability for clinical manufacture.

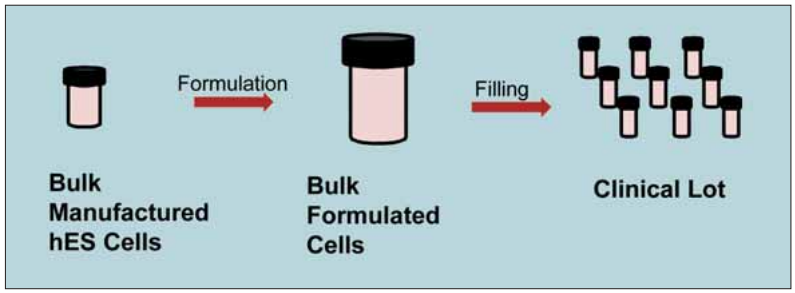

Figure 6: Bulk Manufactured Cells – Bulk Formulated Cells – Clinical Lot.

At the end of this manufacturing phase, the product would be a container with a homogeneous batch of high viability differentiated progenitor cells, free from any contaminating, viable feeder cells.

Batch biosafety testing would be performed on each manufactured batch of harvested cells, generally referred to as the Bulk Harvest.

In all other existing biopharmaceutical areas where cells are used for manufacturing, guidelines direct an organisation to create a cell bank using "cells at the limit of In vitro age" and to characterise this. The concept being that if there is a microbiological or cellular contaminant, then this will have been amplified and is potentially easier to detect than in the MCB. It is also a reference point to measure any genetic stability changes from the start of the manufacturing process i.e. the MCB passage level. Further, it is generally considered for most cell lines, the higher the passage level, the

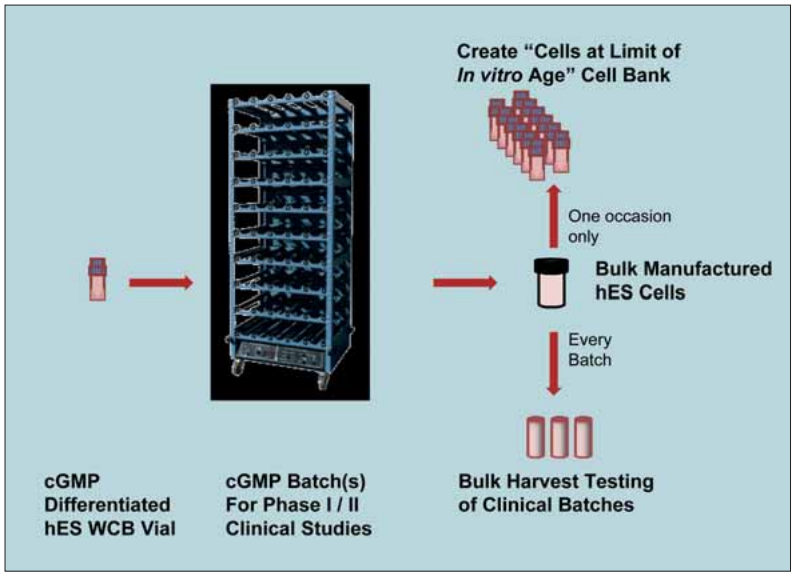

Figure 5: Production of a Clinical Batch - Cells At Limit of In vitro Age & Batch Testing

greater the risk of tumorigenicity becoming an issue.

Bulk manufactured cells require formulation into a suitable buffer for In vivo use and critically, at a known level of bio-potency. So there is an absolute requirement to have some means to measure this function, to ensure batch to batch consistency.

Timelines for delivery of the cells in a healthy condition to the patient will be an issue:

- Do cells remain in culture while batch release testing is undertaken?
- Are the cells used immediately without batch release results available?
- Can the cells be frozen, stored and recovered before clinical application?

Such issues need to be considered and resolved.

Biosafety Concerns Specific to Stem Cell

hES cell lines have a number of specific regulatory safety issues. Considering the 7 biosafety areas identified earlier:-

1. Toxicity. Probably there are no additional issues related to hES cell compared to other human cells in current use..
2. Microbiological Safety. On the whole, the concerns of the regulatory agencies are relatively well described in this area of human cells; guidance can be found in various Points to Consider type documents. Two potential problem areas are:-
   - Confidentiality surrounding the donor of the embryo. This does not allow for the level of scrutiny on their health status that would be desired, which would generally also include follow-up health monitoring after donation in a 6 month to 12 month timeframe, and sometimes beyond.
   - The use of murine feeder cells and the threat of murine retrovirus infection described earlier.
3. Identity. Potentially a problem exists here, as the basis of stem cell therapy is dependent upon differentiation of the cell line. Such changes will cause cells at the clinically differentiated level to have genotypic and phenotypic differences from earlier passage levels such as the MCB. Assays such as isoenzyme analysis and DNA fingerprinting will give some information, but probably the whole solution will be to have identified key markers at key passage levels which will confirm their identity and relationship to each other.
4. Tumourigenicity. Pluripotent hES cells are tumourigenic, they are capable of forming teratomas In vivo. Directing the differentiation of hES cells to a non-tumourigenic progenitor is only going to address this problem if it is possible to demonstrate that there are no pluripotent hES cells remaining, and also no lesser differentiated cells, which still retain tumourigenic potential.

   Probably this issue represents one of the greatest challenges to any organisation planning to enter hES cell therapy clinical trials.
5. Oncogenicity. This is also a very important issue, but it is closely associated with the tumourigenicity of the cells. If tumourigenicity issues are resolved then probably issues relating to oncogenicity can easily be addressed.
6. Genetic stability. Like Identity, this is another challenge related to the differences in cells between earlier passage levels (MCB) and clinical passage levels. Karyology can be used to demonstrate the diploid characteristics of the cells at all passage levels. Other measures of genetic characteristics will probably be required and more consideration on this issue will be needed. Part, or all of the solution, will be to define separately the genetic characteristics of the MCB and Cells at Limit, using genotypic and phenotypic markers, and then justify the changes that are seen.
7. TSE. No immunologically based test currently available has the sensitivity to give sufficient assurance that the hES cells are not harbouring a TSE agent. Concerns will be raised about those hES cell lines that have been developed from embryos in parts of the world where nvCJP is prevalent, the UK being the best example. It will be the local regulatory agency that will have to decide, based on a risk/benefit analysis, on whether or not a clinical trial can proceed. A possible investigation which might address the TSE question is In vivo studies with transgenic mice, engineered to express the human variant of the PRP gene. □

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Stem Cell Treatments – Successful Early Clinical Trials

Alan Trounson, President of The Californian Institute for Regenerative Medicine (CIRM) on prospects of stem cell research and the challenges ahead of the institute.

INTERVIEW | **HUGH ILYINE**

INFO | ALAN TROUNSON

Author

Alan O. Trounson (1946) obtained his PhD from the University of Sydney (AU) in 1974 and worked as a post-doctoral fellow at the Agricultural Research Council's Unit of Reproductive Physiology and Biochemistry in Cambridge (UK) until 1976, when he joined Monash University. In 1983 he achieved the first frozen embryo baby born in Australia, was appointed Director of the Centre for Early Human Development (1985). In 1991 he was awarded a Personal Chair in Obstetrics and Gynaecology/Paediatrics, in 2003 a Personal Chair as Professor of Stem Cell Sciences, became Director of the Monash Immunology and Stem Cell Laboratories (AU). Alan Trounson is a Fellow of the Royal College of Obstetricians and Gynaecologists and an Honorary Fellow of the Australian and New Zealand College of Obstetricians and Gynaecologists, and was awarded an honorary doctorate by the faculty of medicine at the Vrije Universiteit Brussel (BE) and received numerous other awards. Since January 2008, he is President of CIRM.

Compared to 12 months ago, are you more optimistic or less optimistic regarding the stem cell and regenerative medicine field?

Overall I'm optimistic about progress in the field over the last twelve months, California's progress during this time has been terrific, and inspires real confidence for the future. You get a different view in coming here compared to my previous academic research work in Australia. CIRM, with its emphasis on stem cells, connectivity to world leading research institutes and the biotechnology industry, has created great morale and momentum.

How do you see new technologies emerging in the field?

Well, looking at the excellent progress of mesenchymal stem cell (MSC) treatments into the clinic is a good place to start. Even if mechanisms of action are not yet fully understood, clear positive responses have been shown so far in lung, cardiac and graft versus host disease. Others following include MS and possibly diabetes. It's happening now - early clinical trials have been established already.

iPS cells show great promise, but it is still early days. However, the appeal of these cells for new drug discovery as well as personalized medicine is enormous. In the coming months efforts will need to be made to see if retrovirus use can be avoided, and to learn more about where the introduced genes are integrating, as well as their regulatory control. There is some evidence emerging that multipotent adult progenitor stem cells are capable of 'spontaneously' re-programming, which might allow new cell lines to be developed without the addition of retrovirus or extra genes. Although such sources of multipotent cells as the placenta remain somewhat out of focus as far as research efforts, for me they still represent a source of considerable potential for applications to regenerative medicine.

So how do you relate the work to date with human embryonic stem cells (HESC) with these new technologies?

I really see HESC's as part of the continuum of progress in the field. At the early stage we had autologous bone marrow treatments, making use of the native stem cells, which have led to the current Phase II clinical trials using mesenchymal stem cells. We have HESC's just arriving now in preparation for Phase I clinical trials for safety, at the same time as stability and long term culture effects are being studied, so there is still a good deal of work to do here. With iPS cells, we are still some way away from formulating any clinical trials.

As we learn more from these early animal studies and clinical trials, my thinking is that we will combine cells and drugs together in more sophisticated ways for improved clinical outcomes.

What do you see as the future for Somatic Cell Nuclear Transfer (SCNT)?

I still see SCNT as a fundamentally appealing technology, but the iPS cells are also a very good alternative. The major issue for SCNT is the large number of female eggs needed to conduct research due to its low efficiency at this time, though that may change.

The UK Parliament has just approved legislation enabling the creation of SCNT chimaeras using animal oocytes. Can you see this

type of legislation in other countries?

I really don't know. My experience in Australia indicated that it was unlikely to be approved there, and it's hard to see in other countries approval being readily legislated. Perhaps this will change once the reprogramming of cells using this technology has been demonstrated successfully in the UK.

How do you see Cancer Stem Cell Cells (CSC), and is CIRM investing in research in this area?

We are investing in this in early stage research programs in cancer stem cells. It's part of our research portfolio, and we see it as a potentially productive area. To be able to better identify these rare progenitor / stem cells across a range of cancers, may be very important and lead to therapies that target these dangerous cells. Focus on the discovery of selective agents that kill such cancer stem cells and block metastases would be a great result. Of course we need to recognise the enormous investment already made by government agencies, charities and industry over many many years to deliver cancer therapies. CIRM will concentrate its efforts only in the CSC area.

What do you see Alan as some of the unique elements that make up CIRM?

What I think is really great about CIRM is that it is a unique and remarkable public/private effort by Californians. Without the initiatives of Robert Klein and his team, working in consultation with government and public institutions, this wouldn't have happened. At CIRM we now have the funding, the governance and public access in a very open way. Public comments are allowed at the Board meetings. Linking the public aspirations for improved medicines and cell based therapies with the researcher's desires to make these discoveries, and engaging the connectors in the biotechnology industry makes this a truly exciting world class initiative. We have committed CIRM investment in 12 new institutes, that will result in $800million in research space construction, with $271 million from CIRM, and the remainder from Universities, Institutes and Charitable sources. Additionally, CIRM has now approved $260 million in research grants, and we are hopeful that within the next six months we'll have 600 scientists active in Californian stem cell research.

With all this funding, will there be shortage of skilled scientists?

Well, I guess it's possible, but in reality we have an influx of interstate and overseas researchers coming here and other scientists who are now making corrections to their careers. Even some top scientists from interstate and overseas are now interested in working part-time in California. In the coming years there is no doubt that California will be a wonderful place to undertake productive research in stem cells.

Coming up this year we hope also to be in position to provide loans over 6-10 years to California biotechnology companies developing new stem cell products and services. When you look at the number of potential jobs being created and their multiplier effects, I'm confident California will be sufficiently attractive to resource the skilled people it will need.

How do you see CIRM relating to other parts of the US and overseas centres of stem cell excellence?

Our mission at CIRM is to accelerate the discoveries made from stem cell research into valuable regenerative medicines. Our first responsibility is to recognise the Proposition 71 initiative and huge investment made by the people of California, with our priority of improving public health and economic growth here in California. But, we are also ready to collaborate with others outside California. At the moment we are developing memorandums of understanding to collaborate with a number of countries and organizations. Our mission is to get new medicines faster to patients in need. □

INFO CIRM

Author

CIRM was established in early 2005 with the passage of Proposition 71, the California Stem Cell Research and Cures Initiative. The statewide ballot with 59% approval was on November 2, 2004 creating a US$3bn over ten years funding initiative for stem cell research at Californian universities and research institutes. It called for the establishment of a new state agency, The Californian Institute for Regenerative Medicine (CIRM) to make grants and provide loans for research in stem cells, research facilities and development of stem cell therapies. CIRM is governed by a 29 member governing board, the Independent Citizens Oversight Committee (ICOC), composed of representatives from Californian Universities, Research Institutes, biotechnology companies and patient advocate groups.

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Biofuels from Non-Food Biomass

Dramatic price increases for basic food products in recent months, especially of grains, have led to the awareness that biofuels such as ethanol and butanol made from food-grade carbohydrates, but also biodiesels from edible plant oils, are not an energy panacea but indeed carry dire consequences for the global food supply. New bioconversion processes hold great promise to make the production of biofuels more economical and ecological with the use of cellulosic feedstock such as wood and agricultural waste streams. Even hydrogen production is now attempted through biotechnological methods.

TEXT | MICHAEL LINDENMAIER

Ethanol and butanol from non-food biomass such as wood pulp (cellulosic ethanol) as well as agricultural and residential waste streams could offer economically and ecologically sound solutions to the energy and climate crisis. Wood biomass is a highly renewable resource that offers the possibility for sustainable cultivation of fast growing, pest-resistant tree species (soft wood) such as hybrid poplars. Cellulose, the main polysaccharide of wood, and basically of all plant materials, consists of glucose that cannot be metabolized by humans due to its β(1→4) polymeric linkage. Some animals especially Ruminantia (cows, sheep etc.) and other herbivores are able to enzymatically cleave cellulose to obtain energy from the hydrolysate.

From traditional hydrolysis to microbial cellulases and co-fermentation

The challenge of industrial biotechnology for biofuel production from celluloses is to develop the optimal processes for ethanol and butanol bioreactors. In the past, chemical processes such as acid-catalyzed hydrolysis and subsequent fermentation with yeast, followed by distillation have been applied. Historical butanol processes, first designed by Chaim Weizmann during World War I for the production of acetone from corn starch through bacterial fermentation with solventogenic *Clostridia acetobutylicum*, were later used for the generation of butanol from other polysaccharides (see BWE 02-2007, Focus Biofuels p. 12 – 15). Starch, the most important polysaccharide in foods is also a glucose polymer with α(1 → 4) linkage, which can be enzymatically cleaved by digestive enzymes (α-amylases) of all mammals including humans. Modern bioprocesses will lead to better methods for the enzymatic break down of all types of polysaccharides including non-food cellulose and hemicellulose for both ethanol and butanol production (2nd generation biofuels). In some cases, these two-step processes (hydrolysis, alcohol fermentation) could be simplified to one process step through the introduction of cellulase genes into engineered yeast strains or through co-culture of saccharolytic and celluloytic, thermophilic *Clostridia*. Another step into direction of bioprocessed celluloses and hemicelluloses is the improved ability of yeast or *Clostridia* bacteria to use xylose as a substrate for ethanol and butanol production.

Increasing production economics (SSF versus SHF)

The overall efficiency of traditional bioethanol production from corn is still relatively low. Ethanol (made from corn and other grains) used in combustion engines still only saves about 10% in total CO_2 output, which is largely due to the fossil fuel energy used for crop cultivation, transportation and for the process of bioethanol production itself. At this time, drastic efficiency increases of traditional combustion engines used in automobiles are limited. It is therefore paramount to create novel bioprocesses that increase the economics and thus the ecological benefits of biofuels. As figure 1 shows, such types of biofuels generated from waste streams or regional cellulose sources (forests) are environmentally friendly with regard to total CO_2 output. One such approach lies in the Simultaneous Saccharification and Fermentation (SSF) of non-food biomass wherein genetically engineered yeast (*Saccharomyces cerevisiae*) are employed. Improved fermentation systems will eventually increase ethanol levels in biorecators broths and consequently, less energy from fossil fuels will be required to distill off the ethanol or butanol fractions. At this time, Separate Hydrolysis and Fermentation processes (SHF) are still the norm, but in order to make lignocellulosic ethanol production more competitive compared to sugar- and starch based processes, biotechnological innovations will have to lead the way to create novel enzymes or more efficient microbial fermentation bioreactors.

Xylose and the role of fungi

Xylose (in German also «wood-sugar») is after glucose the most abundant pentose in lignocellulose biomass and agricultural residues. Beside its use in the reduced form (xylite) as a natural sweetener, it is of primary interest for sugar fermentation to obtain ethanol from hemicelluloses because it is not directly accessible by conventional yeast cells. With the use of recombinant DNA techniques and targeted mutants of Sacharomycetales (e.g. *Candida* spp.), xylose-metabolizing pathways have already been developed. In this regard, the genes of *Pichia stipitis*, a naturally occurring xylose metabolizer, are of particular interest. The two principal, rate-limiting (anaerobic) pathways are catalyzed by Reductase-Xylitol Dehydrogenase (XYL1, XYL2) or Xylitose Isomerase (xylA), both of which are relatively inefficient if it comes to ethanol production (for an overview of engineered pentose metabolism in yeast, see fig. 2). It will require an orchestrated effort by both molecular biologists and bioprocess engineers to design new yeast strains or mutants as well as optimal bioprocesses for ethanol production. In contrast to yeast cultures, Clostridia bacteria express the required enzymes to metabolize xylol, and as a result, xylol-metabolizing pathways are less of a problem for clostridial fermentation processes.

Another promising approach for biorefineries is the co-fermentation with different fungi strains, mostly in combination with conventional yeast. Such mixed culture media (e.g. with *Tramates* spp.) are capable of fermenting glucose, xylose as well as hemicellulose and cellulose in one process step. Pilot processes were designed using spent sulfur liquor as the substrate, a ligno-cellulose waste product of paper manufacturing. However, similar processes are applicable using other waste streams containing residual sugars, cellobiose or pretreated wood pulp. Even more promising is the discovery of bacterial and fungi cellulases, which are located in so-called cellulosomes. They were first described in *Clostridia thermocellum* and contain several enzymes for the digestion of the usually heterogeneous cellulose phases; they could be regarded as the «industrial organelles» of the future. One could postulate such entities as functional nanostructures since they are viable outside of their bacterial or fungal cells of origin.

The fungi *Trichoderma reeisi*, has been traditionally the most productive source for not only well-characterized, industrial cellulases and hemicellulases, but also for xylanases, which find use especially in the food industry. Cellulases *in-vivo* are complex enzyme structures organized in supramolecular complexes, the cellulosomes. They contain roughly five different enzymatic subunits representing namely endocellulases, exocellulases, cellobiases, oxidative cellulases and cellulose phosphorylases wherein only endocellulases and cellobiases participate in the actual hydrolysis of the β(1 → 4) linkage. Recent work on the molecular biology of cellulosomes had led to the discovery of numerous cellulosome-related «signature» sequences known as dockerins and cohesins. Depending on their amino acid composition and tertiary structures, cellulases are divided into clans and families (see fig.3). In industrial applications, cellulases from other fungi, such as *Penicillum* spp., have shown high saccharification efficiencies.

While current efforts in enzymology and process engineering to make cellulose materials more accessible to yeast fermentation are well under way, such bioprocesses need to be optimized for large-scale industrial reactors if they are to be competitive compared to conventional starch digestion and industrial fermentation of hydrolyzed starches. Membrane reactors that continuously remove inhibitory products and the use of modified cellulases are currently at the forefront of this technology.

Prof. Dr. Edelmann

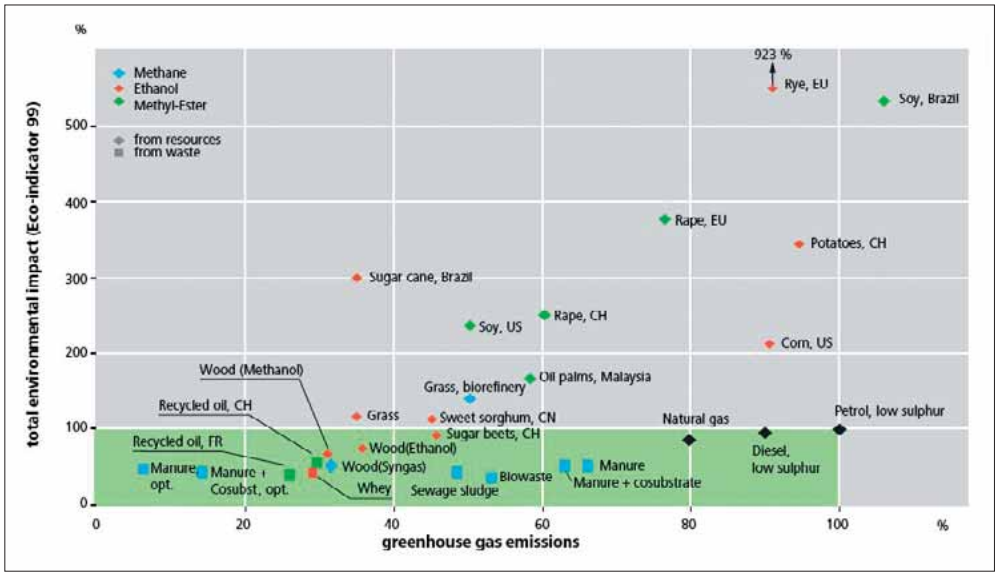

Figure 1: Total environmental impact (vertical) and greenhouse emissions (horizontal) of the biofuels studied, in percent, compared with petrol. Fuels noted within the green area score better results than petrol with respect to greenhouse gases as well as total environmental impact. («Ecological Evaluation of Biofuels», Final Report, BFE, 007, www.empa.ch/biofuels)

Biodiesels and inedible oils and fats

Plant oils (as well as animal fats) are fatty acid esters of glycerol that could be used directly in some diesel engines but their incomplete and inefficient oxidation would produce smoke with environmentally polluting particulates. Thus, plant oils must be chemically interesterified to (trans) alkyl esters of fatty acids (Fatty Acid Methyl Esters = FAME) with a lower flash point. The by-product of this chemical process is

glycerol that needs to be removed to obtain high-grade biodiesel.

Batch processes for biodiesel production from plant oils are sometimes carried out with very little technical know-how. Waste plant oils (e.g. from fast-food restaurants) as well as fats from animal sources generated in meat and poultry processing facilities are usually added to animal feed, but such waste streams can also be converted to alkyl esters. The availability of waste oils and animal fats has been limited and usually does not reach the volume necessary to make a significant impact on the overall biofuel supply. However in the EU, this situation has changed recently with the implementation of new regulations that make it illegal to add waste products from meat and food-processing facilities to animal feed. It can be expected that such waste streams from the food-producing sector will increase in the near future and consequently will lead to increased volumes to make the larger scale production of biodiesel from waste lipids more efficient.

Jeffries Th. W.

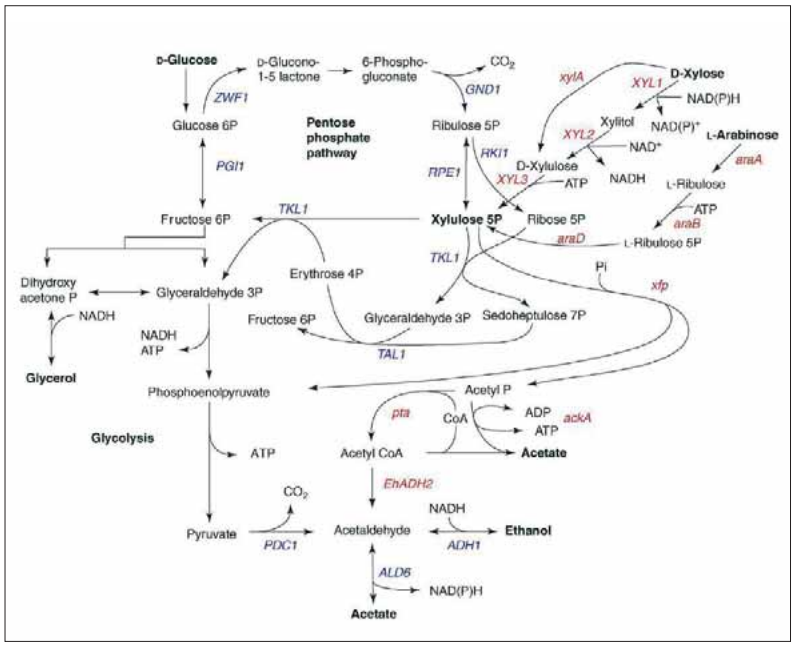

Figure 2: Engineering Pentose Phosphate Pathway (PPP) in yeast.

Non-edible plant oils for biodiesel manufacturing have attracted considerable attention in recent years. There are two plant species especially suitable for that purpose; these are the two Soapnut varieties *Sapindus mukorossi* and *S. trifoliatus* as well as Jatropha (*Jatropha curcas*, L.), both producing oil rich kernels suitable for oil extraction. In biodiesel production, both of these oil types could be converted to 97% FAME with acid-catalyzed transesterification. Another crop with a promising potential for biodiesel production is *Camelina sativa* (Siberian Oilseed), a dry land crop related to rapeseed. Other species that are already used as inedible oil sources for biodiesel production, particularly in India, include the tree species *Azadirachta indica* (Neem), *Calophyllum inophyllum* (Ball Nut) and *Pongamia pinnata* (Indian Beech). While some of these oil rich crops have been touted as the new solution to obtain cheap oil for biorefineries from plant sources, the large-scale availability of these oils is still limited, and the feasibility of large plantations in developing nations such as Mozambique, where they compete with food crops, has been put into question.

The challenge for biodiesel production is the occurrence of free fatty acid (FA) especially in waste oils. These free fatty acids are not converted to alkyl esters during alkaline transesterification but instead, they give rise to amphipolar substances (soaps), which inhibit the separation of the biodiesel from the water phase during downstream processing.

Industrial enzymes; from batch to continuous processing

Interesterification of tryacylglycerols in biodiesel production is mostly achieved with alkaline- or acid-catalyzed chemical batch processes using methanol or ethanol. Biotechnological processes with industrial enzymes (lipases) are now eyed with great expectations. Lipases are known to selectively use fatty alkyl esters as their substrate. Conversion of fatty acid esters to biodiesels is now achieved with fixed-bed bioreactors that use column packings with immobilized *Candida antarctica* lipase. Such reactors are still in the pilot phase, but the advantages are clear; the eluates can be reprocessed with 2nd and 3rd stage columns, increasing the overall biodiesel content, and because of the specificity of the enzymes, contaminants have little influence on the overall enzymatic production of biodiesels.

Oils from algae cells

As prices for raw materials such as food-grade carbohydrates and plant oils are cutting away at the profits of both food and biofuel producers, unusual sources for energy-rich raw materials are increasingly considered. There are a number of algae species that accumulate lipids (similar to fish oils) and even hydrocarbons, however, the problem to harvest these oils from dried algae biomass still poses considerable problems due to the energy used to dry the biomass before oil extraction. Experiments have shown that the direct extraction of algae biomass is more economical than the indirect fermentation of algal biomass to produce either methane or ethanol. Some microalgaes accumulate more lipids in their cells than terrestrial plants, and at the same time effectively absorb CO_2 from the atmosphere.

Most of the research on oil-producing microalgaes was initiated in the 1940's and then again during the oil crisis in the 1970's. At present time, there are number of start-up companies developing open (ponds) and closed systems (bioreactors) for algal bioethanol and biodiesel production. Biofuel pilot plants using photosynthesis only are still quite rare, and there is little certainty that such processes will ever

Bayer et al.

Folds, clans and families of catalytic domains from cellulases and closely related enzymes.

Fold	Clan*	Family	Former cellulase-based classification	Enzyme type (substrate specificity)	Taxonomic distribution	Number of enzymes†	Mechanism
Structures determined, clans established:							
$(\beta/\alpha)_8$	GH-A	1	–	Mainly β-glucosidases and related glycosyl hydrolases	Very broad: bacteria, fungi, plants and animals	71	Retaining
$(\beta/\alpha)_8$	GH-A	5	Family A (five subfamilies)	Mainly endoglucanases	Bacteria and fungi	119	Retaining
$(\beta/\alpha)_8$	GH-A	10	Family F	Mainly xylanases	Bacteria and fungi	59	Retaining
$(\beta/\alpha)_8$	GH-A	17	–	β-Glucosidases, endo-1,3-β glucosidases and lichenases	Fungi and plants	59	Retaining
$(\beta/\alpha)_8$‡	GH-A	26	Family I	Mainly endo-1,4-β mannosidases	Bacteria	14	Retaining
$(\beta/\alpha)_8$‡	GH-A	39	–	β-Xylosidases	Bacteria	7	Retaining
β-jelly roll	GH-B	7	Family C	Endoglucanases and cellobiohydrolases	Fungi	17	Retaining
β-jelly roll	GH-B	16	–	Mainly β-glucanases (lichenases and laminarinases)	Bacteria	71	Retaining
β-jelly roll	GH-C	11	Family G	Mainly xylanases	Bacteria and fungi	65	Retaining
β-jelly roll	GH-C	12	Family H	Endoglucanases	Bacteria and fungi	14	Retaining
Structures determined, clans not established:							
Distorted (β/α) barrel	–	6	Family B	Endoglucanases and cellobiohydrolases	Bacteria and fungi	18	Inverting
$(\alpha/\alpha)_6$	–	8	Family D	Mainly endoglucanases	Bacteria	10	Inverting
$(\alpha/\alpha)_6$	–	9	Family E (two subfamilies)	Mainly endoglucanases	Bacteria and fungi	45	Inverting
β barrel	–	45	Family K	Endoglucanases	Bacteria and fungi	5	Inverting
$(\alpha/\alpha)_6$	–	48	Family L	Processive endoglucanases and/or cellobiohydrolases	Bacteria	6	Inverting
Structures not determined:							
–	–	3	–	Mainly β-glucosidases	Bacteria and fungi	58	Retaining
–	–	44	Family J	Endoglucanases	Bacteria	5	Inverting
–	–	51	–	Endoglucanases and arabinofuranosidases	Bacteria and fungi	9	Retaining
–	–	52	–	β-Xylosidases	Bacteria	2	ND
–	–	55	–	Exo and endo-1,3-glucanases	Fungi	2	ND
–	–	61	–	Endoglucanases	Fungi	1	ND

Figure 3: Cellulose, cellulases and cellulosomes

really make it to the production stage. At this time, the most promising microalgae for hydrocarbon accumulation in aquaculture is *Botryococcus braunii*, a type of algae that grows in stagnant fresh water ponds in Australia and China, producing a thick oily layer on its surface. Solazyme Inc., a synthetic biology company headquartered in San Francisco has now found a process to optimize aquaculture of selected microalgae (diatoms) strains to produce fatty acids in absence of sunlight. Early this year, it has entered a collaborative agreement with Chevron Technologies Inc. to accelerate the development and marketing of algae-based biofuels. The feedstock of this process, using an officially undisclosed type of microalgae, was initially corn syrup or other food-based products, however, the current process can also utilize cellulosic materials, as the company claims. In this way, this type of algae is actually fermenting carbohydrates to plant oils, which can be harvested prior to conversion to alkyl esters. Because their product Soladiesel™ is not produced under phototrophic conditions, there are little or no advantages over traditional oil crops in terms of CO_2 fixation.

The purity challenge

Another concern and limitation to the realization of alternative biofuels is the purity of the resulting product, especially of biodiesels. It is therefore crucial for all biofuel-producing industries to develop proper quality assurance protocols that ensure the quality of alcohol and alkyl ester-based liquid biofuels. This is especially important with regard to the environmental pollutants that often jeopardize the utility of biodiesels. Germany has probably been the leading force if it comes to the standardization of biofuel specifications through the establishments of DIN standards. The cooperative effort of organizations such as «Arbeitsgemeinschaft Qualitätsmanagement Biodiesel e.v.» should be pointed out. Through agreements between the car and the biodiesel manufacturing industry, the DIN EN 12414 norm has recently been established. Such norms

ensure the quality of biodiesels starting from the raw material certifications, to the processing and finally to the transport and distribution of the product.

The rise of the biofuel industry has also increased the demand for laboratory services. Analytical technologies such as GC and FT-IR (Fourier Transform-Infrared Spectroscopy) to determine the content of glycerin are applicable particularly for biodiesel (B100) quality insurance and process control. In order to meet ASTM (USA) and European CEN standards, high quality analyzers, including HPLC to measure the amount of unfermented sugar in bioethanol production, are essential. Other quality issues arise from the content of sulfates since such impurities will endanger the integrity of engine components. Oil refineries, which include the biofuel sector, buy approximately USD 1 billion in laboratory equipment annually.

The future of biogas and biohydrogen

The process to obtain biogas, which is mostly a mixture of methane and carbondioxide, is probably the most highly developed technology to economically generate energy from a variety of solid and liquid waste streams, including residential household wastes, manure, sewage from water treatment facilities but also solid agricultural residues from grain processing that allow anaerobic fermentation with suitable methanobacteria. Another advantage of such processes is the biologically induced degradation of environmental toxins such as chlorinated hydrocarbons and biphenyls whose concentrations are significantly reduced, allowing the residue of such operations to be distributed again on the fields so that nitrogen can be recycled as fertilizers. Furthermore, biogas is a technology that is appropriately used in both the industrial and developing world, allowing for a safe exchange of technical know-how that is exclusively used for peaceful means. Europe today is leading the world in total biogas production and in the design of biogas plants. According to the European Biomass Industry Association (EUBIA), the total biogas production capacity has more than doubled over the last 5 years. Since methane as well as nitrous oxides are very potent greenhouse gases, the collection and utilization of organic waste fractions is of crucial importance to the current fight against global warming.

Another much more futuristic approach to the biological gasification of non-edible biomass or the use of solar energy through photosynthetically active microorganism is the design of hydrogen producing bioreactors. There are two approaches, first the molecular engineering of algae (e.g. *Chlamydomonas reinhardtii*) that use photoenergy and water as the reductant without the need for biomass and secondly the two-stage production of hydrogen through anaerobic digestion of waste biomass sometimes in combination with photosynthesis.

Since hydrogen-powered fuel cells will be the ultimate energy source of the future, increased research activities and investments in this area will eventually lead to a significant industry sector for the biological production of hydrogen. Terrestrial biomass and modern bioconversion processes will therefore play a crucial role in the future of the renewable energy industry. □

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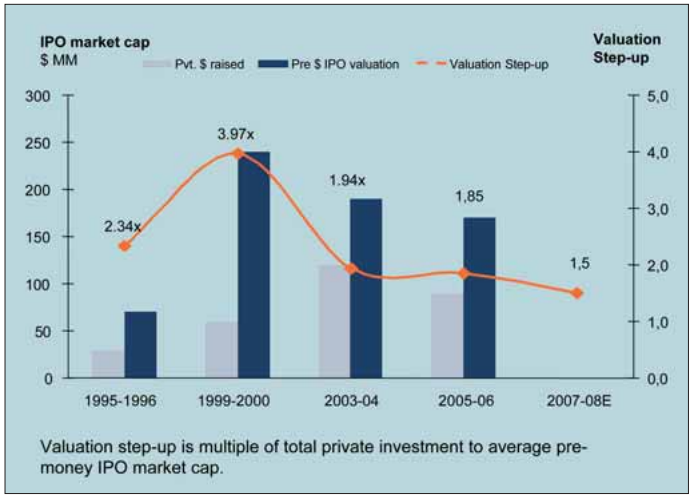
Virtual Asset Development

A promising approach to fund attractive biotechnology projects more efficiently.

TEXT | HUBERT BIRNER, ALEXANDRA GOLL; AXEL POLACK

The pharmaceutical business is presently undergoing a major reconstruction: demands for higher capital efficiency have to be balanced with the need for innovation ideally provided by a biotech industry which has time and money to transform exciting disruptive technologies into products to fight disease. Emerging markets are gaining significance while the established pharmaceutical markets of Western Europe and the U.S. are expected to grow more slowly. As a result, current M&A activities aim to either establish an attractive and innovative product pipeline or reduce structural costs by benefiting from economies of scale.



IPO returns remain disapointing

Undoubtedly, the biotech sector presently experiences major pressure from private and public capital markets and the sector is not rewarded for the years of work that management teams and their investors have put into the development of broad pipelines. In other words, neither are private investors putting a premium in follow-on financings on milestones met by a biotech company since the last private round, nor are public investors putting a premium on biotechnology companies for their efforts to develop a multi-stage and risk mitigating product pipeline. This has the consequence that IPOs, if they happen at all, are valued often only slightly above the last private financing round and that they are considered financing transactions and not a sound basis for the exit of the venture capital investor.

As a consequence, less and less money from private and public investors is coming into the sector and the industry needs to develop new models to finance early stage development assets.

Capital Efficiency is Key

The biggest challenge for the development of early stage biopharmaceutical products is to find the appropriate financial model to do so. We need innovative and cost effective ways to develop pharmaceutical products. And, we need to discover ways to become more independent of the stock exchanges for our biotech investment exits. The reality for most biotech companies is that only a fraction of the investment is spent on the development of those products that eventually determine the value of the whole corporation. Building fully fledged biotech companies is not the only answer for the science innovation question in the future – especially in times when public markets do not value the build up costs of a "risk-mitigating" pipeline pre-IPO.

Capturing the Investment Window

We at TVM Capital like many others Venture Capital groups are constantly evaluating strategies that make our biotech investments more cost effective. One possible solution for the development of early stage biopharmaceutical assets is what we at TVM Capital have coined "Virtual Asset Development" (VAD).

This approach is defined as follows: Only one pre-clinical asset is being developed by a rather virtual biotech company to proof of concept in man (Phase 2a) and with a defined investment amount (ideally not more than $20M in total). The asset/the company is expected to be sold off at the time of reaching proof of concept (ideally proof of concept in man) with or without an earn out model, but certainly without requiring further investment. The legal structure of such small entity is a "small AG", a GmbH or a Ltd. and it has not more than 2 or 3 major institutional shareholders who all share a clear path to exit and the "will to kill" of the project in case a milestone is not met. Another key feature is that a VAD program must have a simple governance structure to allow swift and efficient decision making. The management team, founders and originators of such a project (i.e. academic institutions or biotech companies) will have a clearly defined in-going and out-going equity participation because the capital required to exit (or write-off) the project is committed to the project upfront and not dependent on future financing rounds with unpredictable dilutive effects.

Such a VAD program will be managed by a very small management team in a highly cost-efficient development setup. Most of the development steps will be governed by clear

go/no-go decision points with a simple human proof of concept in mind. The actual development activity will not necessarily be undertaken by full time employees, but is more or less contracted out to fully integrated service provides such as contract research organizations (CROS), medicinal chemistry service providers or regulatory consultants. As the economic outcome of such a program is undoubtedly dependent on the need of various potential buyers to own this program after proof of concept and to pay a substantial price (ideally all upfront), another key success factor of such VAD programs is strong business development know-how when assessing the commercial attractiveness of any asset available for development prior to funding as well as prior to auctioning the asset out after completion of milestones. These skills can either sit directly with the management team or are also outsourced to high profile business development service organisations.

Proteon Therapeutics is one example of TVM Capital's recent investments in relatively virtual and single asset type companies. The company is a privately held biopharmaceutical company based in Waltham, MA and Kansas City, MO (US and is solely focused on developing its drug candidate (PRT-201) – a therapeutic protein - for the improvement of blood flow following vascular surgery procedures from pre-clinical stage through Phase 2a. The Company's initial clinical focus is vascular access for hemodialysis and peripheral arterial disease (PAD).

The scope of VAD

VAD is not the answer for the commercialization of a disruptive new technology ("innovation in science" or platform technology) that no doubt needs a team of dedicated scientists, a well equipped lab, and a fully functional management team.

A recent analysis by TVM Capital of 44 trade sale cases in biotechology between 2002 and 2007 reveals that real platform technology companies have outperformed VAD approaches by roughly 100% and classic multi-product biotech companies by approximately 200%. However, there is limited opportunity to invest in truly revolutionary new platform technology companies with a broad product potential. From this perspective VAD is an attractive model for single assets that are worth developing without an underlying "platform" as this model created on average significantly more value in trade sales than the traditional biotech setups we are used to invest in.

We believe that this potentially smarter approach of dedicating less capital to highly promising VAD projects while keeping all constituents highly aligned can help to bring back "venture type returns" for investors like TVM Capital. However, it is needless to say, that in order to achieve such returns, a portfolio of VAD programs is necessary to manage attrition inherent in drug development. And to reiterate, creating VAD type companies is one possible future strategy for Venture Capitalists to make money – in an ideal world, they are part of a portfolio that still contains full fledged earlier and later stage biotech companies. □

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INFO | **AUTHORS**

Hubert Birner (1966) obtained his PhD in biochemistry from the Ludwig-Maximilians-University, LMU München (DE) and his MBA from Harvard Business School. He was an Assistant Professor for Biochemistry at LMU and started his industry career as management consultant in McKinsey & Company's European Health Care and Pharmaceutical Practice. In 1998, he moved to Zeneca to become Head of Business Development Europe and Director of Marketing Germany. In 2000 he joined TVM Capital where he is General Partner for life sciences in the firm's Munich office. Hubert Birner currently serves as Chairman of the Board of Direvo Biotech AG, Köln (DE), Argos Therapeutics Inc., Durham NC (US), and Spepharm Holding BV, Amsterdam (NL). He is also a member of the Board of Directors of Evotec AG, Hamburg (DE), Jerini AG, Berlin (DE), Proteon Therapeutics, Inc, Kansas City, MO (US), BioXell SA, Milano (IT), and represents the interests of TVM Capital with Ardana Bioscience plc, Edinburgh (UK).

Alexandra Goll (1956) holds a pharmacy degree from Freie Universität Berlin (DE) and obtained her PhD from Philipps-Universität Marburg (DE) in 1986 where she performed post-doctoral studies at the Department of Pharmacology. From there she moved on to Hoffmann-La Roche AG Basel (CH) to become Global Business Leader for HIV and CMV responsible for strategic marketing and business development for Virology. In February 1998 she joined TVM where she is today General Partner , made over 15 investments (e.g. Actelion Ltd; EUSA Inc.; Medigene AG) and serving on the Board of Directors of Biovertis AG (AT), Cerenis Therapeutics (FR/US), Wilex (DE) and others.

Axel Polack (1957) studied medicine at Universität Freiburg (DE). He obtained postdoctoral lecture qualification ("Habilitation") in 1993 and was appointed assistant professor at Ludwig-Maximilians-Universität München (DE) in 1995. In 1998 he became Managing Director of technology transfer company, ITN GmbH (now Ascenion) and joined TVM Capital in 2000 where he is today General Partner serving on the boards of Noxxon Pharma (DE) and Genetix Pharmaceuticals (US) as well as representing the interests of TVM Capital with f-star (AT).

«Cells alone won't help»

In an interview with BioWorld Europe, Dr. Harald Stallforth, Management Board R&D Aesculap AG, explains strategy in corporate technology funding of regenerative medicine from the perspective of a global medtech company and leveraging R&D with other parts of B. Braun.

INTERVIEW | WOLF G KRONER

What are the reasons Aesculap invested as part of B. Braun Melsungen in TETEC, a German tissue engineering start-up, in 2000?
We see ourselves as provider of medical technology systems – what we term 'therapeutic cascade' - which we want to deliver to the hospital for all indications within a specific focus like knee surgery. Starting from knee or knee injuries, we aim to offer the entire portfolio of knee therapies, be it cruciate ligament replacement or total knee endoprothesis.

Wouldn't a licence to TETEC's autologous chondrocyte transplantation technology have been cheaper?
B. Braun is family-owned, and our overall strategy is to directly command the development of products, of established as well as future technologies.

Or, you could have started your own in-house R&D project -
Well, such a decision depends on how close a new technology is to the established core technology of your company. In our case, the biological questions of regenerative medicine are far off from what we usually deal with here at Tuttlingen, which basically is mastering metals and manufacturing instruments. There are other crucial advantages of having a stake in a separate company. For instance, shifts in technologies sometimes come about quite suddenly. A small firm is much more flexible compared to a big one, where you have your standards and routines.

B. Braun's capital share in TETEC is 72,3 %, why not 10% or a small strategic stake leaving the rest to a private VC?
First of all, we want the original inventors be involved in growing the company. Second, we have a major and long-term interest in this specific technology.

So far, TETEC is your only investment in regenerative medicine. It might appear logical to invest also in other tissue engineering firms and, in addition, to expand international reach.
It is true, we have been approached by several other competitive entities. However, it is our firm belief that TETEC is the competence partner in this area, and it would be difficult to find anyone other matching it. We are also convinced that TETEC is 'enough by now', so to say. It is close to us at Tuttlingen as well as to NMI, the Natural and Medical Sciences Institute at Tübingen University, with which we closely co-operate. However, in the end, even us, we have limited resources. After nine years of operations TETEC still costs a lot of money.

How that? In 2006 it had revenues of 1,5 million Euros and an operating loss of only 40.000 Euros?
You are right, if you compare the financials of TETEC to other companies in their class this certainly is very competitive. But we continue to finance the build-up of their technology. For example, we currently are investing into a new production plant and administration building for TETEC which will cost us in between 6 to 7 million Euros. But you should also be aware that investing into a company is not simply providing capital. It is also providing support, for example by transferring some of Aesculap's R&D projects to them.

So, how do you leverage R&D in other parts of B. Braun on this investments?
Simply by bringing people and projects into contact with each other and fostering communication across the lines. We are the headquarter, and we certainly have several ideas where to go, and how to develop technologies into products. We are targeting first orthopaedics with cartilage and disk replacement, then we have neurosurgery, spine surgery, or vascular systems –

- which Braun declared as priority area to grow. However with 15 employees in TETEC you might run into capacity problems linking tissue engineering with vascular systems.
This is exactly why they have to grow, and why we continue to invest. But

INFO | AUTHOR

Harald Stallforth (1953) studied Mechanical Engineering at Technische Universität, München (DE), obtained his PhD in Engineering from Rheinisch Westfälische Technische Universität, Aachen (DE) in 1980, and earned a degree in law in 1982. That year he joined Aesculap, where he is today on the Management Board in charge of Research & Development.

Arsculap Implantatefabrik

the big challenge is regulatory approval. Right now, we decided to apply for European market authorisation which will take some two to three years. At that time, the company could well have a turnover of maybe 5 million Euros and employ 30 to 40 people. Currently, we are also evaluating to invest into FDA approval for their cartilage replacement. It's incredible how many resources are needed to do this, but, if successful, this will mean further major expansion of TETEC.

A critical success factor is the acceptance of these technologies by doctors and surgeons -

As one of the bigger medtech companies one of our key advantages is a well-organised sales force to bring these products into the hospitals. And here our sales representatives are in permanent discussions with orthopaedic and trauma surgeons. Nevertheless, acceptance is an issue to deal with. New surgical techniques have to be implemented in clinical routine.

Would you say, Aesculap Academy with subsidiaries in several European countries is a stratagem to commercialise tissue engineering products?

It is important, indeed. We frequently offer training courses in our continuous education centres. Training is mandatory and part of regulatory approval. But, first of all, it's a question to get into contact with the surgeon.

Genzyme also invests into regenerative medicine technologies. This company has successfully built around tissue engineering a supply chain. For example, delivery of tissue products straight from manufacturing to bedside. B. Braun operates a hospital logistics firm. Is there a role for SteriLog?

SteriLog is not involved in this at all. But, in principle, you are right. We have a big advantage over our competitors. We command the entire process chain which is a very important building block in our strategy. We can deliver everything including surgical instrumentation. A company only dealing with biologic or replacement material, is not of great help to the surgeon who has to organise the entire treatment. A surgeon needs surgical instrumentation, training and the biomaterial. Here, we are in a position to provide the complete solution, not only tissue engineering products, which basically are just cells. Cells alone won't help. You also have to have scaffolds like collagen, gelatine, synthetic or other. We have overall know-how in biomaterials, biological as well as synthetic biomaterials.

A few years ago you remarked, you won't recommend a biotech investment to any medtech company. Why?

We have learnt in the past, especially with TETEC, that future always takes a longer time than expected. Investing into biotech requires not only an initial funding to be increased thereafter by more capital, you also need to provide sustainable support and have some stamina in order to reach your goals and finally make a profit. When we invested into TETEC in 2000, people expected that by 2008 this treatment will have become routine in hospitals. While there are always some customers interested, this does not add up to a real market. Just compare the numbers: Today Aesculap sells around 120.000 hip and knee prostheses per year out of Tuttlingen. During the same time TETEC sells around 1.000 cartilage replacements. It's a long way to move such an entirely new technology into the market. □

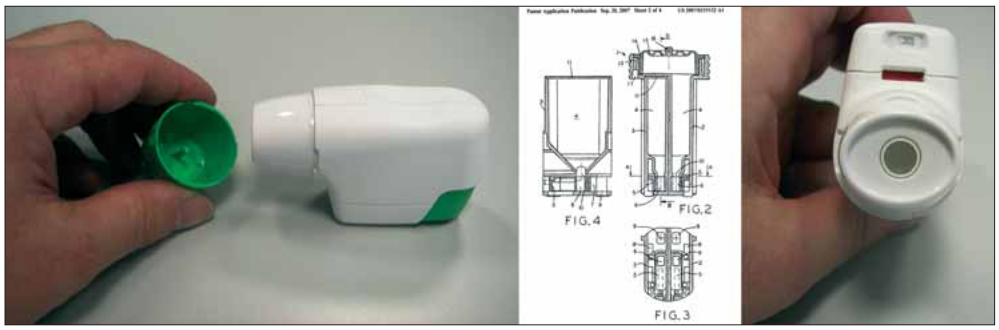
From Hope to Patients' Relief

A report on Spanish Almirall's R&D combining medicinal chemistry with biotechnological and engineering know how.

TEXT | **WOLF G KRONER**

Get a capsule out from the blister pack. … Open the inhaler once. .. Open it twice. … Load the capsule, only one at a time otherwise the inhaler breaks. …Inhale! Actually, you should inhale twice. … After you've inhaled open the device. … Check if the capsule is empty. … Take the capsule out. … Clean it. … Close it. Put it away." Single-dose, dry powder-inhalers currently on the market require this cumbersome four-step administration of a Chronic Obstructive Pulmonary Disease (COPD) drug, explains Carsten Niederländer, Director of Pharmaceutical Development with Almirall Prodesfarma. This is especially true for the chronically ill. Think of a child with asthma not to mix up medicines. Imagine a typical 75-year old patient with COPD who needs easy-to-use devices. Consider the time it takes a nurse whose tasks are to teach self-administration and supervise compliance (*). Almirall Sofotec designed its "Genuair" to be a One-Press-and-Inhale Device. It already contains 30 doses for a one month supply, is easy to use, and does not need clean-up.

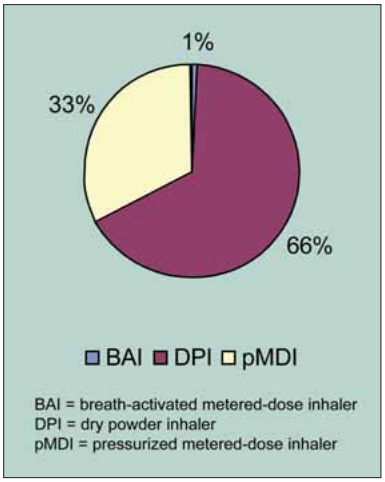
Wolf G Kroner; US PTO



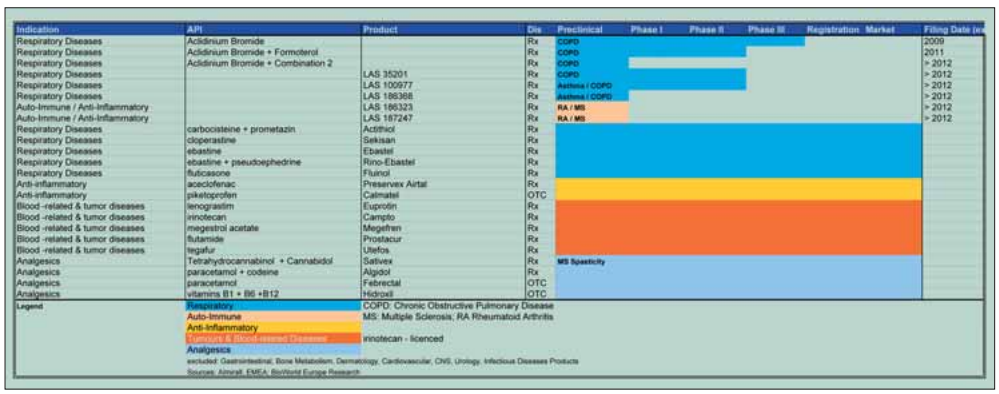
One-Press-and-Inhale Device. Improved to meet FDA Requirements and to be applied worldwide

The device has been proved to effectively deliver an inhalable drug. Thus, in Almirall's Phase II study of LAS100977 a drug candidate for the treatment of COPD and asthma, mean FEV1 and FVC values (**) were reported to be significantly increased and improving lung function over 24 hours. The onset of the bronchodilatory effect was within 15 minutes, peaked at 1 to 2 hours, and persisted for up to 32 hours with determinate doses. With a one-time charge the device can deliver in between 15 and 200 doses and since the takeover by Almirall from Reckitt Benckiser, the device has been further developed to meet regulatory standards worldwide.

Right from the start Sofotec's inhalers (original trade name "Novolizer") were developed for out-licensing. Initially, Sofotec's inventors targeted Altana's COPD-drug candidate ("Roflumilast"). Whilst the new "Genuair" is not yet on the market, several other pharma companies offer Almirall Sofotec's inhaler to doctors and patients. This is already generating sizable royalties for Almirall and expected to increase even more the company's revenues, because the proprietary inhaler is a platform delivery technology applicable to a multitude of respiratory disease indications. However, while such a device is critical for success in the market, certainly, an inhaler alone won't suffice for treatment. You still need an effective drug.



Shares per total (France, Germany, Italy, Spain, UK, US) 2007 asthma/ COPD sales (USD) per inhaler type.



Grupo Almirall Cuentas Anuales Consolidadas (IFRS) 31-12-2007

Almirall Group	2007 m€	2006 k€	diff	diff %
Turnover total	1.439 €	1.114 €	325	29.2
sales	792 €	758 €	34	4.5
- thereof by Almirall	708 €	678 €	30	4.4
operating profit	129 €	146 €	-17	-11.4
cash flow*	190 €	421 €	-231	-54.9
Expenses total				
expenses employees	207 €	186 €	22	11.6
cost of capitalized R&D	17 €		17	
Other Revenues	125 €	96 €	29	29.9
co-promotion of products	29 €	28 €	1	3.9
co-promotion agreements	28 €	28 €	0	1.4
co-development agreements	58 €	31 €	27	84.3
out-licensing	1.1	2.2	-1.1	-50.4
royalties	1.5	0.9	0.6	65.2
public subsidies	1.5	1.4	0.0	1.9
other	6.1	5.0	1.1	21.8
Other				
utilization tax incentives for R&	42 €	29 €	13	46.1
net sales / regional				
Spain	521 €	517 €	4	0.8
Europe & Middle East	179 €	157 €	22	14.0
Americas, Asia, Africa	74 €	65 €	9	13.4
Other	19 €	19 €	0	-2.0
revenues Almirall sales	708 €	678 €	30	4.4
revenues royalties (mainly For	66 €	61 €	5	8.7
Founded	1943			
Major Shareholders	Grupo Plafin, SA (45,09%), Todasa, SA 24,66%; Fidelity International Ltd. 6%			
Employees	3074 (ca.15% RD) 2.975			
Key Investments 2006/07	Hermal (DE) - Reckitt Benckiser, Sofotec (DE) - Meda Pharma, Shire (IE) Allergy Portfolio			

* due to major investments

Encouraging Results From Worldwide Clinical Trials

According to WHO (2008a, b) some 210 million people have chronic obstructive pulmonary disease worldwide. In 2005 more than 3 million people died of COPD worldwide. It is the fourth leading cause of death. Only 10% of patients are in high-income countries. Major causes are tobacco smoke and especially in low-income countries air pollution including the one from biofuels (eg. Qiwen 2007). Zhong et al. 2007 found that 32.8 millions COPD patients and 15 millions with Asthma in China alone. However, it is still in the Western World where patients have satisfactory access to essential medicines and pharmaceutical companies get a better return on their R&D investments. In the respiratory field, Eastern Europe and Russia are attractive markets, because COPD and asthma are increasing above the European average (Górecka 2007). Once affected, COPD cannot be reversed.

Inhaled anti-cholinergic drugs like ipratropium bromide have been used for many decades for the treatment of different respiratory obstructive diseases such as asthma and. Their mechanism of action is by blocking muscarinic receptors in the lung, thus producing a bronchodilation that improves respiratory airflow and decreases the air that remains trapped in the lung. A series of phase I to III studies are being carried out by Almirall with their novel once-daily anti-cholinergic aclidinium bromide. The largest two are the ACCLAIM/COPD I&II trials, wherein more than 1.600 moderate to severe COPD patients, recruited across 23 countries worldwide have been treated with aclidinium bromide or placebo over a one year treatment period. In phase I and II studies completed to date, Almirall's once-daily aclidinium bromide has shown positive and promising results. The compound shows high affinity and long residence time at the M3 receptor, resulting in a potent and sustained bronchodilatation, reports Gonzalo de Miquel, Global Medical Director R&D with the company. Aclidinium is rapidly cleared from plasma minimizing risks of systemic anti-cholinergic side effects.

Last stage clinical development is rapidly progressing. The first approval is expected in 2010. However, the major roll-out of future fixed-dose combinations of aclidinium bromide with other compounds (like formoterol, or inhaled steroids) will be in 2012, and – if all works well – ahead of major competitors. □

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Wolf G Kroner

José Luis Díaz Villarig, Sr. President of the Department of External Investigations, shows Almirall's hope.

Wolf G Kroner

Coriell's Genotyping and Microarray Centre

Turning Personalized Medicine into Reality

Personalized medicine is becoming tangible for common citizens as scientists, physicians, payors, diagnostics and drug developers as well as regulatory bodies work together. The Greater Philadelphia Region is a hot bed for a large-scale evidence-based initiative.

TEXT | **WOLF G KRONER**

Imagine you visit your doctor. Maybe you have a common disease, for instance hypertension. To the best of his or her knowledge the doctor prescribes you a drug that brings relief to millions of other patients with the same condition. In the case of hypertension this may be Coumadin. After some weeks of taking this drug you start coughing up blood. The doctor recalls that in about 10% of patients taking warfarin causes serious adverse effects due to genetic variations in polymorphisms of CYP2C9. A genetic test which costs some 350 Euros confirms this. Now imagine the opposite. The doctor does not prescribe you a drug, because regulatory authorities have issued warnings of causing it serious side effects. However, you have read about scientific evidence that the drug actually works better than other drugs, albeit only in patients with a determinate genetic make-up. You want to know it by yourself and spend 500 Euros on a genetic test that finally confirms that you could greatly benefit from this drug. AstraZeneca's gefitinib for treatment of non-small cell lung cancer is an instance. In 2005 the FDA issued a warning for Iressa, but actually it works very well in 10% of NSCC-patients. Finally, take the example of a patient whose underlying condition is misdiagnosed, symptoms do not ameliorate or translate into other ones. The patient looks up many doctors, swallows many different pills, receives different treatments. Finally, the real cause is discovered. This can happen if haemochromatosis is taken as kidney failure. In many cases genetic testing is the starting point for an effective treatments. A genetic profile once in life makes many future laboratory tests unnecessary, it helps physicians to improve medical decisions for treatment, and healthy citizens to match their lifestyle to known health risks. "Personalized medicine means using the information about significant differences in the individual genome, that is, knowing ahead of time, and trying to mitigate or even eliminate risk, before the consequences are manifest, says Mike Christman, President and CEO of Coriell.

Wolf G Kroner

Within a few months more than 3.000 citizens enrolled to know their individual genetic profile

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Mike Christman, Coriell

Medically Actionable Genomic Variants

The Coriell Institute for Medical Research, Camden NJ (USA) started «Coriell Personalized Medicine Collaborative» (CPMC) in 2007. It offers healthy citizens and patients alike to sequence their DNA, identify gene variants that are biomarkers for a health condition requiring medical treatment. In starting the service about ten to twenty conditions will be screened, says Christman including complex diseases like type II diabetes and coronary artery disease, but also diseases like hemochromatosis once considered to be caused by one gene, but identified through genetic diagnosis as caused by mutations each involving a different gene (Pietrangelo 2004), and strongly affected by teratogenic factors like hepatitis, cancer or alcohol abuse. Adult individuals register at Coriell's website. They have to watch a presentation, sign a consent form and sub-

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John Sheridan, Cooper Hospital

mit a saliva sample by postal mail. Thereafter they receive a secure access to an online portal where to complete a medical and family history questionnaire, can access results of their DNA sequenced and receive an annual update. Coriell plans to monitor the effects of genetic information on subjects health. Rigorous data protection has been put in place to ensure confidentiality.

In contrast to direct-to-consumer gene test providers CPMC provides for genetic counselling (internet-based and telephone service), requires necessary additional diagnostic data, and has set up a reputed advisory body backed by scientific staff to monitor and evaluate the literature in order to add more diseases for genomic screening. While the medical establishment in Europe regularly scorns 'internet' medicine, CPMC demonstrates that the internet is but a tool, that can be put to use to offer

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Russell E. Kaufman, WISTAR

quality medical services at reduced cost to a larger population while enhancing individual autonomy: Wherever you are in the world you can access your personal data and allow a doctor to view your medical record.

Partnering: Sharing risks and benefits while always being responsible for final results

Currently, CPMC partners are hospitals and other research institutes in the Greater Philadelphia Region. Fox Chase Cancer Center is one of the first US national centres dedicated only to cancer with a large program in cancer risk and prevention. It has "the largest pipeline of not yet FDA approved drugs,and maintains strong relationships not only with the regions pharma champions GSK and AstraZeneca, but also with others like Genentech and Novartis" says Michael Seiden, the centre's Pre-

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Michael Seiden, Fox Chase Cancer Center

sident. While running its own platform of 'omics'-technologies In addition, Fox Chase can bring in its worldwide pathology program network and expertise in biomolecular imaging. Cooper Hospital is another important stakeholder of CPMC. It has more than 25.000 patient admissions, 56.000 emergency room visits per year and a network of over 75 clinical practices in the region. In 2006 its operating revenues were $667m. The hospital is currently investing some $300m in modernizing and constructing new facilities (including a stem cell research centre). Cooper

Wolf G Kroner

Jim Dwyer, Virtua Health

promotes not only patients to enroll in CPMC, but as an employer also encourages its 5.300 employees to enroll. The hospital is not interested in knowing the genetic profile of his employees and will not be able to track it back to the individual. Notwithstanding John Sheridan, Coopre's CEO is convinced that the orga-

nisation will financially benefit. Employees who change their lifestyles knowing better their health risks reduce social security costs. Another CPMC partner is Virtua Health, a multi-hospital organisation in New Jersey which specialises in women's health (7.752 deliveries p.y.), pediatrics, cancer, cardiology, orthopaedics and geriatrics, and prides itself "to put patients first". 7.200 employees and 2.000 physicians work in this hospital system which earns yearly net revenues of $1bn with an operating margin of 10.4% placing it first in the state of New Jersey. Jim Dwyer, Virtua's Chief Medical Officer is convinced that genome-based medicine is already feasible, but he sees the need to make physicians familiar with the new genomic technologies and decision-making based on genetic data. Participation in CPMC will help Virtua to learn how to improve current health care, how to cut cost and earn higher returns.

Bringing together different parts of the value chain requires «team science',» underlines Russell E. Kaufman, President of the Wistar Institute which is a designated national cancer centre with special expertise in vaccine development. Its 33 laboratories are dedicated to three research areas: gene expression and regulation, immunology, and oncogenesis at the molecular and cellular level. Kaufman observes a profound change in the relationship between researchers within health care organizations in the Philadelphia region. «During the intense beginnings of managed care, many academic medical institutions were competing against each other for patients, and were spending huge amounts of money but not getting return on their investments. The researchers in those institutions were discouraged from collaborating. Now, they partner with each other on many different levels.» Wistar recently entered a partnership with Coriell to co-market its cell lines, and this may well expand into a broader partnership in CPMC. Future partners of this initiative are insurance companies and managed care organisations whereas CPMC will be at distance to initiatives like Google Health or Microsoft, said Mike Christman, when it comes to manage the medical records of enrolled subjects.

Building upon entrepreneurship

Up to september 2008 some 3.000 volunteers have registered. At the end of 2009 it will be 10.000. Enrollment is free of charge, but costs are in the range of 1.000 dollars per individual. CPMC partners have managed to mobilize funding for the starting version of the collaborative, i.e. a large-scale research project for personalised medicine. Thus, the initiative is about to overcome limitations of present genetic profiling for complex disease. In many cases sample numbers are still too small to warrant individual genetic risk interpretations (Janssens et al. 2008). CPMC links genotyping data to scientific research as many clinical laboratories do, but by establishing an account for each genetic profile accessible to and managed by the sample donor, it offers a tangible return to those who consent to support research. «The promise of the Personalized Medicine Collaborative is to use information now that we have the technology to profile individual genomes, and make it available to your physician for clinical decision making» says Mike Christman.

The Greater Philadelphia Region, which spans Pennsylvania (PA), New Jersey (NJ), and Delaware (DE), appears particularly promising for such an initiative. There are biomedical research institutes with a global imprint, in addition to those mentioned the Center for Integrative Genomics at Princeton University (NJ) or Fraunhofer Center for Molecular Biotechnology in DE. The area has a powerful health care infrastructure with 5 medical schools, 120 hospitals, and 374 physicians for each 100.000 inhabitants. PA alone received nearly $1.5bn NIH funding in 2005. Last not least, there is a favourable political and regulatory climate. The State of New Jersey was the first in the US to fund stem cell research allocating $10m between 2005 and 2007, and until recently having the strictest law governing genetic data protection. This links up to collaborations with diagnostic and pharmaceutical companies. major European players are headquartered in the area: GSK, Sanofi Pasteur, or Siemens Medical Solutions in PA, AstraZeneca, Agilent or Avecia manufacturing microbially-derived biopharmaceuticals and DNA medicines in DE. □

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What is the Value of a Therapy?

In discussing Roche's approach to personalised medicine, Prof. Knowles tells his reasons for joining the company, overcoming obstacles in technology, managing R&D, and future plans in bringing personalised medicine about.

INTERVIEW | **WOLF G KRONER**

Your colleague, Dr Schwan, says that he intends to push Roche forward in personalised medicine.

Yes, we at Roche are strongly committed to systematically explore personalised health care, PHC approaches as part of our R&D effort and make PHC happen. My personal reason for joining Roche, over ten years ago was precisely because at that time Roche had two important characteristics. First of all, because Roche had been clearly a strong supporter of innovative technologies. The company had already set up the partnership with Genentech a long time before anybody else had thought about investing in the biotech sector. Secondly, Roche had established a group to work on PCR and had acquired patents in that area – both very visionary decisions. Finally, I felt that because Roche was combining pharmaceuticals and diagnostics, it was one of the few companies capable of actually developing the concept we know today as personalised healthcare.

INFO | JONATHAN KNOWLES

Prof. Jonathan Knowles (1947) studied at Magdalen College School Oxford (UK). He was awarded a 1st Class Honours Degree in Molecular Genetics from the University of East Anglia in 1969, and obtained his Ph.D. in Genetics of Mitochondria from University of Edinburgh in 1973. Thereafter he hold several fellowships at Edinburgh University, CNRS Centre de Génétique Moléculaire Gif-sur-Yvette (FR), Max-Planck-Institut Tübingen (DE), Department of Microbiology and Immunology, University of Denver, CO (US), and University of Helsinki. In 1979 he lectured as Visiting Assistant Professor at Albert Einstein College New York (US). From 1980 to 1989 he worked at the Biotechnical Laboratory VTT Helsinki (FI). He moved on as Director of the Glaxo Institute for Molecular Biology Genève (CH) where in 1995 he was nominated Research Director responsible for all preclinical research at the four European sites. Since 1997 he is Head of Research F. Hoffmann-La Roche Ltd, since 1998 Board Member of Genentech, and since 2003 Board Member of Chugai Pharmaceuticals. He was nominated Honorary Professor by Universität Basel (CH) and has been a distinguished Member of Scientific Advisory Boards and Review Committees at many institutions throughout the world. Since 1998 he is Member of EMBO Heidelberg (DE). He served on EuropaBio's Board of Management. Since 2004 he is Chairman of the Research Directors' Group of EFPIA, the European Federation of Pharmaceutical Industry Associations, and in addition elected Chair of the Governing Board of the Innovative Medicines Initiative, a public-private partnership between the pharmaceutical industry and the European Communities represented by the European Commission.

'Personalised medicine' is a buzz phrase with many meanings –

Let's be clear about what it means. Identifying patients in clinical trials who are more likely to respond to a treatment gives you a much better chance of successful outcome. This is not a totally new concept. What is new is bringing all the knowledge gained in molecular biology over the last fifty years into the development of new drugs, and there it will have a very strong impact.

So you not only apply genetic diagnostics, but also, say, imaging?

Oh absolutely. Our aim is to apply the whole panoply of diagnostics. Obviously, we have a strong focus on in vitro diagnostics within Roche, but other areas are starting to emerge now.

And Ventana?

The recent acquisition of Ventana is absolutely in line with our personalised healthcare strategy. Ventana is clearly focused on tissue-based in vitro diagnostics, so now we have it on board we are well positioned to drive personalised healthcare especially in oncology.

You mentioned tangible results for genetic diagnostics from the deCODE partnership. Human geneticists and other medical specialists are sceptical, saying it's research and still too early to apply these kinds of genetic tests to patients. What is your response?

I would agree with that. Ten years ago, a key question for us was understanding the relative contribution of genes to different diseases, but it wasn't possible to conduct the appropriate experiments back then. Now it's becoming much easier. In most of our clinical studies we are actually looking for genetic risk factors that influence response to a certain treatment. I believe genetic risk factors by themselves are unlikely to be very predictive, but if you combine them with additional information such as the serum levels of different inflammatory components, for instance, then I believe we will achieve increasingly higher levels of predictability. We will never reach hundred

percent, but the closer you can get to understanding which patients benefit from which therapy, the more beneficial and safe for the patient as well as cost-effective it will obviously be.

Do you think it will soon be possible to apply this research?

The contribution of genetic factors to conditions such as inflammatory diseases is now being investigated in large, late-phase studies, some of which involve several thousand patients. And I am confident that the results of these trials will be increasingly applied in clinical practice.

There is a gap between diagnostics development, which takes some 7 years, and drug development which takes about 12. Will you be able to close this gap with clinical trials?

Yes. A very important point is to acknowledge the role of diagnostics in the future of medicine, and I think we at Roche are well prepared to systematically implement that role. The strategically important point is that we have diagnostics and pharmaceuticals under the same roof, which helps us to create interfaces and synergies that are harder to create through partnerships with external companies, for example. Moreover, we have made great progress in the last couple of years. We have undergone major restructuring of our R&D organisation.

Can you give me an example?

We created what we call disease biology areas. There are five of them: the central nervous system, inflammation, oncology, virology and metabolism. Each of these DBAs includes a team of four people who cover the entire value chain. They are the Head of Research, the Head of Clinical Research and Exploratory Development (CRED), who is accountable for all the biomarker programs, the Head of Clinical Development and the Head of Strategic Marketing. These teams take all decisions on projects from the beginning of discovery through to the end of the patent life cycle in their respective therapy areas.

Throughout the drug's entire life-time?

They have the autonomy of decision making until the end of proof of concept and make proposals to the strategic portfolio committee which makes decisions as to how we manage our late phase portfolio. Let me give you a further example of our organisational matrix. Each of our products has a life-cycle team. The leader of the life-cycle team reports to the Head of Strategic Marketing, who is a member of the Disease Biology Area. So all project members report to one member of this team from the beginning of discovery right through to market launch, and it takes all the decisions across the entire organisation.

With some 215,000 patients enrolled in clinical trials around the world, you can create a large pool of biomarkers.

We're currently running a state-of-the-art trial with Tarceva which should finally resolve a lot of questions about biomarkers. In this particular case the trial is investigating the mechanism of the epidermal growth factor receptor in lung cancer. This is a good example of how Diagnostics and Pharmaceuticals are working together very closely to support a product that's already on the market and to understand how it could be even better targeted and more effectively used.

What is the impact on patient stratification in terms of detecting new responders – say for example people affected by Maturity Onset Diabetes of the Young, MODY?

We've invested a great deal in creating a molecule to address the MODY 2 gene, which is a glucokinase. This has taught us two things. The first is that this molecule – actually there are a number of them – regulates glucose pretty effectively in all patients. So the fact that you identify the target from a small subset – it's about 3% of diabetics – doesn't necessarily mean that the mechanism can't also be used in other patients. The second is that there is no one-to-one correlation between how you get to the target and where the drug is useful. As regards stratification let me give you the example of a special oncology trial we're running in conjunction with Plexxikon where we're only including patients who have a B-Raf(V600E) mutation ,because the drug is far more effective in patients with this B-Raf mutation. When we recruit patients for this trial, we conduct a genetic analysis of their tumour before we include them. A second example is another ongoing trial of an MDM2-targeting compound. This drug will only work in patients with a normal wildtype p53 gene –a gene that controls cell death and growth. Again, we only include patients who have been genetically tested for a normal p53 gene. Various other studies, some of which involve established products such as Pegasys and Tarceva, are also in progress and we hope these will improve our understanding of how these drugs can be used to maximum effect.

What is the role of early disease prevention in pharmaceutical R&D?

The very important question that payers are asking themselves is 'what is the value of a therapy?' The statins are probably a good example here. Statins are prescribed to a large number of people on the basis of a statistical calculation. You can calculate that you are saving lives and that at the same time it is cost-effective to give statins to very large numbers of people.

One option could be to diagnose susceptibility so that costly therapies can be discarded…

This presents a very interesting opportunity. Let's take type 1 diabetes as an example. Type 1 diabetes is only diagnosed at a point when some 90% of the beta cells creating insulin

have been already destroyed. Unless you regenerate beta cells – which is an alternative strategy, of course – the diagnosis comes rather late. We're not running any trials yet, but we are thinking about how we might do so.

The company also needs to change its mindset to not thinking from the perspective of a mass indication where a huge number of patients are affected by a particular disease.

We are very ambitious when it comes to the effectiveness of the medicines we want to create. When we create a new medicine, we aim to continue our tradition of producing very effective first-in-class medicines. Our most important product, MabThera/Rituxan has demonstrated a high efficacy in Non-Hodgkin's Lymphoma, with 70 to 80% of the patients who have the medicine included in their treatment responding. The complete response rate for Herceptin in adjuvant breast cancer with HER2 positive patients is about 50%. So our products are extremely effective, and we want to make sure this remains the case by doing everything we can to identify the patients who really respond. In my view, focusing on a large patient numbers limits efficacy, and this is something that I feel is relevant to diseases such as asthma, Alzheimer's or diabetes. The only way to ensure efficacy is to say: These are the patients that need this special treatment.

Is this something Roche has learned from orphan drug companies?

No, I don't think so. I'd say we learnt it from MabThera/Rituxan. It also derives from the realisation that bringing major efficacy to even a small patient population clearly generates value for society and patients as well as for Roche and its stakeholders.

Let's turn to CEPT. As we all know, it's a drug that prevents atherosclerosis.

We believe it actually prevents cardiac events. It also doesn't have the defects that competitor compounds have.

But it might be a very long time in coming. Commenting on the torcetrabip failure, clinicians close to the FDA have said that it might take seven or eight years to move the CEPT inhibitors forward…

It depends on the number of patients. The smaller the number of patients, the longer you will have to wait to get statistical results in clinical trials, so we plan to include several thousand patients. In addition we will be choosing patients who have already had one myocardial infarction and are therefore at greater risk of having a second. The morbidity/mortality study we have designed is a cardiovascular event driven trial. Its duration will depend on patient recruitment and the event rate in the study.

Obviously it's supposed to treat hypercholesterolemia.

Total cholesterol levels are only one of the factors that need to be studied. There is also the number of triglycerides, and, most importantly, the LDL/HDL ratio. Statins lower LDL, the 'bad lipid'. Our molecule goes further by raising HDL, which is the 'good cholesterol'. If you raise the good lipid while simultaneously decreasing the bad one, you will see an even more dramatic effect.

By studying atherosclerosis you end up in different medical fields such as hypercholesterolemia or hypertriglyceridemia. You discover the many inter-relationships between these diseases. I do not think that the current theory of disease is adequate in terms of disease classification. A lot of interesting work is now being done to improve our understanding of vascular disease in the context of plaques and imbalanced lipids. However the imaging of plaques is still quite difficult. I think the next five years will see technological advances that will allow us to diagnose plaques and gain a better understanding of the mechanisms.

The way I understand in vivo imaging, the best way to do it is to have some sort of nanobodies carrying the contrast agent.

It could be a nanobody, but it could also be an antibody. I think it could also be, for instance, the corticotropin releasing factor, which is a measure of vascular inflammation.

My guess is that Siemens has broader imaging capabilities.

Siemens clearly has the imaging expertise in terms of making 'big machines' – the physics of imaging. As far as what we might contribute to imaging agents goes, we have significant world-class expertise in creating antibodies that are not immunogenic. We also have some pretty good expertise in the development of different types of markers, and we have a lot of expertise in creating diagnostic reagents. Also we are constantly creating new agents and antibodies in the context of tissue histopathology, particularly now that we have Ventana on board. So I believe it is possible that we may be able to make some significant contributions to imaging reagents – in other words to what you measure.

So, again, personalised medicine's time has come?

Ten years ago no one gave much thought to personalised healthcare, either inside or outside Roche,– to say nothing of academia. It has taken some time to understand where we need to go. Ten years ago, we set up our pioneering collaboration with deCODE precisely to answer the question of whether there are significant risk factors for common diseases. The answer was a definite 'yes'. For the last five years therefore, one of our main strategic focuses across the Roche Group – that's Genentech, Chugai, and our Pharmaceuticals and Diagnostics Divisions – has indeed been to start implementing what we call 'personalised healthcare' so that we can develop better and more effective medicines. □

BioUetikon Reaches Out For Global Business

The Irish bioprocess service contractor is a good example how the marriage of excellent science, skilled management, and dedicated foreign investment are creating wealth and accelerate expansion beyond local networks.

WOLF G KRONER | INTERVIEW

Do-it-yourself or Buy, is a question small biotechnology companies increasingly have to answer when they move forward their drug candidate. Contract manufacturers offer to move forward drug candidates at greater speed, with financial risks better calculable for investors, and solving capacity problems in upscale before they pose a serious concern to the drug developer. Irish BíoUetikon at Dublin City University (DCU) campus has been staking its future on this trend.

It began in 1994 when researchers around Martin Clynes at DCU's National Cell and Tissue Culture Centre were approached by Dutch API manufacturer Diosynth, then part of Akzo Nobel, to develop a process for its cell lines producing follicle stimulating hormones. "We had the basic skills, they needed" recalls Clynes, "and within about a year we were producing recombinant human proteins for the medical market. It was give and take, Diosynth helped the researchers to bring up facilities to GMP-standards. By contrast, says Clynes, he and his team realised that Diosynth were sooner or later to build up their own bioprocessing unit, and they at Dublin could not really exploit their success as a springboard to other contracts without turning fully commercial. In 1998, he spun-off Archport with DCU as the only shareholder. Diosynth had been using nearly all capacities while others queued up for services. Archport decided to invest into new laboratory infrastructures completed in 2002. In 2005 it became clear that the scientists were about to lose out in the race to turn profitable despite an increase in turnover of more than 4 percent against 2004. By 2006 Archport had borrowed more than 4m secured by DCU and invested some 3,6m in laboratory facilities and equipment. But earnings (2005: 378.000 with pre-tax loss of 565.000) did not rise as quickly as expected.

In August 2006 German API manufacturer CU Chemie Uetikon, part of Swiss CPH Chemie + Papier Holdings, became the helper in the hour of need. According to company files CU acquired an 80% share in Archport Ltd. It was a win-win deal (see interview). The acquisition of Archport, saved CU some six years of building up the bioprocess expertise on its own, believes Professor Martin Clynes, who moved to BioUetikons Advisory Board and is currently Head of DCU's National Institute for Cellular Biotechnology. While retaining Archport's science expertise and extensive network among Irelands pharma manufacturers, CU Chemie Uetikon streamlined the firm's business approach. Thus, Archport was renamed BioUetikon and Mike Mulcahy was hired as Managing Director in 2007. He is an experienced business, marketing and sales person who understands quality-conscious pharma manufacturers and also the challenges faced by small biotechs with limited resources, but passionate science.

Leveraging Expertise and a Worldwide Customer Base

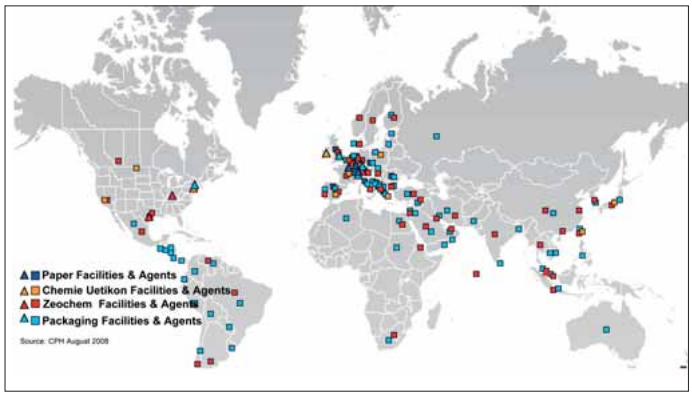
Last April, BioUetikon was officially launched, by the then Prime Minister of Ireland, Taoiseach Mr. Bertie Ahern. While the company is continuing as fee-for-service contract manufacturer it is set to rapidly expand beyond the existing customer network in Ireland. Mike Mulcahy is determined to leverage the CPH network and CU Chemie Uetikon's global customer and technical support base beyond present customers in Ireland and the UK.

BioUetikon

Former Irish Prime Minister Bertie Ahern is supporting national biotech champions and insisted to attend the launch as An Taoiseach, with Mike Mulcahy (r).

A core group of experienced employees remained throughout the period of transition, and now engineers and scientists are able to handle multiple projects at the same time offering upstream and downstream development, process optimisation, and cGMP production. There are 3 separate cell culture suites and a matching 3 downstream processing rooms which allow several projects to run at the same time. 7 cold stores

BioUetikon

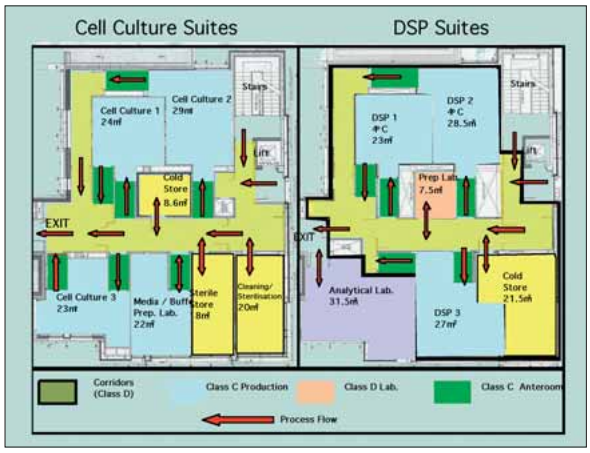


CPH network

extending over 100 m² operate around the facility.

While current facilities extend some 1.600 m² located in the DCU R&D building, Mulcahy is already considering the addition of a larger facility outside the university campus. BioUetikon has various bioreactors available for development and GMP production. For a commercial recombinant protein production project like Diosynth's one a 10 L-bioreactor operating in perfusion mode was sufficient. Other bioreactors include a 100 L working volume stainless steel and 20/50L single use options. Investment in single use technology in 2008 will increase available scale to 250L batch or fed-batch. "By end of the year, it's at 250 L, but we can incorporate fermenters up to 1.000 L to produce GMP materials" says Mulcahy. BioUetikon is balancing scientific challenges with the need to speed up customer projects. Those benefitting most are small biopharma as they regularly lack the expertise in up-scaling and the time to build it in-house. "A start-up might have a good scientific foundation, they might have experienced senior executives with solid financial backing, and project management to develop a pharmaceutical" Mulcahy knows from experience, "but they need people who can help to implement their plans". BioUetikon's customers often need process scale up and optimisation first as there is no resolved process. Clients can also tap into the well established quality system and in-house microbial testing, validation, and other quality assurance services. Mulcahy explains that a crucial part of the added-value BioUetikon offers, is how closely it can integrate people and know-how to projects. This enables clients, he says, to improve development of their candidate products on its way to the market. "Outsourcing manufacturing of clinical samples can easily save a company 12 months set up-time and considerable overhead compared to if it were to establish GMP compliant production by itself" argues Mike Mulcahy. Early collaborating with a CMO considerably decreases development risks. In addition, it speeds up the process and therefore can be more convincing to potential investors to provide a biotech start-up with capital they ask for. Successful customers like Fusion Antibodies and Life Scientific in Ireland or Orthomimetics in Cambridge (UK) already are vivid examples for these claims.

BioUetikon



CPH Facilities

In August 2006 German API manufacturer CU Chemie Uetikon, part of Swiss CPH Chemie + Papier Holdings, became the helper in the hour of need. According to company files CU acquired an 80% share in Archport for 200.000 and took over guarantees for loans and other credits. It was a win-win deal (see interview). The acquisition of Archport, saved CU some six years of building up the bioprocess expertise on its own, believes Martin Clynes, who moved to BioUetikons Advisory Board and is currently Head of DCU's National Institute for Cellular Biotechnology. While retaining Archport's science expertise and extensive network among Irelands pharma manufacturers, CU Chemie Uetikon streamlined the firm's business approach. Thus, Archport was renamed BioUetikon and Mike Mulcahy was hired as Managing Director in 2007. He is an experienced marketing and sales person who understands quality-conscious pharma manufacturers as well as small biotechs with scarce resources, but passionate science.

Leveraging Expertise and a Worldwide Customer Base

Last April, BioUetikon was officially launched, and indeed it has been more than publicly announcing a change in the firm's name and a completed transition to a new shareholder. While the company is continuing as fee-for-service contract manufacturer it is set to rapidly expand beyond the existing customer network in Ireland. Mike Mulcahy is determined to leverage the CPH network and CU Chemie Uetikon's global customer and technical support base beyond present customers in Ireland, UK and Sweden.

While the number of ten employees has not changed since 2000, engineers and scientists are now able to handle multiple projects at the same time offering upstream and downstream development, process optimisation, or cGMP production of clinical samples. There are 3 separate cell culture suites matching 3 downstream processing rooms which allow to run several projects at a time. 7 cold stores extending over 100 m² operate round the hour.

While current facilities extend some 1.600 m² located in the DCU R&D building, Mulcahy is already eyeing to re-locate to a larger facility outside the university campus. For the Diosynth project a 10 L-bioreactor was sufficient. Meanwhile available fermentation batch size is 100 L. "By end of the year, it's a 100 L, but we can incorporate fermenters up to 1.000 L to produce clinical materials" says Mulcahy. BioUetikon is balancing scientific challenges with the need to speed up customer projects. Those benefitting most are small biopharma as they regularly lack the expertise in up-scaling and the time

to build it inhouse. "A start-up might have a good scientific foundation, they might have some senior executives where they get funding, and project management to develop a pharmaceutical" Mulcahy knows from experience, "but they need people who can implement technically plans". While BioUetikon's customers typically need process optimisation first not having a resolved process, the company claims doing better than other CMO's which also do process development in parallel to optimisation, have in-house microbial testing, validating, and quality assurance services. Mulcahy explains a crucial part of the added-value BioUetikon offers is is consulting and coaching clients enabling them to improve development of their other candidate products and save needed monies next time they return. "Outsourcing manufacturing of clinical samples can easily save a company 12 months and wage for two full time employees, if it were to establish GMP compliant production by itself" argues Mike Mulcahy, as early collaborating with a CMO decreases development risks. In addition, it speeds up the process and therefore is more convincing investors to provide a biotech start-up capital they ask for. Successful customers like Fusion Antibodies in Belfast or Orthomimetics in Cambridge (UK) already are vivid examples for these claims. □

«We are competing worldwide»

BioUetikon will complement its range of services. Heinz Sieger, the executive managing director of CU Chemie Uetikon, the mother company of BioUetikon Ltd. answered BioWorld *EUROPE*s questions.

WOLF G KRONER | INTERVIEW

In 2004 CU Chemie Uetikon was still a rather traditional chemical company. Why did you decide venturing into biotechnology?

We are a very successful fine chemical manufacturer and we want to head our sector in the future. During these days we realised that biotechnology was arriving in pharma industry at a much faster pace than anyone could have expected. Today, some 10 to 12% of API's on the market are already manufactured by biotechnological processes.

You didn't build up an R&D unit in-house?

Innovation doesn't consist only of inventing something new. Innovation also means integrating something you do not have. You may notice that even today, there aren't so many API contract manufacturers worldwide able to put biotechnology to work. At the time, we decided to simply add it to the broad spectrum of technologies we already master.

You're based in Southern Germany near CPH Chemie + Papier, the Swiss holding company. Why coming a long way to Ireland?

You're right. Ironically, it is nearly easier to travel from Ireland to the States than from Lahr to Dublin. However, there were not these opportunities available in Germany we found here in Ireland.

To acquire an existing company and start right ahead?

Before choosing BioUetikon, then Archport, we evaluated a dozen of biotechnology companies throughout Europe. None of these were as advanced and had such a collaboration network. In addition, we received a lot of support by Irish authorities. The presence of Taoiseach, the President of the Irish people at the launch underlines the willingness to continue support in the future. I should add, Dublin City University, which is a longstanding shareholder of this company, should really be regarded an exemplar of industry orientation throughout academia in Europe.

What will be the role of BioUetikon in CPH?

It is part of the fine chemistry business «Organo». We are competing worldwide, with Asian API manufacturers in particular. For the next years, BioUetikon will complement the range of services we offer to those of our customers interested in biomolecules and bioprocess optimisation. Mike Mulcahy is a talented manager, he and his team will rapidly grow BioUetikon. And, when we are growing as planned we intend to expand from the present laboratory building limited in size beyond the DCU campus. □

INFO | HEINZ SIEGER

HS

Heinz Sieger (1947) obtained his Ph.D. in chemistry from Universität Bonn, DE. Thereafter he worked in positions with increasing responsibility in the Chemical Industry for Specialities. In 1995 he joined CPH Chemie + Papier Holding where he leads the Organo business unit and is executive managing director of CU Chemie Uetikon, Lahr (DE), the mother company of BioUetikon Ltd. Since 2006 he is Board Member of CEFIC, the European Chemical Industry Council, UK.

The xCELLigence System

Real-time cell-based toxicology testing might replace animal testing for product release and drug safety.

TEXT | JAMES O'CONNELL, YAMA ABASSI, BIAO XI, XIAOBO WANG, AND XIAO XU

The xCELLigence System, co-developed by Roche and ACEA Biosciences, was used to conduct in vitro testing of selected microbial toxins (e.g., *Clostridium botulinum* toxin type A, BOTOX®, and the closely related *C. difficile* toxin), and *in vitro* cardiotoxicity testing of drug candidates. Currently, laboratory animals are required to be used in the release of pharmaceutical products such as botulinum toxin type A and in the prediction of cardiotoxicity of new drugs. Such tests consume very large numbers of laboratory animals. We believe that the xCELLigence System can essentially replace the animals used in these types of testing. Furthermore, as the xCELLigence System becomes linked to other systems, such as the Roche 454 System and the Roche Nimblegen Arrays, we expect that animal testing will be reduced significantly in pharmaceutical development in general.

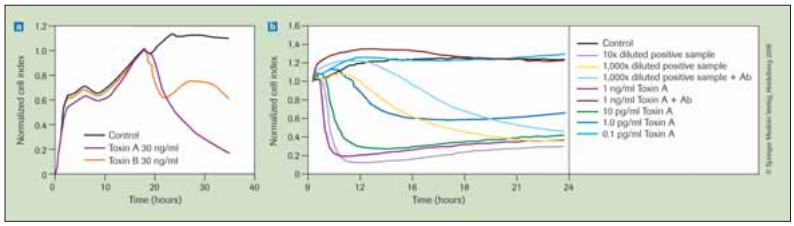


Figure 1: Cell-based assay for C. difficle toxin A and B testing on the xCELLigence System. a) Cytotoxic effect of toxin A and B. The toxic effects of both toxin A and B were tested using a cell-based assay on the xCELLigence system. Cytotoxic kinetic patterns are different for both toxins b) Sensitivity and specificity of the toxin detection using fecal samples. The system is able to detect the toxin in fecal samples with great sensitivity (1 pg/ml) and excellent specificity.

Background

Corporate statement from Allergan on botulinum toxin type assays: «BOTOX® (botulinum toxin type A, Allergan Inc.) is, first and foremost, an important medicine that over the last 15 years of clinical use has helped millions of patients with serious medical conditions worldwide. In the U.S., BOTOX® is approved for the treatment of four debilitating conditions, including two eye disorders that can lead to functional blindness, cervical dystonia which is a painful movement disorder affecting the head and neck, and excessive underarm sweating. Worldwide, it is approved for twenty different indications in more than seventy-five countries, and the therapeutic use of BOTOX® accounts for the majority of all BOTOX® use.» Currently all pharmaceutical manufacturers are required by the Food and Drug Administration (FDA) in the United States and by other worldwide health regulatory agencies to protect patients and consumers by assuring product safety and efficacy through animal testing and other methods. Botulinum toxin type A is unique among medical therapies in that it is a biological product, which means that it is derived from natural sources – in this case, from the bacterium *C. botulinum*. When manufacturing biological products, testing is particularly critical to ensure the consistent safety and efficacy of each batch of product.

The safety and efficacy of botulinum toxin type A is assessed by using the LD50 Test (Lethal Dose 50%). In the case of botulinum toxin type A, mice are injected with the active ingredient – a form of the same toxin that causes Botulism food poisoning – and experience differing levels of muscular paralysis. Those given a high or powerful dose eventually die from suffocation after their respiratory muscles become paralyzed. In addition, over the last ten years Allergan, the botulinum toxin type A manufacturer,has invested more than 40 million U.S. dollar in the development of alternative assays which they hope will be able to replace animal-based assays in the manufacture of the product.

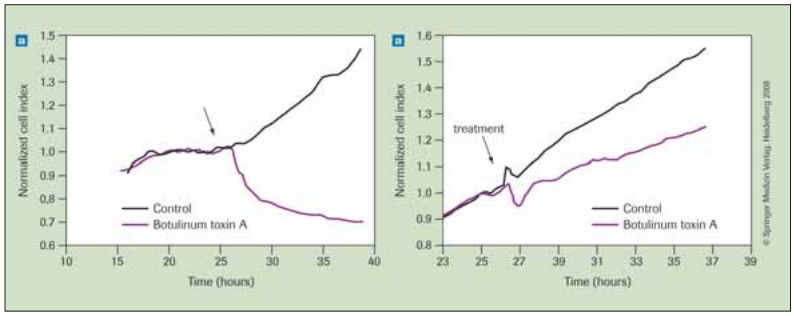


Figure 2: Dynamic monitoring of toxic effect of botulinum toxin on CNS cell lines using the xCELLigence System. Botulinum toxin A at a concentration of 6.67 µg/ml was tested on the A172 glioblastoma cell line (ATCC) and the SH-SY5Y neuroblastoma cell line (ATCC).

The xCELLigence System

The xCELLigence system allows for label-free dynamic monitoring of living cells. The core of the system is the integrated microelectronic sensor contained in each well of the 96-well E-Plate. Application of a low-voltage AC current allows the microsensor to detect minor changes in the ionic environment of the well which is related to changes in cell number, changes in cell morphology, and the strength and quality of attachment of the cells to the bottom surface of the microwell. The system is ideally

suited for cell-based applications such as:

- Cellular quality control
- Cell proliferation
- Cytotoxicity
- Cell adhesion and spreading
- Receptor-mediated signaling
- Barrier function

xCELLigence Assays Designed to Reduce Animal Testing in Pharmaceutical Development:

Microbial toxin assays

This cell-based assay on the xCELLigence System provides kinetic information which is not available with other technologies. *Clostridium difficile* is resistant to most antibiotics and its toxins A and B cause colitis. The real-time monitoring of the live cells using the xCELLigence System shows the time dependent cytotoxic effect of toxin A and B and the unique cell death patterns associated with each specific toxin (Figure 1 a). This provides a great predictive value for the *in vitro* cell-based assay.

In addition, we have used the xCELLigence System to identify *C. difficile* toxins A and B directly from stool samples using cell culture and specific toxin neutralization with highly specific toxin A and B antibodies. The fecal samples were obtained either from subjects infected with *C. difficle* (positive samples) or fecal samples spiked with purified toxin A – which were added to the cells as a control. The sensitivity was determined by the fecal samples spiked with the serially diluted toxin. The specificity was determined by the neutralization of the toxic effect by specific antibody. The test as performed with the xCELLigence System is extremely sensitive – in the pg/ml range – and highly specific (Figure 1b).

We also were able to use the system to detect the cytotoxicity of botulinum toxin, the active compound in the over 1 billion U.S. dollar/year drug. Currently, there is no *in vitro* assay approved by the U.S. FDA (and other international regulatory bodies) for the release of botulinum toxin A. However, using the xCELLigence System, we were able to detect toxin-specific cytotoxicity in two CNS cell lines, A172 glioblastoma cell line and SH-SY5Y neuroblastoma cell line. Cells were treated with botulinum toxin at a concentration of 6.67 _g/ml, and the toxic effect was continuously monitored in real-time on the xCELLigence System (Figure 2).

Our preliminary results from this in vitro cell-based botulinum toxin test indicate the possibility of replacing the required animal test with a real-time labelfree cell-based assay on the xCELLigence System. We are currently screening additional cell lines to be used in this important assay.

Cardiotoxicity assays

Similarly, another important area that the xCELLigence System could significantly impact is the cardiotoxicity assessment of drug candidates, which currently requires the extensive use of laboratory animals. A major effort has been underway to replace these tests with *in vitro* tests, but the most substantial shortcoming of current *in vitro* cardiotoxicity assessment methods is a lack of adequate predictivity. The predictivity can be significantly improved by using a real-time, label-free cardiomyocyte-based assay on the xCELLigence System. Based on our preliminary study with mouse stem cell derived cardiomyocytes, the real-time, continuous monitoring of cardiomyocytes in response to exposure to test compounds allows for detection of early, transient ion channel- or receptor-mediated effects and long-term cardiomyocytotoxicity in the same living cell population (Figure 3). In our experiment, mouse stem cells were seeded onto the 96 well E-plate and then differentiated to cardiomyocytes (CorAT cells, Axiogenesis) in differentiation media. The differentiation was monitored in real-time. Once the stem cells differentiated into specific cardiomyocytes, the compound emetine was added in different concentrations. The cardiotoxic effect was then continuously monitored in real-time for an additional 24 hours (Figure 3).

In addition, with the unique features of the automatic data acquisition and high-throughput assay format, the xCELLigence System can be used in secondary screening as well, which makes the prediction and prioritization of cardiotoxicity possible even in the early stage of drug discovery.

Conclusion

We believe that the combination of the xCELLigence System with other high information content systems such as the Roche 454 and Roche Nimblegen Systems will bring a new level of accuracy and information to *in vitro* testing that will significantly reduce the number of animal tests required in pharmaceutical development. □

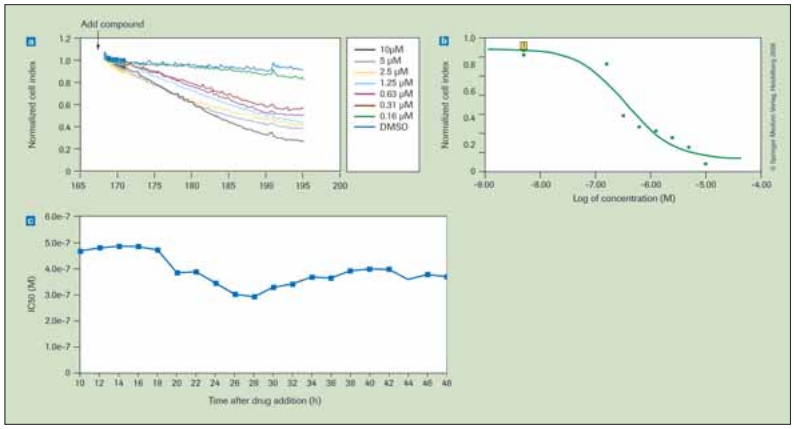


Figure 3: Monitoring of emetine-mediated cardiotoxicity using mouse stem cell-derived Cardiomyocytes (CorAT cells) on the xCELLigence System. (a) Dose-dependent cytotoxic kinetics of emetine (b) Dose-dependent cardiotoxic effect at 24 hours of compound treatment (c) Time-dependent IC50 values during compound treatment. IC50 is a quantitative measure indicating how much of a particular substance is needed to inhibit a given biological process or component of a process (i.e., an enzyme, cell, cell receptor or microorganism) by half.

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Biofuels Analysis using Reagent-Free Ion Chromatography

Biofuels are a type of alternative fuel created from biomass – harnessing the solar energy stored by plants in the form of carbohydrates. A key class of biofuels is the biologically produced alcohols such as ethanol, methanol, propanol, and butanol. While the production of ethanol as an alternative to gasoline has increased rapidly, some scientists believe that methanol will be the more important alternative fuel. Methanol can be produced from non-food sources including coal and natural gas. It is harder to ignite than gasoline and burns cooler, making it safer for the consumer. Methanol is also a better precursor to other chemical processes than ethanol.

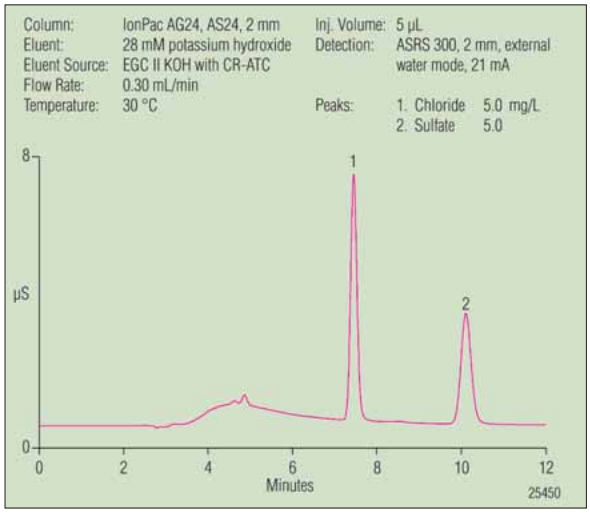


Figure 1: Separation of 5 mg/L chloride and sulfate in HPLC grade methanol on the IonPac AS24 column.

Fuel methanol or ethanol that is contaminated with soluble anions such as chloride and sulfate can form plugging deposits and cause corrosion in automobile engines. If sulfate and chloride concentrations exceed the limits defined in ASTM Specification D 4806, then the ethanol may be rejected as unacceptable for use in automotive spark-ignition engine fuel. The ASTM International has proposed that a new ion chromatography (IC) method be adopted to measure this critical product specification for ethanol intended for automotive spark-ignition engine fuel use.[1]

Ion chromatography (IC) with suppressed conductivity detection is the simplest approach for determining inorganic anions in alcohol fuels, enabling the detection of the target anions with a single injection, fast analysis times, and minimal or no sample preparation. Dionex provides a wide range of solutions for the determination of anions from parts per billion (ppb) to parts per million (ppm) concentrations in alcohols. The use of IonPac AS24 or AS18 columns on a Reagent-Free ion chromatography system makes it simple for the analyst to get accurate and reproducible results.

Figure 1 shows the direct injection of an untreated methanol sample – the simplest approach for determining chloride and sulfate. This procedure eliminates costly and time consuming sample preparation commonly used to determine anions in solvents. Separation of chloride and sulfate at 5 mg/L each in methanol was obtained using the optimized conditions with the AS24 column and an electrolytically generated potassium hydroxide eluent. The AS24 column allows fast (<12 min) runs, and excellent retention time stability.

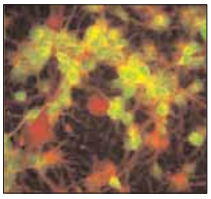
The Dionex Reagent-Free ion chromatography systems combined with our best-in-class columns and chemistries provide customers in the alternative fuel industry with convenient methods to meet the rigorous demands required by the ASTM for production of quality biofuels. Whether you are analyzing ethanol, methanol, propanol or butanol, the Dionex Reagent-Free ion chromatography system gives you consistent, specific, and reproducible results for the determination of corrosive ions in biofuels. □

References

1 ASTM Specification D 4806, ASTM International, 100 Barr Harbor Drive, West Conshohocken, PA 19428.

MilliTrace™ Constitutive GFP Reporter Neural Stem Cell Lines

New cell lines allow robust, in vivo monitoring of stem cell behavior.



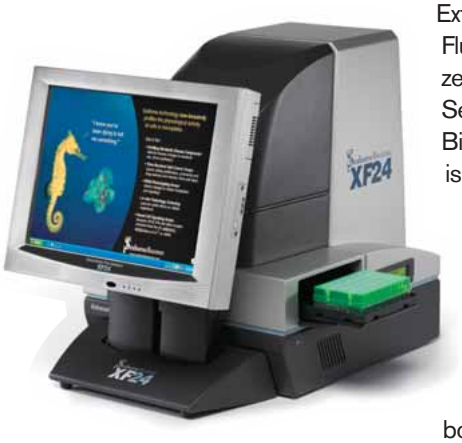
Millipore Corporation, a Life Science leader providing technologies, tools and services for bioscience research and biopharmaceutical manufacturing, recently announced the availability of their MilliTrace primary rodent neural stem cell (NSC) lines that express green fluorescent protein (GFP) constitutively. GFP expression in these stem cells is the best way for researchers to monitor the behavior of specific populations of cells as they proliferate, migrate, and differentiate into various cell lineages, depending on developmental context. The MilliTrace cell lines are the first commercially available, GFP-expressing, karyotypically normal stem cell lines, and are supplied with optimized expansion medium.

Many immortal cell lines display unstable or aneuploid chromosomes; however, for stem cell lines to yield physiologically relevant results, it is important that the cells display normal chromosomes. Unlike human NSC cultures, rodent NSCs undergo prolonged self-renewal in culture without losing chromosomal stability. Researchers at Millipore have been able to isolate and propagate monolayer cultures of NSCs from two different regions of the rodent brain: the adult rat hippocampus and the embryonic mouse cortex. Even after introduction of constitutively expressing GFP, the NSCs from these tissues show normal karyotype, stability over more than 10 passages, and multipotency. Both cell lines can be readily differentiated into neurons, astrocytes, or oligodendrocytes, using appropriate cues.

Validated for high levels of GFP expression, stem cell marker expression, and multipotency, MilliTrace GFP Reporter Neural Stem Cell Lines can improve reproducibility and data quality for a variety of applications. Researchers can use MilliTrace cell lines to study cell-cell interactions in co-culture studies or contribution of NSCs to the stem cell niche in vivo. MilliTrace cell lines also enable high-throughput screens to discover agents that affect stem cell maintenance and differentiation. □

www.millipore.com

Mitochondrial Measurements



The XF24 Extracellular Flux Analyzer from Seahorse Bioscience is a better way to profile bioenergetics. By measuring both mitochondrial respiration and glycolysis simultaneously and in real-time, the XF24 provides physiologically relevant insight into the effect of drug treatments, gene transfections and toxins on cell metabolism.

Key features of the XF24 Flux Analyzer:

- Simultaneously measure O_2 consumption, extracellular acidification and CO_2 production
- Measurments of a single population of cells over a period of minutes, hours or even days
- Non-invasive assay in microplate format with re-use of cells

Mitochondrial integrity displays a key component of cell physiology. Under typical *in vitro* cell culture conditions, oxygen consumption rate (OCR) is a direct measurement of mitochondrial respiration and extracellular acidification rate (ECAR) is dominated by lactic acid production formed during glycolytic energy metabolism. Measuring both parameters simultaneously enables a more comprehensive assessment of cellular energetics and provides a valuable monitor of mitochondrial functionality. In addition, the optional detection of CO_2 production monitors the pentose phosphate pathway. Recognition of the value is underscored by the growing number of investigators using the Seahorse XF24 to achieve these metabolic measurement.

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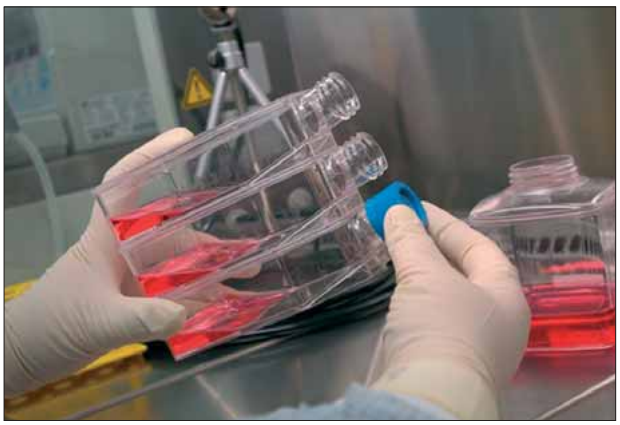
CELL pro Danube – production of cellular therapeutics with highest quality



CELL pro Danube Ltd. founded in 2006 is a highly innovative life-science start up enterprise producing up-to-date cellular therapeutics against cancer on highest quality level. The company has set up a new, highly sophisticated GMP-laboratory for aseptic processing, modification and storage of human cells in the biotechnology innovation centre of Krems a. d. Donau near Vienna, Austria. The company has two separated GMP-clean room areas of class 100, preparation rooms of class 10.000 and storage areas as well as quality control laboratories all equipped with state-of-the-art devices (e.g. -20, -80°C and -150 °C deep freezers, high performance LAFs, FACS, ELISA, PCR and so on). Intensive R&D co-operations with universities, public institutions and other companies ensure most current and best scientific input and know-how in the field of cellular biotechnology. Focusing its activities on the production and modification of autologous dendritic cells for cancer therapy CELL pro Danube is one of the most sophisticated and developed laboratories in Austria for human cell therapies. Highest clean room requirements based on Annex I of the EU-GMP guideline are the source and fundament of best quality practices. "To cure cancer" is the vision of the company and the team around Wolfgang Huber PhD, General Manager of CELL pro Danube. To reach this enthusiastic goal CELL pro Danube is permanently adapting and modifying its cellular therapeutics to provide the patients with the best medicine.

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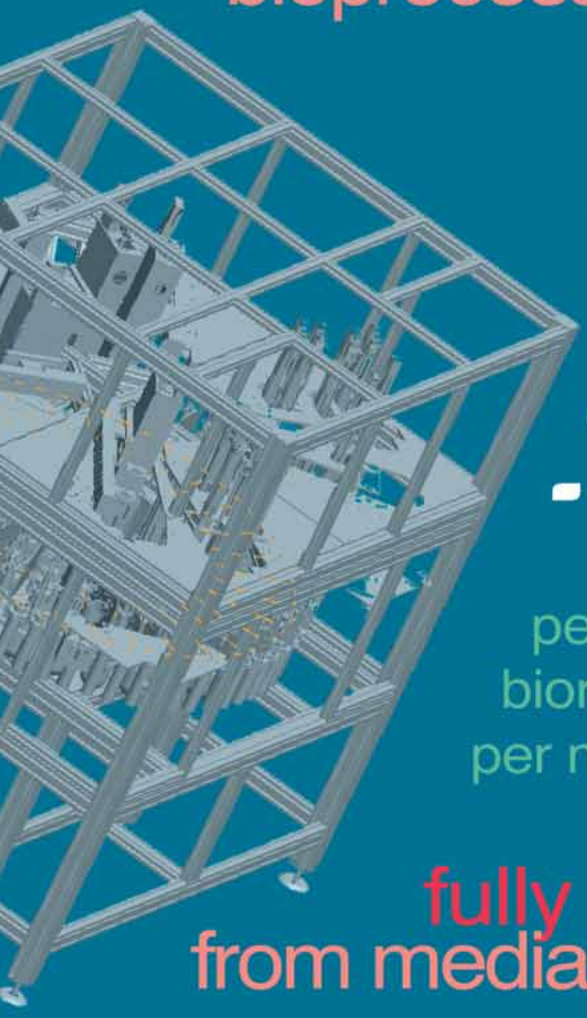

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Small Molecule Kinetics Characterization

With the launch of the Octet® RED System for label-free, real-time kinetic analysis of drug compounds, FortéBio introduced a second system for the analysis of molecular interaction. The new system brings unparalleled ease-of-use, sensitivity, throughput and affordability to the analysis of small molecules, peptides and proteins for research, development and bioprocessing. Octet RED is based on ForteBio's proprietary Bio-Layer Interferometry (BLI) technology, in which optical biosensors measure multiple interactions in parallel, without the use of chemical labeling. The Octet RED System's high sensitivity and rapid data acquisition capabilities enable analysis of low molecular weight molecules that interact extremely quickly, which is critical for comprehensive kinetic characterization of drug candidates.

In addition to small molecule characterization, the Octet RED systems allows also for full analysis of protein and peptide interaction as it is possible with FortéBio's first system, the Octet QK.

The degree to which a drug compound binds to a therapeutic target helps predict a drug candidate's potency and effectiveness, and is a significant component of drug discovery and lead optimization. A label-free method of analysis is increasingly preferred because it avoids label interference with the binding activities of the therapeutic target. The faster and more affordable way which the Octet RED systems displays, allows accelerated validation of drug screening as well as accelerated development of biologicals.

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Next Generation Modular Microplate Washer Series Platform

The unique modular design of this new MDS Analytical Technologies / Molecular Devices washer platform allows you to configure the system for your current microplate format and applications, while at the same time provides an affordable upgrade path when your assay requirements change. The systems' 96- and 384-well wash heads are interchangeable to extend the capabilities of the system on a single instrument platform. The interchangeable heads install in seconds, without the need for tools, calibration or alignment.

The AquaMax system supports a variety of biological assays, including ELISA, immunoassays, cytotoxicity assays, and many cell-based assays. Control of dispense pressure, aspiration position & speed, overflow washing and plate soaking allows to optimally adapt the AquaMax to different assay requirements.

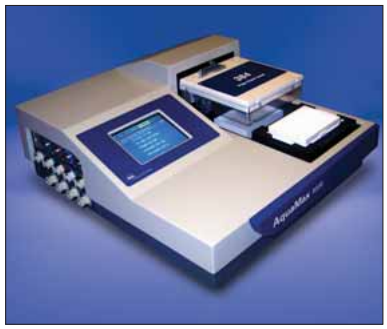
Key Benefits:

- Maximum Efficiency
- Maximum Cost saving
- Maximum Flexibility
- Maximum Speed
- Maximum User Friendliness

The system washes all wells in 96- and 384-well microplates simultaneously. Intuitive, touch-screen programming gives you a high degree of control but preserving convenience. A comprehensive cleaning routine prevents and eliminates clogging, the primary cause of microplate washer failure. The unique reverse-flush feature cleans the instrument head, not just the probes.

AquaMax is completely self-contained and does not require external pumps or computers, and thus is space saving. AquaMax washers are available in two different configurations: AquaMax 2000 has two fluid inlets and AquaMax 4000 has four fluid inlets. Both configurations can be equipped with user-exchangeable 96-well or 384-well heads.

In addition, AquaMax is compatible to StakMax plate stacker for automated plate feeding of up to 50 microplates and can also be easily integrated into robotic platforms.



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454 Sequencing at the Forefront of Bone Marrow and Stem Cell Therapy Research

The Blood Centre Linz, Austria has purchased a Genome Sequencer FLX System from Roche, for use in research on rejection reactions in Bone Marrow and Stem Cell transplantation. Using conventional Sanger sequencing it can take weeks to analyse large DNA regions. The Genome Sequencer FLX System can achieve this task within hours, improving efficiency and reducing costs. The ultimate goal of the research undertaken with the Genome Sequencer System is to create a significant reduction in the high rate of rejection reactions in transplantation.

Typically, the odds of finding a suitable stem cell donor is 1 in 500,000. Every year in the German speaking countries, approximately 12,000 people require urgent transplantations. The success of these transplantations is largely dependent on an exact tissue matching based upon HLA typing. The human leukocyte antigen system (HLA) is a major component of the immune system in humans and is fairly unique from person to person. In transplantation, any cell displaying an HLA type other than the patient's is «non-self» and is attacked by the immune system as an invader, resulting in the rejection of the tissue bearing those cells. Because of the importance of HLA in transplantation it is subject to extensive research, the HLA loci are among of the most frequently typed by serology or PCR relative to any other autosomal alleles.

With the Genome Sequencer from Roche Diagnostics, the researchers at the Blood Centre Linz will concentrate on making the HLA typing application significantly quicker and more efficient in order to ensure a significant decrease in graft rejection by a better matching recipients to donors.

«The continuous decoding of genetic characteristics offers unprecedented insights and opportunities in the area of medical research. There is enormous potential in the area of bone marrow and stem cell therapy, which is especially applied in the case of various forms of leukaemia,» declares the medical director of the Blood Centre Linz, Christian Gabriel. Werner Watzinger, Administrative Director of the Blood Centre Linz, added: «The purchasing costs of the Genome Sequencer FLX System were covered with the help of government aid money. We are very proud to be the first non-university institution in Austria which operates such a system.»

«454 Sequencing has quickly been adopted worldwide for a broad spectrum of applications because of its high quality result s and its improved efficiency over other technologies. The amplicon sequencing application is a sensitive method for detecting genetic variation, including SNPs (single nucleotide polymorphisms), insertions, and deletions, in target genomic regions. The accurate detection of genetic variation can be combined with phenotypic information which is especially relevant for medical research,» underlines Dr. Manfred Baier, Head of Roche Applied Science.

«The usage of the 454 Sequencing system is a milestone in DNA analysis. Institutions like the Blood Centre Linz can carry out their research projects far more efficiently and advance at a faster rate. We see a great opportunity to improve the scientific basics of transplantation medicine and we are looking forward to having the honour of supporting a team of experts in this field here in Austria,» adds Andrijka Kashan, General Manager of Roche Diagnostics Austria.

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Dr. Burkhard Ziebolz
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Von Standard Reparaturen bis hin zu GLP-Anforderungen, bietet das Socorex Service Center schnelle und zuverlässige Wartungsarbeiten und Kalibrationen an, basierend auf einer lang-jährigen Erfahrung und dem professionellen Know-how in der Herstellung von Flüssigkeits-dosierinstrumenten. Das bestehende Angebot wurde auf ein All-Marken-Service für Pipetten und Dispenser erweitert. Socorex hat für jedes benötigte Niveau an Wartungsarbeiten eine sachgerechte Lösung, sowie spezifische Antworten auf individuelle Kundenbedürfnisse. Alle Bearbeitungen werden in einer Zeitspanne von drei bis fünf Werktagen oder in nur 48-Stunden mit dem «Express Service» effizient durchgeführt.

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www.eupa2009.org

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VIII European Sympo-sium of the Protein Society
www.proteinsocietyzu-rich2009.org

Bioregenerative Engineering – Principles and Applications

By Shu Q. Liu, Member of the Biomedical Engineering Faculty at Northwestern University.
Wiley-Interscience, ISBN 978-0-473-70907-7

Regenerative medicine is a new field with a tremendous potential to enhance the quality of human life. This wonderfully arranged and illustrated book by Shu Q. Liu and published by Wiley Sciences gives the reader an in-depth look into the world of stem cells, and how they might be applied to generate for example vital organs from a primordial cell line. The generation of stem cells from a variety of cell types, not just embryonic stem cells, has kept up with new insights from molecular biology, which tries to decipher the genetic regulation of cell differentiation.

The clear structure of this well-written text book and the broad coverage of bioregenerative applications is primarily targeted to educate graduate and undergraduate students and professionals in the fields of bioengineering, molecular biology and cell biology as well as medical doctors and biomedical scientists.

A concise bibliography after each chapter facilitates the location of particular articles and abstracts for further reading. Particular emphasis has been placed on comprehensive diagrams and tables. Black and white as well as colour images illustrate the different cell types and histological features of e.g. neurodegenerative and regenerative processes.

In addition to the presentation of basic concepts in stem cell bioengineering, the functionalities of embryonic cells and the regeneration of adult cells and tissues are pragmatically discussed. Practical considerations for organ regeneration and the aspects of new biomaterials in the production of vital tissues are explained in addition to the detailed molecular principles of cell differentiation. The pathophysiological concepts are well presented, as they require focused study to solve the problems of bioregenerative processes of organ systems. Throughout the book, the clear writing style contributes to the readability of this challenging topic. It is clearly recommended as advanced reading for medical scientists and doctors in clinical settings as well as medical and engineering scientists in research environments.

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