Ovarian cancer stem cells: essential targets?
Graham Kelly, CEO, Novogen & Gil Mor, Department of Obstetrics Gynecology and Reproductive Sciences, Yale University School of Medicine

Hot melt extrusion processing: recent trends
Dennis Douroumis, Reader in Pharmaceutical Sciences, University of Greenwich and Director, Centre for Innovation in Process Engineering and Research

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- **Quality standards and enabling new developments**
  Guido Rasi, Executive Director, European Medicines Agency

- **The therapeutic potential of RNA directed epigenetic regulation of transcription**
  Kevin Morris, Department of Molecular and Experimental Medicine, The Scripps Research Institute

- **Drug resistance mechanisms in disease treatment strategies**
  Bhupinder Bhullar, Lab Head, Novartis

- **Drug targets in the Ubiquitin System**
  Kamyar Hadian, Head of Assay Development and Screening, Helmholtz Zentrum Muenchen, Institute of Molecular Toxicology and Pharmacology

- **Heat shock proteins: A high content assay opportunity to measure cellular stress response**
  Christophe Antczak, Senior Research Scientist, Balajee Somalinga, Senior Research Scientist, Hakim Djaballah, Director, HTS Core Facility at Memorial Sloan-Kettering Cancer Center

- **Validating molecular targets in preclinical drug development**
  Isabella Gashaw, Khusru Asadullah, Martin Bechem and Karl Ziegelbauer, Bayer Healthcare

- **NMR as a tool for chemokine inhibitor discovery**
  Brian Volkman, Medical College of Wisconsin

- **Oncology biomarkers in clinical practice – a new horizon**
  Nick La Thangue, Semira Sheikh and Heidi Olscha, Department of Oncology, University of Oxford

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The case of Tamiflu and transparency

The recent announcement that Tamiflu may not work in quite the way advertised back in 2005, when governments worldwide stockpiled the drug under the threat of a bird-flu epidemic, has left the media questioning clinical trial data availability. To the world at large, it seems to have come as a shock that methods and results of clinical trials are not available to the doctors who prescribe the drugs, researchers who use the released data and ultimately patients, who are advised to take these drugs under the expectation that doctors and the medical community can be trusted. How can we trust a community who deals with undisclosed data and legal confidentiality? Roche broke no laws by not providing the data, which is essentially what has upset the wider community. Roche, unfortunately, will become the poster-child for how clinical data is disseminated to the world. The industry is moving towards a more open, upfront way of operating, and in the past, secrecy was understood and accepted. But today, transparency is absolutely key, not to save money but so that patients can trust those who have trained and dedicated their lives to helping them.

The other issue that has emerged is the length of time it has taken for these results to be released and for the data to be analysed. The Cochrane Collaboration requested the results back in 2009 and have finally published their results as a research article in BMJ, who joined the team in seeking out the truth about the data. It ended a five year crusade after they received the data, all 160,000 pages of it, last year from Roche and GlaxoSmithKline, whose undisclosed data and legal confidentiality? Roche broke no laws by not providing the data, which is essentially what has upset the wider community. Roche, unfortunately, will become the poster-child for how clinical data is disseminated to the world. The industry is moving towards a more open, upfront way of operating, and in the past, secrecy was understood and accepted. But today, transparency is absolutely key, not to save money but so that patients can trust those who have trained and dedicated their lives to helping them.

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Coming up in the next issue of European Pharmaceutical Review:
# Future prospects: Using self-amplifying mRNA vaccines to facilitate a rapid response to pandemic influenza
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# Polymorph myths
Tony Thrush, Senior Research Associate, University of Southampton
# Risk profiling and proactive response to bio-contamination in GMP classified and controlled areas
James L Drinkwater, Chairman of PHSS – Pharmaceutical and Healthcare Sciences Society
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During the 1960s, manufacturers were required to demonstrate the safety of food and colour additives. The ‘Delaney Clause’ prohibited the use of any additives known to cause cancer in man or in animals. The FDA was required by law to apply a ‘zero-risk tolerance’ approach to food additives. By the early 1970s, it became clear that this approach was untenable and would lead to a large scale ban of animal feed products. There was also a much clearer understanding that exposure to small quantities of food additives does not materially increase the risk of contracting cancer, i.e. there is an acceptable risk / benefit ratio, which prompts the question: Is zero risk attainable or even desirable?

Over the last 50 years, the UK government’s general approach towards the responsibility of the regulatory agencies towards risk management has been to maintain risks ‘as low as reasonably practicable’ (ALARP). There is then a balance between the risks encountered versus the expended effort (time, cost and effort). If the expended effort is disproportionate to the perceived benefits accruing from reduction of risk then the necessary ‘duty of care’ has been fulfilled and additional measures are not required. Hence risk resolution should be viewed from a perspective of ‘safe enough’ or acceptable rather than from an absolute perspective of safe or not safe. This led to the Tolerability of Risk (ToR) approach, which subdivides risk into unacceptable, tolerable or broadly acceptable; where tolerable does not equate to acceptable, but instead reflects a ‘willingness by society as a whole to live with a risk so as to secure certain benefits in confidence that the risk is one that is worth taking and that it is being properly controlled’. That is, where an acceptable risk / benefit balance has been attained and the cumulative benefit to the patient outweighs the inherent risk.

Therefore, clinical studies for oncology products typically involve patients whose disease state is progressive, often fatal. Therefore, as the clinical outcomes are so poor, the ‘tolerable risk’ can be higher. For example, in oncology products, a case can be made for managing mutagenic impurities according to the guidelines for standard impurities (ICH Q3A/Q3B), rather than the much more restrictive guideline for mutagenic impurities (ICH M7).

Delaney was in agreement with this pragmatic approach, reflecting that, “Should the issue of genotoxic impurity limits in pharmaceuticals be resolved from the perspective of historical precedent (the zero risk tolerance mindset), or from a more carefully considered cancer-risk avoidance and risk management view point that weighs the new regulatory burdens created against the benefits conferred by pharmaceutical products to patients?”

Delaney contended that the ICH M7 guidance often ignores the reality that pharmaceutical syntheses frequently needs to use highly reactive intermediates (DNA reactive, i.e. mutagenic). He estimated that about a quarter of all reactive intermediates used in synthesis would likely be mutagenic. By focusing on the need for avoidance (i.e. risk averse), the guidance sets an inappropriate expectation for chemists to expend greater efforts to utilise synthetic options that are often considerably less efficient, employing ‘non-green’ chemistry, without materially improving patient safety. Therefore, the degree of risk that can be tolerated is predicated on prevailing circumstances, the proximity of that risk to the end user, especially if additional controls can be introduced after the process that is being assessed and prior to the product being provided to the patient.

In conclusion, it seems apparent that a consensus across Industry and regulators on risk assessment / mitigation strategies is required. Otherwise, different perceptions of risk will prevail and risk will continue to be in the ‘eyes of the beholder’.

**References**


APRIL 2014

Stem Cell Summit 2014
Date: 23 – 25 April 2014
Location: Boston, MA, USA
e: amber.kempf@gtcbio.com
w: www.gtcbio.com/conference/stem-cell-summit-overview

Drug Discovery Chemistry
Date: 23 – 25 April 2014
Location: San Diego, California, USA
e: chi@healthtech.com
w: www.drugdiscoverychemistry.com

PEP-CON 2014
Date: 25 – 28 April 2014
Location: Dalian, China
e: Sally@bit-pepcon.com
w: www.bitlifesciences.com/PepCon2014/default.asp

Strategic Protein Formulation & Drug Delivery
Date: 26 – 30 April 2014
Location: Barcelona, Spain
e: Daniel.evans@Insideintelligence.com
w: http://sop.insideintelligence.com

BTS Annual Congress 2014
Date: 28 – 29 April 2014
Location: London, UK
e: meetings@thebts.org
w: www.thebts.org

ISPE Europe Annual Conference
Date: 28 – 30 April 2014
Location: Frankfurt, Germany
e: ahyaan.raghuvarshi@mci-group.com
w: www.ispe.org/2014-europe-annual-conference

Genomics Research 2014
Date: 29 – 30 April 2014
Location: San Francisco, USA
e: enquiries@SELECTBIO.com

Biomarkers & Diagnostics World Congress 2014
Date: 30 April – 2 May 2014
Location: Philadelphia, USA
e: chi@healthtech.com
w: www.biomarkerworldcongress.com

MAY 2014

Drug Delivery & Formulation Americas Summit 2014
Date: 1 – 2 May 2014
Location: San Diego, USA
e: enquire@wtgevents.com
w: www.ddfsummit.com

CHI’s Biologics Formulation and Delivery Summit
Date: 5 – 7 May 2014
Location: Boston, USA
e: chi@healthtech.com
w: www.healthtech.com/biologics-delivery

Annual International Conference on Pharmaceutical Sciences
Date: 5 – 8 May 2014
Location: Athens, Greece
e: atiner@atiner.gr
w: www.atiner.gr/pharmako.htm

PEGS
Date: 5 – 9 May 2014
Location: Boston, USA
e: chi@healthtech.com
w: www.pegsummit.com

Europe Drug Discovery Summit
Date: 5 – 9 May 2014
Location: Berlin, Germany
e: info@gtcbio.com
w: www.gtcbio.com/conference/edds-overview

Protein Kinases in Drug Discovery
Date: 8 – 9 May 2014
Location: Berlin, Germany
e: info@gtcbio.com
w: www.gtcbio.com/conference/protein-kinases-drug-discovery-europe-overview

Drug Design & Medicinal Chemistry
Date: 8 – 9 May 2014
Location: Berlin, Germany
e: info@gtcbio.com
w: www.gtcbio.com/conference/medchem-overview

Interpack
Date: 8 – 14 May 2014
Location: Dusseldorf, Germany
e: interpack@messe-duesseldorf.de
w: www.interpack.com

European Lab Automation
Date: 13 – 15 May 2014
Location: Barcelona, Spain
e: enquiries@SELECTBIO.com

Combating counterfeit medicines: a major and growing challenge to patient safety. Going global
Date: 15 May 2014
Location: London, UK
e: events@jpag.org
w: www.jpag.org/?p=meetings&r=41

2014 AAPS National Biotechnology Conference
Date: 19 – 21 May 2014
Location: San Diego, USA
e: SmithT@aaps.org
w: www.aaps.org/nationalbiotech

World Pharma Congress
Date: 21 – 23 May 2014
Location: Boston, USA
e: chi@healthtech.com
w: www.worldpharmacongress.com

JUNE 2014

2nd Annual Discovery Chemistry & Drug Design Congress 2014
Date: 2 – 3 June 2014
Location: Geneva, Switzerland
e: info@oxfordglobal.co.uk
w: www.discoverychemistry-congress1.com

NGS 2014
Date: 2 – 4 June 2014
Location: Barcelona, Spain
w: www.iscb.org/ngs2014

Stem Cells and BioProcessing
Date: 3 June 2014
Location: London, UK
e: enquiries@euroscicon.com
w: www.regonline.co.uk/builder/site/Default.aspx?EventID=1248356

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Treatment with once-monthly Abilify Maintena® (aripiprazole) significantly reduces hospitalisation rates for patients with schizophrenia compared with daily oral antipsychotics

H. Lundbeck A/S and Otsuka Pharmaceutical Europe Ltd. have announced results from the final analysis of a mirror-image study showing statistically significant reductions in total psychiatric hospitalisation rates in patients diagnosed with schizophrenia who were switched from oral antipsychotics to Abilify Maintena once-monthly 400 mg – a prolonged-release suspension for intra-muscular injection of aripiprazole. These findings were presented as a late breaker poster at the 4th Biennial Schizophrenia International Research Society (SIRS) conference in Florence, Italy on 5 – 9 April. In this multi-centre, open-label, North-American mirror-image study, patients with schizophrenia who had been treated with oral antipsychotics as standard of care, were switched to Abilify Maintena once-monthly 400 mg and followed for six months in a naturalistic community setting. The results from the Abilify Maintena treatment period were compared retrospectively with the treatment period on oral antipsychotics prior to the switch in the same patients and setting. The study primary endpoint showed that Abilify Maintena once-monthly 400 mg significantly reduced the rates of psychiatric hospitalisation tenfold during the last three months versus prior oral antipsychotic. Abilify Maintena once-monthly 400 mg also significantly reduced the rates of psychiatric hospitalisations at six months compared with prior oral antipsychotics. The study found Abilify Maintena once-monthly 400 mg to be well tolerated, consistent with previously reported treatment emergent adverse events with oral Abilify. The most common treatment emergent adverse events with greater than five per cent incidence observed during the prospective treatment period with Abilify Maintena were insomnia and akathisia. “Our ability to reduce the risk of relapse and rehospitalisation is critical in facilitating improvement in psychosocial and vocational functioning. With each relapse patients can lose hard won gains and find it more and more difficult to progress towards recovery,” said study investigator John M. Kane, MD, Chairman of Psychiatry, The Zucker Hillside Hospital, and Vice President, Behavioral Health Services, North Shore-LIJ Health System.

EUROPEAN MEDICINES AGENCY

EMA and Australian regulator strengthen collaboration in the area of orphan medicines

In a meeting at the European Medicines Agency (EMA) on 4 April 2014, the EMA’s Executive Director, Professor Guido Rasi, and the National Manager of the Australian Therapeutic Goods Administration (TGA), Professor John Skerritt, announced that the two regulators have agreed to share the full assessment reports related to marketing authorisations of orphan medicines, which are intended to treat rare diseases. If the same marketing-authorisation application is received in parallel by EMA and TGA, the two regulators have the possibility of scientific exchange to facilitate the evaluation of the medicine. Both regulators will still reach their own conclusions about the suitability of each medicine to be authorised in their respective markets. The agreement will reinforce collaboration and work-sharing between the two regulatory authorities and will contribute to accelerating access to new medicines for patients with rare diseases in Europe and in Australia. Global collaboration on orphan medicines and rare diseases is particularly important in view of the small number of patients worldwide and the need for the limited number of studies performed to benefit patients regardless of where they live. Under the framework of greater international work-sharing, the EMA and TGA also collaborate and exchange information in a number of other areas, including through a mutual recognition agreement on good manufacturing practice (GMP) for medicines, their work to rationalise international GMP inspections of active substance manufacturers, and as part of multilateral relationships involving other regulatory partners.

CANCER RESEARCH UK

Cancer Research UK drug launched in its first clinical trial

A new cancer drug, based on further development of a discovery originally made by Cancer Research UK, has entered a clinical trial to target a wide range of cancers. The drug, RG7813, delivers a cytokine (engineered IL2) to a specific part of the carcinoembryonic antigen (CEA) protein which is exposed only on the surface of cancer cells, resulting in a narrowly-targeted treatment. Cancer Research Technology, the commercial arm of Cancer Research UK, licensed the monoclonal antibody called PRIA3 to Roche. PRIA3 was discovered in Sir Walter Bodmer’s laboratory when he was director of the Imperial Cancer Research Fund. Roche has subsequently engineered the antibody and incorporated it into its proprietary immunocytokine drug platform to generate the final drug candidate. Clinical trials have then been initiated. Sir Walter Bodmer, head of the Cancer and Immunogenetics Laboratory at the University of Oxford, said: “It’s very exciting to see that a drug based on our monoclonal PRIA3 antibody is now going into the clinic. By combining these discoveries we make in the lab with the latest developments in immunotherapy, we’re expanding our arsenal of cancer drugs at a greater pace – which will ultimately benefit more patients, sooner.” Dr Phil L’Huillier, Cancer Research Technology’s director of business management, said: “We’re delighted to see the trial launch of this promising new drug, which harnesses the power of the immune system, and that potentially could treat a range of cancers. This drug is particularly exciting because it homes in on a new target only accessible on the surface of cancer cells, increasing its potency while sparing healthy cells. We hope that the early clinical trials prove this is a safe and effective new treatment for cancer patients – ultimately saving more lives from the disease.”

SHIRE

Shire submits INTUNIV® (guanfacine extended release) Marketing Authorisation Application to EMA

Shire plc has announced the acceptance of submission of a Marketing Authorisation Application (MAA) by the European Medicines Agency (EMA) for their once-daily, non-stimulant guanfacine extended release for the treatment of Attention Deficit/Hyperactivity Disorder (ADHD) in children / adolescents aged 6 – 17 years. “The regulatory submission of guanfacine extended release brings Shire one step closer to providing an alternative to stimulant medication for the treatment of ADHD,” said Perry Stemberg, Senior Vice President of the Neuroscience Business Unit at Shire. “Every patient has different needs and we hope to be able to extend our range of treatment options which will help the healthcare professional to offer personalised management of ADHD.” Evidence for the submission is based on three pivotal studies investigating the short and long term safety and efficacy profile of guanfacine extended release.
Penn Pharma enters into manufacturing agreement with BioAlliance

Pharmaceutical manufacturer Penn Pharma has announced an agreement with BioAlliance Pharma to manufacture one of their leading development products in Penn’s new contained manufacturing facility which opened last year. Penn Pharma has been chosen by BioAlliance, an international specialist based in France, for the development and manufacture of Validive®. The product, which is developed for the prevention and treatment of oral mucositis induced by radiotherapy and/or chemotherapy in cancer patients, received Fast Track status this month from the US Food and Drug Administration (FDA). Penn Pharma, based in South Wales, will be involved in the development of the final dosage form, Phase III clinical supplies and future commercial supply needs. Judith Greciet, Chief Executive at BioAlliance Pharma, said: “We have chosen to continue the development of this leading product with Penn Pharma due to their expertise and capability in solid dose contained manufacturing. Validive® is a mucoadhesive tablet based on our Lauriad® technology that delivers the anti-inflammatory active clonidine, directly onto the site of inflammation in the oral cavity. The FDA’s decision to grant Fast Track approval to Validive® reflects how serious oral mucositis is, the vital need for an effective treatment, and the potential Validive® has to address an unmet clinical need.”

New Metrohm brochure and website: Pharmaceutical analysis

No other industry is as thoroughly regulated as the pharmaceutical industry. The active ingredients are legion and so are the standards to test for them. Metrohm’s new brochure Pharmaceutical analysis is a highly valuable compendium to get an overall view of the most important parameters determining the quality of pharmaceuticals and the methods best suited to check them. Pharmaceutical analysis draws on Metrohm’s comprehensive expertise in assuring the quality of pharmaceuticals. Using the US Pharmacopeia as a reference, the new brochure covers basic general parameters such as pH value, conductivity and water content as well as the determination of specific active ingredients, excipients and impurities. Methods described include near-infrared spectroscopy (NIRS), potentiometric, conductometry and Karl Fischer titration, ion chromatography, stability measurement, voltammetry, at line and online process analysis. Pharmaceutical analysis is an invaluable tool for anyone who is involved in the professional quality assurance of pharmaceutical products. Metrohm’s complete know-how on pharmaceutical analysis is also available at pharma.metrohm.com

Update on Phase III clinical trial of investigational MAGE-A3 antigen-specific cancer immunotherapeutic in non-small cell lung cancer

GlaxoSmithKline plc has announced its decision to stop the MAGRITI trial, a Phase III trial of its MAGE-A3ii cancer immunotherapeutic in non-small cell lung cancer (NSCLC) patients, after establishing that it will not be possible to identify a sub-population of gene-signature positive NSCLC patients that may benefit from the treatment. Data from the trial announced on 20 March 2014 showed that it did not meet its first or second co-primary endpoints as it did not significantly extend disease-free survival (DFSiv) when compared to placebo in either the overall MAGE-A3 positive population (first co-primary endpoint) or in those MAGE-A3-positive patients who did not receive chemotherapy (second co-primary endpoint). Glaxo continued with the MAGRIT trial to investigate the third co-primary endpoint of DFS in a gene signature positive sub-population, which was designed to identify a subset of MAGE-A3 positive patients that may benefit from the treatment. However, the pre-planned independent third-party analysis of a proportion of the data (to identify a gene signature classifier) has concluded that assessment of the third co-primary endpoint is not feasible due to an insufficient treatment effect. The trial will be stopped and Glaxo will now gain access to the un-blinded data, in order to conduct a full assessment of the findings and understand learnings for other aspects of immunotherapy development within GSK.

Where do you start when developing a new medicine?

A pioneering public-private research initiative between GSK, the European Bioinformatics Institute (EMBL-EBI) and the Wellcome Trust Sanger Institute is to harness the power of ‘big data’ and genome sequencing to improve the success rate for discovering new medicines. The ‘new Centre for Therapeutic Target Validation (CTTV) will aim to address a wide range of human diseases and will share its data openly in the interests of accelerating drug discovery. The CTTV aims to use the almost daily advances in cutting-edge genetic research to help researchers in that crucial first step in exploring new medicines – finding where to start. Target validation is about clearly defining the role that a biological process plays in disease before developing a new drug to tackle it. Currently, an estimated 90 per cent of compounds entering clinical trials fail to demonstrate the necessary efficacy and safety requirements, never reaching patients as medicines. This is often because the biological target for a drug is not well understood. Dr Ewan Birney, Associate Director and Senior Scientist at EMBL-EBI, has been appointed as Interim Head of the CTTV. Dr Birney, who brings with him significant knowledge about genomics and bioinformatics, will develop a work programme to steer the research activities of the centre’s scientists. “The Centre for Therapeutic Target Validation is a transformative collaboration to improve the process of discovering new medicines,” says Dr Birney. “The pre-competitive nature of the centre is critical: the collaboration of EMBL-EBI and the Sanger Institute with GSK allows us to make the most of commercial R&D practice, but the data and information will be available to everyone. It is truly exciting to apply so many different areas of expertise, from data integration to genomics, to the challenge of creating better medicines.”
DAIICHI SANKYO

Daiichi Sankyo and UC San Francisco announce collaboration in drug discovery research for neurodegenerative diseases

Daiichi Sankyo Co., Ltd. and University of California, San Francisco have announced that they have established a drug discovery collaboration focused on developing novel therapeutics and molecular diagnostics for multiple neurodegenerative diseases. Under the terms of the agreement, Daiichi Sankyo will provide its compound library to the UCSF Institute for Neurodegenerative Diseases (IND), and both parties will perform high-throughput compound screening and optimisation together. The project will bring together the drug development capabilities of Daiichi Sankyo with the expertise of world-renowned neuroscientists at UCSF, in a collaborative effort to create multiple drug discovery programs in debilitating disease areas such as Alzheimer’s, Parkinson’s, Creutzfeldt-Jakob disease and fronto-temporal dementias – all conditions for which there currently are no effective therapeutics available. Daiichi Sankyo will provide research funding and milestone payments and royalties for successful clinical progression and commercialisation of new products. Daiichi Sankyo will receive the option to enter into an exclusive license agreement to develop and commercialise promising compounds.

BOEHRINGER INGELHEIM

FDA approves Pradaxa® for treatment and reduction in risk of recurrent deep vein thrombosis (DVT) and pulmonary embolism (PE)

Boehringer Ingelheim has announced that the US Food and Drug Administration (FDA) has approved Pradaxa® (dabigatran etexilate) for the treatment of DVT and PE in patients who have been treated with a parenteral (injectable) anticoagulant for five to 10 days, and to reduce the risk of recurrent DVT and PE in patients who have been previously treated. “Venous thromboembolism is the third most common cardiovascular disease after myocardial infarction and stroke. About one-third of patients with a DVT or PE will suffer a recurrence within 10 years,” said Samuel Z. Goldhaber, MD, Director of Brigham and Women’s Hospital’s Thrombosis Research Group and Professor of Medicine, Harvard Medical School. “Dabigatran has an established efficacy and safety profile for stroke risk reduction in patients with non-valvular atrial fibrillation. This new FDA approval expands dabigatran’s indications to include treatment and the reduction of the risk of recurrence of DVT and PE.” The FDA approval is based on results from four robust Phase III clinical trials involving almost 10,000 patients that demonstrated the efficacy of Pradaxa® 150 mg twice daily in the treatment and prevention of recurrent DVT and PE. Trial data also showed a 92 per cent reduction in the risk of recurrent blood clots versus placebo. Results showed that DVT or PE patients taking Pradaxa® experienced significantly lower rates of bleeding, resulting in a favourable overall safety profile.

AMGEN

Amgen provides update on Phase III study of talimogene laherparepvec in patients with metastatic melanoma

Amgen has announced top-line results from the primary overall survival (OS) analysis of a Phase III trial in melanoma, which evaluated the efficacy and safety of talimogene laherparepvec for the treatment of unselected stage IIIB, IIC or IV melanoma compared to treatment with subcutaneous granulocyte-macrophage colony-stimulating factor (GM-CSF). Results showed that, while the primary end point of durable response rate was met (as previously reported), the secondary endpoint of OS was not met, although there was a strong trend in favour of talimogene laherparepvec. The estimated OS hazard ratio and improvement in median OS were similar to what was previously reported at the interim OS analysis. Talimogene laherparepvec is an investigational oncolytic immunotherapy designed to selectively replicate in tumours and to initiate an immune response to target cancer that has metastasised, or spread to other areas of the body. “We remain encouraged that the study met its primary endpoint of achieving durable responses in patients with metastatic melanoma,” said Sean E. Harper, MD, Executive Vice President of Research and Development at Amgen. “We missed statistical significance on the secondary endpoint of overall survival but the strong trend in survival benefit supports further research of talimogene laherparepvec to better understand its role in melanoma, both as a single-agent and in combination with other therapies.” The global, randomised, open-label Phase III trial enrolled patients with unselected stage IIIB, IIC or IV melanoma. Patients were randomised 2:1 to receive either talimogene laherparepvec every two weeks through direct tumour injection or GM-CSF subcutaneously for the first 14 days of each 28-day cycle, for up to 18 months. The most frequent adverse events observed in this trial were fatigue, chills and pyrexia. The most common serious adverse events include disease progression, cellulitis and pyrexia.

B&W TEK

B&W Tek announces agreement with LabWare, Inc for automation and reporting

B&W Tek, Inc., an advanced instrumentation company that delivers lab quality Raman spectroscopy solutions through user-friendly mobile platforms, is pleased to announce its newest strategic partnership with LabWare, Inc., the global leader of Laboratory Information Management Systems (LIMS) and instrument integration software products. B&W Tek and LabWare have joined together to distribute a pre-configured Raman spectroscopy template solution that allows for seamless and easy automation of data acquisition from B&W Tek’s NanoRam handheld Raman spectrometer into LabWare’s Enterprise Laboratory Platform allowing customers to reduce traditional cost and time associated with the implementation of Raman analytical technology. LabWare’s Enterprise Laboratory Platform combines the award-winning LabWare LIMS™ and LabWare ELN™, a comprehensive and fully integrated Electronic Laboratory Notebook application, which enables companies to optimise compliance, improve quality, increase productivity and reduce costs. The preconfigured template solution leverages LabWare’s LabStation instrument integration engine to securely parse and map data generated by the NanoRam into the corresponding LabWare sample record. The Raman spectroscopy template solution is fully 21 CFR compliant, providing the framework for immediate benefits and flexibility to be configured to suit individual needs. “It was our goal to expedite the way companies and individuals inspect their incoming raw materials and finished products,” says Jack Zhou, COO of B&W Tek, Inc. “To do so, we sought out LabWare Inc.’s expertise in laboratory automation and reporting to provide a process in which our NanoRam can help our customers reduce non-value added work and focus on increasing productivity. Together with LabWare’s automation framework and the NanoRam’s wireless capabilities, we have given our customers the opportunity to cut the cord and deliver real time results remotely in a secure, modern way.”
**ICON BIOSCIENCE**

**Icon Bioscience announces David S. Tierney, MD as President & Chief Executive Officer**

Icon Bioscience, Inc., a specialty biopharmaceutical company focused on utilising its Verisome™ drug delivery platform to develop unique intraocular eye-care therapeutics, has announced that David S. Tierney, MD has joined the Company as President & Chief Executive Officer. Icon Bioscience noted that Dr. Tierney is an accomplished healthcare executive with a proven record of achievements leading the growth of both pharmaceutical and medical device companies. Additionally, Dr. Tierney possess significant experience in successfully developing and commercialising drug delivery platforms, a particularly valuable asset with regard to Icon’s Verisome technology. “We are delighted to have David join our team,” said Vernon G. Wong, MD, Chairman and founder of Icon Bioscience and inventor of Verisome. “David possesses a wealth of expertise in medical science, clinical and regulatory affairs, and in overseeing the advancement of healthcare companies through the product development phase to commercial operations, key elements in support of Icon’s exciting growth outlook.” David Tierney commented, “Icon is on the threshold of an important new growth phase as is evident from the Company’s robust product pipeline with its lead product candidate in late-stage development.” He further noted, “I expect Icon’s Verisome technology to drive a transformational change in ophthalmic pharmaceuticals and am thrilled to have the opportunity to participate and contribute to that prospect.”

**SANOFI**

**Sanofi appoints Dr. Anne C. Beal to the newly created position of Chief Patient Officer**

Sanofi has announced the appointment of Anne C. Beal, MD, MPH, to the newly created position of Chief Patient Officer. “The appointment of a Chief Patient Officer at Sanofi, the first for a top 10 biopharmaceutical company, shows our commitment to go further in meeting the needs of patients,” said Pascale Witz, Executive Vice President, Global Divisions and Strategic Development at Sanofi. “Interactions with patients are a source of strength for the company and Dr. Beal’s appointment will help ensure the patient perspective advances our approach to meeting the unmet needs of patients.” Dr. Beal’s responsibility will be to further elevate the perspective of the patient within Sanofi so the company’s future healthcare offerings can better incorporate the unique priorities and needs of patients and caregivers in a variety of Sanofi activities, ranging from early stage R&D through to on-market availability of novel healthcare solutions. “I am incredibly honoured to join Sanofi as the first Chief Patient Officer,” said Dr. Anne C. Beal, M.D., MPH, Chief Patient Officer at Sanofi. “I will use my experience as a physician, researcher, philanthropic leader, and advocate for patient access to high quality care to infuse the patient perspective into Sanofi’s work that will advance our ability to deliver health care solutions that matter most to patients and those who care for them.”

**SMITHERS AVANZA**

**Smithers Avanza appoints Michael Dorato as Executive Vice President**

Smithers Avanza has announced that Dr. Michael Dorato has been appointed as Executive Vice President, Smithers Avanza Development Services, effective 7 April 2014. Dorato will report to Smithers Group President and CEO, Michael Hochschwender. As the leader of the Development Services group at the Smithers Avanza facility in Gaithersburg, Maryland, Dorato has responsibility for the business, operations, scientific services, and quality teams. Dorato has nearly 35 years of experience in the pharmaceutical and CRO industries, and holds a PhD and MS in Pharmacology from St. John’s University. Prior to joining Smithers, he served as Vice President and CSO of Global Discovery Services at Covance and Executive Director of Toxicology at Eli Lilly and Company. In these positions, Dorato directed teams in safety and efficacy lead optimisation, investigative and regulatory toxicology, discovery and late phase pathology, animal welfare, molecular and anatomical imaging, in vivo pharmacology, veterinary medicine and model development. “With his background and expertise in leading toxicology and pharmacology business units, it is clear that Dr. Dorato understands the technical and operational aspects of running a toxicology facility. I am confident that our clients will benefit from his scientific knowledge and that his business leadership experience will lead to growth and new opportunities to service our market,” said Hochschwender, President and CEO of The Smithers Group.

**MERCK**

**Merck announces appointment of Robert M. Davis as Chief Financial Officer; succeeds Peter N. Kellogg, who has served in the role since 2007**

Merck has announced the appointment of Robert M. Davis, 47, as Executive Vice President and Chief Financial Officer, effective 23 April 2014. Davis, who will also oversee corporate strategy and corporate business development, will succeed Peter N. Kellogg, 58. After having made significant contributions to the company as Merck’s CFO since 2007, Kellogg will work closely with Davis to ensure a seamless transition and will leave Merck on May 16. “Rob is an accomplished executive with significant financial and operational expertise, including as a CFO, and will be an exceptional addition to our team,” said Kenneth C. Frazier, Chairman and Chief Executive Officer, Merck. “Rob’s broad, global business and healthcare experience, which encompasses commercial, R&D, quality, regulatory, manufacturing and supply chain, will be an asset to us in implementing a significantly streamlined, more flexible cost structure and operating model, while enabling us to focus on our highest-potential growth opportunities.” Davis is Corporate Vice President and President of Baxter’s Medical Products business where he oversaw the successful integration of that company’s Global Medication Delivery and Renal businesses, along with corporate manufacturing and R&D functions, into a single, integrated division, improving R&D productivity and eliminating significant costs. Prior to his current role, Davis served as Corporate Vice President and President of Baxter’s Renal business, as Corporate Vice President and Chief Financial Officer from May of 2006 through May of 2010, and as Treasurer from 2004 through May of 2006. Davis joined Baxter as Treasurer in 2004 after more than 14 years at Eli Lilly and Company. “It is a tremendous opportunity to join Merck at such an exciting time,” said Davis. “Merck’s long-standing commitment to science, innovation and improving global health is unsurpassed among pharmaceutical companies.” Kellogg joined Merck in 2007 as Executive Vice President and Chief Financial Officer. He played a major role in driving the execution of Merck’s merger with Schering-Plough and in designing the company’s capital structure to enhance shareholder returns, including its 2013 accelerated share repurchase program.
ARIA PHARMACEUTICALS

ARIAD announces appointment of Hugh Cole, Senior Pharmaceutical-Industry Executive, as Chief Business Officer

Hugh M. Cole to the position of senior vice president and chief business officer. Mr. Cole will be responsible for global business and corporate development, licensing and strategic planning. He will report to ARIAD’s Chairman and Chief Executive Officer, Harvey J. Berger, MD, MD. Mr. Cole is a seasoned pharmaceutical executive with more than 25 years of industry experience and a deep background in rare and orphan pharmaceutical markets. Prior to joining ARIAD, he spent seven years at Shire Pharmaceuticals, most recently as senior vice president, strategic planning and program management and as a global franchise head, and before that, as vice president, business development. Previously, he held senior positions in business and corporate development at Oscent Pharmaceuticals (formerly Genome Therapeutics) and at Millennium Pharmaceuticals and its affiliates. Mr. Cole led numerous successful acquisitions both at Shire and Oscent. “Hugh is a thoughtful and experienced executive who brings great value to our leadership team,” stated Dr. Berger. “He will be integral in seeking business and corporate development opportunities for ARIAD, including a commercial partnership for Iclusig® in Japan, as well as helping to further maximise our oncology pipeline.”

INOVIO PHARMACEUTICALS

Inovio Pharmaceuticals continues building its senior team with the appointment of VP of Quality

Inovio Pharmaceuticals, Inc. have announced the appointment of E.J. Brandreth as Vice President of Quality. He will be responsible for all quality and compliance functions for a company that is moving forward with numerous late and early stage clinical trials and scaling up manufacturing and other operational areas to support its broad pipeline of immunotherapies and DNA vaccines. He will report to Inovio’s Chief Operating Officer Dr. Niranjan Sardea. Prior to joining Inovio, Mr. Brandreth had a successful career transitioning start-up clinical biotech operations into successful commercial entities; he supported the growth of several pharmaceutical companies in the quality and compliance areas. He was most recently Senior Vice President, Quality and Regulatory Affairs at Ajinomoto Althea, and previously held similar positions with IDEC, BioMarin and Favirile, Inc. He holds an MBA from the University of Phoenix and a BA in Biology from UC San Diego.

KITE PHARMA

Kite Pharma announces the appointment of Arie Belldegrun, M.D., as President and Chief Executive Officer

Kite Pharma, Inc., a clinical-stage biopharmaceutical company focused on developing engineered autologous T cell therapy (eACT™) products for cancer, have announced the appointment of Arie Belldegrun, MD, FACS, as President and Chief Executive Officer. Dr. Belldegrun, who is also a founder of Kite, will continue in his current role as Chairman of the Company. “Dr. Belldegrun has had a distinguished 18 year tenure in the life sciences industry, having been closely involved with the founding and advancement of successful private and public biopharmaceutical companies,” commented David Bondeman, speaking on behalf of Kite's Board of Directors. “We are very pleased that he will take on his new role at Kite as the Company continues its momentum and further advances its broadly enabling platform in cancer immunotherapy.” Prior to founding Kite, Dr. Belldegrun was the founding Vice Chairman of the board of directors and Chairman of the scientific advisory board of Cougar Biotechnology, acquired by Johnson & Johnson in 2009 in a USD 970 million transaction. Previously, Dr. Belldegrun founded Agenys and served as Chairman of the board of directors. He also currently serves as Chairman of Arno Therapeutics, Inc., and as a board member of Teva Pharmaceutical Industries Ltd., SonaCare Medical, and TheraCoat. In addition, Dr. Belldegrun is Professor of Urology, holds the Roy and Carol Doumani Chair in Urologic Oncology, and is Director of the UCLA Institute of Urologic Oncology at the David Geffen School of Medicine at UCLA. Dr. Belldegrun completed his M.D. training at the Hebrew University Hadassah Medical School in Jerusalem, his post-doctoral in immunology research at the Weizmann Institute of Science (Israel) and his residency in urologic surgery at the Brigham and Women's Hospital, Harvard Medical School. He also completed a research fellowship in surgical oncology and immunotherapy under Steven A. Rosenberg, MD, PhD, at the National Cancer Institute/NIH.
Traditionally, antimicrobial drugs have been delivered by oral or parenteral administration in the form of tablets / capsules and injections, respectively. Occasionally, topical antimicrobial formulations such as creams, ointments and gels have been employed to augment systemic administration. There is a large body of literature regarding systemic antimicrobial drug delivery and these will probably remain the mainstay of antimicrobial drug delivery for a long time to come. However, these are plagued with several challenges. Firstly, drugs administered via the oral and parenteral routes mainly involve a general ‘overkill’ approach, where body tissues are exposed to significantly high concentrations of very potent drugs, rather than that required at the site of action. This makes the delivery non-specific and therefore presents a high risk of unwanted side effects and even potential toxicity. Further, drug administration via the oral route requires higher than the dose required due to first pass metabolism in the liver and destruction in the stomach for acid labile drugs. Antimicrobial drugs, especially broad spectrum antibiotics, administered orally also tend to destroy communal (‘friendly’) bacteria naturally present in the stomach and therefore create a favourable environment for more harmful but generally non virulent microorganisms to cause problems, which sometimes result in fatalities. In addition, for conditions such as infected diabetic foot or venous ulcers, systemic administration is largely ineffective due to reduced blood circulation at the extremities, especially in diabetic patients. Finally, it is largely unnecessary to administer antimicrobial drugs for local infections via oral tablets or parenteral injections when smaller local doses may be better.

As a result, novel approaches to antimicrobial drug delivery have been explored to address the above limitations of traditional antimicrobial delivery. Some of these novel drug delivery systems also include modifications of traditional (oral and parenteral) antimicrobial delivery.
drug delivery including controlled delivery systems. These allow prolonged maintenance of therapeutic doses in the bloodstream, therefore avoiding the need for repeated doses, which in the case of painful injections results in patient non-compliance. A possible solution to overcoming microbial resistance is improving drug action at the target site (microorganisms) by employing innovative drug delivery technologies, in other words identifying novel drug delivery systems that can ensure therapeutically effective drug concentrations that ensure high efficacy and reduced toxicity. In the remainder of this review, we will focus on some of the novel delivery systems for delivering antimicrobial drugs with a major focus on topical antimicrobial delivery.

**Micro and nano delivery systems**

Micro and nano-structured biomaterials such as microparticles, nanoparticles and liposomes have unique physicochemical properties such as ultra small and controllable size, large surface area to mass ratio, high reactivity and functionalisable structure. These properties can be applied to facilitate the administration of antimicrobial drugs, thereby overcoming some of the limitations in traditional antimicrobial therapeutics such as oral tablets and injections. In recent years, encapsulation of antimicrobial drugs in nanoparticulate systems has emerged as an innovative and promising alternative that enhances therapeutic effectiveness and minimises undesirable side effects of the drugs. Liposomes are spherical lipid vesicles with a bilayered membrane structure consisting of amphiphilic lipid molecules. Liposomes were initially introduced into the cosmetic market by Dior in 1986. In 1995, Doxil (doxorubicin liposomes) became the first liposomal delivery system approved by the Food and Drug Administration (FDA) to treat acquired immune deficiency syndrome related Kaposi’s sarcoma. At the micro level, developed alginate based microparticles as oral sustained drug delivery carriers for the treatment of tuberculosis in order to improve patient compliance. These microparticles exhibited sustained release of the drugs isoniazid, rifampicin and pyrazinamide for three to five days in plasma and up to nine days within the target organs which was significantly higher than the control by direct administration of the free drugs.

**Topical antimicrobials are compounds which are used to either kill or control the growth of microorganisms**

**Wound antimicrobial delivery**

Liquid antimicrobial formulations such as saline solutions have poor retention at the wound site and are normally only useful as cleansing agents prior to covering with a normal dressing. Topical creams, ointments and gels provide adequate and relatively sustained and apparently non-toxic levels of antisepsics and demonstrated benefit over saline irrigation. However, their use is associated with messiness and also eventually loses their viscosity due to absorption of local fluid and has therefore fallen out of use.

Commonly used topical antiseptic agents for wound infection include iodine-releasing agents (e.g. povidone iodine), chlorine-releasing solutions (e.g. Dakin’s and sodium hypochlorite solutions), hydrogen peroxide, chlorhexidine, silver-releasing agents and acetic acid. In modern wound care practice, iodine, silver and broad spectrum germicidal agents such as neomycin, bacitracin, polymyxin, streptomycin, gentamycin and/or combinations are used to control and treat bacterial infection in chronic wounds.

As a result, new medicated dressings loaded with antimicrobial agents have been reported in the literature and also available commercially. Local delivery of these antibiotics in the form of dressings is more convenient over systemic administration since they deliver a higher concentration of the drug directly to the desired area and are less frequently implicated in causing bacterial resistance. Antimicrobials are incorporated into these dressings to either treat or prevent microbial infections which are one of the major causes of delayed healing in chronic wounds such as are common diabetic foot ulcers surgical and accident wounds where the incidence of infections can be high due to reduced resistance resulting from extreme trauma. For example, povidone-iodine has been used with fabric based dressings. Moist wound dressings loaded with silver for control of wound infection include hydrocolloid, polyurethane, foam, film and silicone dressings.

Treatment of dermal depth burn wounds using antimicrobial releasing silicone gel sheets which promotes epithelisation of superficial burns has been described by Sawada et al who also developed a chitosan-polyurethane film dressing incorporating minocycline for treating severe burn wounds. More novel formulations have been reported for use in the delivery of drugs such as tetracycline. Sudheesh et al also reported on novel 6-chitin/nanosilver composite scaffolds for wound healing applications using 6-chitin hydrogel loaded with silver nanoparticles. The composite scaffolds showed antibacterial activity against Escherichia coli and Staphylococcus aureus and were also haemostatic. Bishweshwar and co-workers proposed the use of electrospray nylon-6 nanofibres loaded with silver nanoparticles as wound dressings for infected wounds. Boateng and co-workers and Pawar et al have recently shown preliminary evidence of potential synergistic antimicrobial activity of streptomycin in combination with diclofenac (in vitro) loaded into freeze-dried polymeric wafer dressings. The drug loaded dressing was active against three main infection causative agents, Escherichia coli and Staphylococcus aureus and Pseudomonas aeruginosa. Broad spectrum antimicrobial agents (e.g. streptomycin, neomycin) have been used to prevent as well as treat infections common in chronic diabetic and venous ulcers and burn injuries. Labovitiadi et al prepared freeze-dried wafers
from a single polymer and loaded with four broad spectrum anti-
microbial compounds individually against methicillin-resistant
Staphylococcus aureus.

As noted in the introduction, it is our view that the delivery of
antibiotics to local wound sites is better than systemic administration
for several reasons. Antibiotic doses needed to achieve sufficient
systemic efficiency often result in toxic reactions such as cumulative cell and organ
toxicity of the aminoglycosides in the ears and kidneys. The use of lower antibiotic
doses within the dressings also reduces the risk of systemic toxicity
considerably. In addition, local delivery from dressings can overcome
the problem of ineffective systemic antibiotic therapy, resulting from
poor blood circulation at the extremities in diabetic foot ulcers.

**Vaginal antimicrobial delivery**
The vagina has been studied as a favourable site for the local and
systemic delivery of drugs, specifically for female-related infections.
Traditionally, the vaginal cavity has been used for the delivery of locally
acting drugs such as antibacterial, antifungal, antiprotozoal, antiviral,
labour-inducing, spermicidal agents, prostaglandins and steroids. In
the past decade, advances have been reported in the delivery of
microbicides, which are formulations loaded with active pharmaco-
cutical ingredients that can prevent the transmission of sexually
transmitted diseases such as gonorrhoea, syphilis and acquired
immune deficiency syndrome. To date, there are approximately
60 antimicrobials in different stages of development. Antimicrobial
drug delivery via the vaginal route can provide better protection than
standard prevention tools such as condoms, and provide greater
control over the risk of exposure to sexually transmitted diseases.

**Light mediated antimicrobial delivery**
Light-mediated killing or photodynamic antimicrobial chemotherapy
against many pathogens has been extensively investigated in vitro
studies. A wide range of organisms from the Gram-positive
Staphylococcus aureus and the Gram-negative Pseudomonas
aeruginosa have been proven as susceptible to photodynamic
antimicrobial chemotherapy with a number of different photo-
sensitizers in vitro. Multidrug-resistant strains are just as susceptible as their naïve counterparts. Both enveloped and non-enveloped viruses have demonstrated susceptibility, in addition to fungi and protozoa. Topical application of a photosensitizer followed by irradiation is a viable application of photodynamic antimicrobial chemotherapy in treatment of infection, due to the accessibility of the target site. Several studies have investigated the use of photodynamic antimicrobial chemotherapy in the treatment of acne, warts, infected wounds and the onychomycosis-infected nails. In these studies, the method of drug delivery depended on a number of different factors, but particularly the photosensitizer used. Genina et al carried out a clinical study involving 140 patients suffering from either acute or chronic maxillary sinusitis, comparing methylene blue-mediated photodynamic antimicrobial chemotherapy of the infected sinus with traditional treatment as the control group. If the delivery of photosensitiser were optimised, photodynamic antimicrobial chemotherapy could prove a viable treatment regimen for oral candidiasis. A study by Teichert et al investigated the potential of methylene blue-mediated photodynamic antimicrobial chemotherapy in the treatment of oral candidiasis in an immunosuppressed murine model, mimicking the conditions in an immunodefective human. Donnelly et al investigated the potential use of photodynamic antimicrobial chemo-therapy in treatment of Pseudomonas aeruginosa induced cystic fibrosis lung infection.

In summary, antimicrobial delivery will continue to evolve with the emergence of innovative and more efficient ways of achieving therapeutic efficacy with the minimum amount of toxicity or side effects.

References

5. Lipsky BA, Huey C. (2009) Topical Antimicrobial Therapy for Treating Chronic Wounds, Clinical Infectious Diseases, 49(10), 1541-1549
Synopsis:
This webinar will give an overview of the recently released UK Pharmaceutical and Healthcare Science Society (PHSS) Technical Monograph #20 – Bio-contamination, characterisation, control, monitoring and deviation management in controlled / GMP classified areas. The concept of Risk Profiling and Proactive Response (RPPR) to bio-contamination in GMP classified areas, focusing on preventing contamination and not just monitoring for compliance will be presented. How trending environmental monitoring data holistically across all supporting areas can detect an increasing risk of a contamination event in Grade A zones will be examined. In particular, the value of using real-time airborne viable particle detection as part of holistic monitoring strategy for risk escalation will be discussed.

Many organisations are pro-actively exploring Rapid Microbiological Methods (RMMs). Real-time airborne viable particle counting is an RMM that is generating great interest. The potential opportunities of improved product quality and efficiencies, whilst maintaining patient safety are too significant to ignore. Cases where this technology is providing value today in a pharmaceutical manufacturing environment will be presented. Included will be a brief discussion of critical performance characteristics of real-time viable particle detectors which should be considered when selecting an instrument to monitor GMP classified areas.

Speakers:
James Drinkwater
Chairman, PHSS

Tim Russell
Product Specialist – Contamination Control, TSI

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With the largest network of harmonized bio/pharmaceutical GMP product testing labs worldwide, Eurofins BioPharma Product Testing is everywhere you are.

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Eurofins’ US and European BioPharma Product Testing labs have operated individually and successfully for decades, but there are quality and data access advantages of these sister labs now partnering to serve clients, as Timothy S. Oostdyk, President, Eurofins Lancaster Laboratories and Senior Vice President, Eurofins BioPharma Product Testing Group, explains: “While a global presence is very valuable, what is essential to international clients is the ability for all laboratories to operate under the same policies, procedures and guidelines. This kind of consistency requires a significant investment of resources and technology, which we at Eurofins are making in order to achieve full harmonisation.”

Today, nearly 2,000 employees of Eurofins BioPharma Product Testing operate under the same Global Quality Policy Manual and utilise the same CAPA/Exceptions Management System and Document Management System. “Starting in 2014, clients working with any of our laboratories will also be able to review project data and reports 24 hours a day, seven days a week with our global online portal, LabAccess.com. Whether a client is working with our laboratory in Lancaster, Munich or Paris – or all three for various projects – project information such as reports, test results, status of samples and project information for any project within the Eurofins BioPharma Product Testing network will be available through this single, online portal,” says Oostdyk.

By working with one harmonised international biopharm GMP product testing provider, Eurofins’ clients will see various enhanced capabilities perquisites. “It is very important that our laboratories operate with a consistent level of scientific expertise, and offer a consistent, market leading product to our customers. Therefore we routinely and deliberately drive collaboration on services, regulations and new technology across our network. Most recently, our scientists in Pennsylvania have been actively working with our team in Munich to launch our European Viral Safety and Clearance services, an exciting addition to our rapidly growing service offerings in the EU,” reveals Oostdyk.

“Active collaboration is also ongoing between the US and Copenhagen to set up harmonised service offerings in trace metals testing to address the emerging requirements in this area, including newly designed labs and protocols. We have also harmonised our rapid sterility methods in the US and Ireland and collaborate regularly to support products being released in both the US and EU. Further, our scientists at Eurofins IDmyk in France are bringing enhanced microbial identification capabilities to the network.”

These types of active collaborations drive consistency and excellence in science and services. Importantly Eurofins BioPharma Product Testing has also harmonised its service models to offer all global clients the same flexible service models with the ability to tailor programs to maximise cost effectiveness and meet project needs. “This includes our traditional fee-for-service model, our managed hours program, full time equivalent program, and our award-winning Professional Scientific Staffing™ program, which has grown to include more than 35 locations in seven countries,” explains Oostdyk.

This harmonisation and collaboration among the global Eurofins BioPharma Product Testing sites will enhance the clients’ overall service experience. Oostdyk elaborates: “With the most comprehensive range of large and small molecule testing services available worldwide, coupled with 14 state-of-the-art facilities in nine countries, we have the proximity backed by our global breadth of harmonised capabilities to deliver a personalised local lab experience to our customers.

“We support all functional areas of bio/pharmaceutical manufacturing, including method development, microbiology, process validation and quality control. And we provide testing for nearly all stages of the drug development process, ranging from pre-clinical through post-product approval, including: testing of all starting materials, process and product related impurities, method development and validation, stability and release testing, process/facility validation, virus clearance and safety, and testing of packaging components. We make it our business to effectively balance delivering the most complete range of harmonised testing services on a global platform, while meticulously treating each client as if they were our only one. When our clients are delighted, we’ve achieved our goal.”

As the largest global network of harmonised BioPharma GMP product testing labs, Eurofins BioPharma Product Testing’s fundamental philosophy is to help clients efficiently allocate their research and manufacturing expenditures by strategically engaging them to meet their unique outsourcing needs. Eurofins’ clients develop and release products in many different markets and require the same consistent level of expertise, quality, best practices, and service options to be delivered across its network of 14 harmonised GMP labs, located in nine countries.

For more information, please visit: www.eurofins.com/biopharm
Also on offer are five co-located conference tracks:

- **Advances in Automation & Robotics** will cover robotics in biobanking, drug discovery and development, and highlight solutions for efficient compound library management.
- **High Content Analysis** will provide an insight into novel 3D cell based screening methods, the use of model organisms, and live cell imaging approaches. Focus will also be given to the evolving use of microfluidics in this field.
- **Advances in Cellular Assays & Cell Culture** will focus on bio-processing technologies; highlighting technology trends, end-user application segments, and opportunities in the application of new approaches for addressing bottlenecks in bio-processing.
- **Advances in NGS & Big Data** will present the latest research covering the growing use of NGS in the clinic and in diagnostics, as well as developments in sequencing platforms and methods. Focus will also be given to novel solutions to the NGS data deluge, addressing issues such as storage and analysis.
- **Advances in qPCR & dPCR** will showcase new developments in qPCR technology, concentrating on improvements to qPCR design, the acquisition of accurate data, and efficient data analysis. The use of dPCR in the clinic will also be discussed, covering applications such as infectious disease diagnosis and cancer detection.

**Training courses**

Appreciate the experimental advantages of growing cells in 3D culture and understand the practical limitations of performing cell based assays in an automated environment in Professor Anthony Davies’ short course; New Techniques and Strategies for Implementing 3D Cell Based Assays in an Automated Drug Discovery Environment, on 13 May.

Learn how appropriate statistics are selected and applied correctly to get the most out of your qPCR data in the two-day course; Statistical Analysis of Real-time PCR Data/ Gene Expression Profiling with Real-time PCR, led by Professor Mikael Kubista on 12 – 13 May.

**Opening Reception**

The Catalan Experience Opening Reception will take place from 17:00 – 19:00 on 13 May, enabling visitors to engage with this year’s sponsors and exhibitors without the pressures of getting back to the conference sessions. The evening will be an enjoyable way to experience the culinary and cultural highlights of Barcelona and the Catalonia region.

The evening will also incorporate the European Life Science Awards ceremony, celebrating the very best of the industry’s achievements. Visit www.LifeScienceAwards.com for more information.

For registration enquiries, please email r.davidson@selectbio.com or call +44 (0)1787 315115. For sponsorship and exhibition inquiries, please email exhibitors@selectbio.com or call +44 (0)1787 315110.
Hot melt extrusion processing for the development of sustained release products

Hot Melt Extrusion (HME) has attracted increased interest for the development of pharmaceutical dosage forms over the last decade. It is a versatile processing technology which produces extrudates in the form of solid dispersions and solid solutions. Among the various applications, HME has been adopted for the development of sustained release dosage forms by using a wide range of pharmaceutical excipients such as polymers or lipids. In this review, we highlight the development and recent trends of sustained release dosages manufactured by extrusion processing.

HME processing is widely used in the food and plastic industry, but it has found ground for pharmaceutical applications such as increasing solubility / dissolution of water insoluble drugs, taste masking of bitter substances, sustained / controlled release formulations, transdermal / transmucosal films and implants. It is a “process of pumping raw materials with a rotating screw under elevated temperature through a die into a product of uniform shape.” By using the appropriate downstream process equipment, it can effectively produce granules, pellets, spheres or even tablets and capsules (calendering). However, the major advantage of HME over conventional formulation techniques (e.g. granulation, spray-drying) is the capability to operate as a continuous manufacturing technology with fewer production steps, absence of organic solvent / water, decreased environmental implications, high throughput and reduces cost in waste, manpower and footprint. Lastly, HME can be coupled with process analytical tools (PAT) such as NIR / Raman spectroscopic probes for the design, analysis and control of the manufacturing process by measuring critical quality and performance attributes. Thus, a better understanding of the extrusion processing and quality of the finished products can be achieved in compliance with pharmaceutical regulatory authorities.
The development of sustained and controlled release (included under the term prolonged release) aims to control the release rate of the active substance leading to controlled drug adsorption in the gastrointestinal tract. This type of oral dosage offers several benefits including control of the therapeutic dose for absorption at the desired delivery rate and hence stable plasma levels of the drug with a therapeutic effect over longer periods. The reduction of the side effects allows minimisation of the dosing frequency, which in turn improves the therapy, cost-effectiveness and patient compliance.

**Sustained release polymeric matrices prepared via HME**

The investigation of polyethylene oxide (PEO) as a sustained release polymeric carrier for chlorpheniramine maleate (CPM) was firstly reported by McGinity et al by extruding rod-shaped tablets. It was found that the PEO molecular weight, drug loading percentage and the inclusion of the PEO influenced the drug dissolution rates. The PEO erosion and the drug diffusion through the created swollen gel on the tablet surface controlled the release patterns of the matrix tablets while CPM was molecularly dispersed within the PEO matrix.

Another interesting approach for the manufacturing of ibuprofen sustained release mini-matrices (multiple unit dosage forms) was introduced by Verhoeven et al who combined xanthan gum (XG), a hydrophilic polymer and ethylcellulose (EC). By altering the XG/EC ratio the drug release rate was modified and increased with increasing XG concentrations. The particle size of XG was another critical attribute as the drug release was fast with coarser XG sizes but slow and reproducible with fine particles. Clinical trials with dogs showed the high Cmax and Tmax for 30 per cent XG compared to those for 20 per cent XG. These values of the extruded mini-matrices were similar to the Ibu-Slow® formulation, suggesting that they behaved as single unit dosage form due to the immediate swelling upon contact with the GI-tract fluids.

Similar polymer combinations applied for metoprolol tartrate to produce zero order release mini-matrices. On this occasion, the authors investigated the effect of XG concentration (1 – 20 per cent w/w), the ratio of the plasticiser (DBS, dibutyl sebacate) and the extrusion processing parameters. The optimisation of these particular formulations allowed the extrusion at low temperatures (60 – 80°C) with smooth extrudates independently of the XG concentrations (Figure 1). Another interesting outcome was that the extrudate quality was not affected by the feed and screw rates but it was also possible to apply lower barrel temperatures when these rates were increased. Some drawbacks of the developed polymeric formulations were the stability at accelerated conditions due to the hydrophilic nature of XG and the low flexibility of XG to further optimise the drug release.

An advanced approach has been used to develop a multilayer (core/coat) dosage form via co-extrusion, where the core provided sustained drug release and the coat immediate drug release. Here, two extruders connected through a co-extrusion die and the two melts were combined to form two concentric layers, a core and a coat. A wide range of thermoplastic polymers was screened for the purposes of the study and eventually a combination of polycaprolactone (core) and polyethylene oxide (coat) to prepare solid dispersions of Metoprolol tartrate (MPT) and hydrochlorothiazide (HCT) respectively. The HCT polymeric coat was designed to dissolve rapidly while the caprolactone core remained intact and MPT release was diffusion controlled. A clinical trial was conducted in dogs and showed no significant differences in the bioavailability (Cmax, Tmax and AUC) between the extruded matrices (filled in hard gelatine capsules) and the Zok-Zid® commercial product.

**Figure 1:** Mini-matrices containing 30 per cent (w/w) MPT, XG, EC and DBS (EC/DBS 2:1, w/w)®

**Figure 2:** X-ray tomography renderings of EVA40 matrices with 50 per cent of MPT. Axial (A and C) and radial (B) cross-sections before dissolution, axial cross-section after 24 hours dissolution, axial (F) and radial (E) cross-sections after 72 hours dissolution. Black spots (A and D) indicate pores. The colour scale used in (B, C, E and F) represents the pore size (maximum opening), blue representing small pores, red representing larger pores.

Sustained release formulations are possible to extrude by using different ethylene-vinyl-acetate grades (EVA®). Polyethylene is a semicrystalline polymer with alternating crystalline lamellae (with different types of crystals) and amorphous domains while the incorporation of VA co-monomer units (9 – 40 per cent) into a polyethylene backbone chain induces differences in physicochemical properties. The main advantage of EVA is that it can be processed in the absence of a plasticiser while the drug release can be tailored by polymer grades, drug loading. A unique feature of EVA is the porous network created once the extrudates are exposed to the dissolution
medium with the drug being released by means of percolation. The crystalline drug is released leaving an empty porous carcass (Figure 2, page 24). Further investigations demonstrated excellent stability of EVA and no effect on the Gi ecosystem following oral administration.

![Photographs of the final shaped products](image)

**Figure 3:** (a–g) Photographs of the final shaped products; from a–g: corn starch, maize starch, pea starch, potato starch, tapioca starch, amylose-free starch and wheat starch

More recently, a continuous processing approach was reported for the extrusion of paracetamol / starch formulations. The novelty of the process lies on the cutting equipment (rotary fly-cutter) adopted from the plastic industry and can operate in both a continuous and start-stop mode. In order to optimise the process, particular attention was paid to screw configuration, temperature profiles and adjustment of cutting / extrusion speeds. The process was optimised for various starch grades and as shown in Figure 3, the extrudates showed regular shape with glossy surface.

**Lipid extrusion for sustained release formulations**

Solid lipids are natural and biodegradable excipients used for pharmaceutical applications due to low cost and non-toxic properties. Lipids can be used for sustained release of water soluble drugs, solubility / dissolution enhancement of water insoluble drugs, taste masking, floating formulations and decrease of gastric irritation. Extrusion processing was initially introduced by Breitkreutz et al for taste masking applications and later was introduced for sustained release formulations.

An interesting feature of lipids is that they can be extruded at temperatures below their melting point through a process known as ‘cold extrusion’. Cold extrusion was adopted by Keleb et al who used low temperatures for the extrusion of polymeric formulations at ambient temperature. Hence, cold extrusions can prove advantageous for the processing of thermal labile drugs. The lipid composition plays a key role on the physicochemical properties of the extrudates. It has been proved that the softening of certain lipids instead of complete melting can avoid the formation of low-melting, metastable polymorphs. Lipids can undergo phase transformations (change of the colour indicates recrystallisation) and produce porous structures if extruded above their melting points. The extrusion of lipids below the melting point usually results in slower release rates compared to those of extruded lipids above the melting range. However, this is not always the case due to the occurrence of the ‘blooming effect’ where sharp fractal structures are observed with lower extrusion temperatures leading to faster drug release rates. Thus it is critical to identify the optimal processing parameters. Furthermore, the stability of lipid-extruded formulations under accelerated conditions is very important for successful formulation development.

Several studies demonstrated that stability depends on the lipid composition, the applied heat energy, shear / friction forces. Heterogeneous lipid compositions show long crystallisation times while homogeneous compositions crystallise faster. In addition, the length of the fatty acid chain length of the lipids has a significant impact and longer chains present slower drug dissolution rates. The contact angle of the extruded surfaces increases with increasing chain length of the lipid leading to decreased wetting ability and hence decreased dissolution rates.

The die diameter has also proven to be a key factor for the development of extruded formulations. It influences the final drug loading, the release rates, the shape / morphology of the extrudates, taste masking and causes electrostatic charging. Small die diameters can increase drug loading up to 80 – 90 per cent and the drug release rates. However, in another study it was shown that the release rates depend on the lipophilicity / hydrophobicity of the drug substance. The use of small dies can cause electrostatic charging and thus it is important to add an antistatic agent (e.g. PEG) to eliminate these problems. Interestingly, small dies facilitate effective taste masking as the suspended drug particles evade the resistance of the die plate walls and form a thin layer of soften lipid on the surface of the extrudates. The larger die diameters result in irregular shapes and increase intersections of the milled extrudates.

The drug release rates from extruded lipid matrices can be tailored by incorporating various excipients such as PEG, PVP and inorganic excipients. The addition of hydrophilic matrices increases the release rates as a function of the molecular weight whereby they dissolve quickly and form an interconnected pore network, which enhances the drug release. Similarly, the addition of inorganic excipients can facilitate drug release but to a smaller extent as its solubilising capacity is hindered. The addition of hydrophilic materials can cause stability issues and hence the appropriate selection of lipids is important. The presence of the aforementioned excipients in lipid extruded matrices showed excellent stability under accelerated conditions. Recently, Robleg et al introduced vegetable calcium stearate (CaSt) as a thermoplastic excipient for the development of sustained release paracetamol pellets. CaSt is a mixture of insoluble calcium salts of various fatty acids, mainly consisting of stearic and palmitic acids and minor proportions of other fatty acids. The drug release was diffusion controlled and left a porous network on the pellet surface.
two-step extrusion process in a continuous manner as it was shown by Witzleb et al. (Figure 4, page 25) and compared with extrudates produced batchwise. The comparison of the two approaches showed larger, coarser and smaller fine fraction with a small extent of agglomeration for the continuous mode compared to the batchwise extrudates. However, the dissolution patterns of both extruded samples showed almost no difference (f2 > 70). Furthermore, some recent trends in lipid extrusion introduced a) a two-step extrusion process and b) extrusion of pre-mixed formulations. In the first occasion, the drug is initially extruded with a hydrophilic polymer (e.g. PEG) followed by milling and subsequently in the second extrusion step the milled extrudates are processed with the lipid matrix. This process can successfully lead to tailoring of drug release profiles by adjusting the percentage of the hydrophilic excipient. In the second process, Vithani et al. extruded pre-mixed batches (complete tablet formulations) with various drug loadings to produce sustained release formulations of sodium diclofenac. The high drug loaded extruded tablets compared with compressed tablets of the same formulations (only the drug/lipid were extruded) and showed sustained drug release rates for more than 12 hours (Figure 5).

Conclusions

HME processing is a versatile technology, which can be successfully used for the development of sustained release pharmaceutical dosage forms. Due to the process flexibility, a wide range of polymers, lipids or co-processed materials can be extruded to tailor the release profiles according to the desired specifications. A careful consideration of the processing parameters and understanding of the investigated formulations it is possible to apply hot or cold extrusion and develop stable extrudates in the form of granules, pellets or tablets. HME is a process that can easily be scaled-up in a continuous mode but also to integrate PAT approaches for quality control and process monitoring.

Dr Dennis Douroumis is a Reader in Pharmaceutical Sciences at the University of Greenwich and the Director of Centre for Innovation in Process Engineering and Research (CIPER). His research focuses on the development of oral solid dosage forms, nanomedicine and medical devices. He is member of the Board of the Academy of Pharmaceutical Sciences (APS) and is an Editorial Board member of more than five international journals including the Wiley’s Editorial Board for the series in ‘Advances in Pharmaceutical Technology’. He has published more than 120 peer-reviewed publications, including 50 full papers, four book chapters and two books (Wiley – Blackwell) on ‘Hot Melt Extrusion Pharmaceutical Applications’ and ‘Drug Delivery Strategies of Poorly Water Soluble Drugs’. The CIPER Centre in collaboration with Thermo Scientific (Karlsruhe) specialises in continuous manufacturing of various solid dosage forms such as increase solubility of water insoluble APIs, taste masking of bitter drugs (paediatric products), controlled / sustained release formulations and continuous crystallisation. HME processing involves the use of QbD approaches and PAT tools (in-line NIR) for the development of continuous manufacturing.

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Electrospray mass spectrometry is often coupled to liquid chromatography (LC-MS) and tandem mass spectrometry (MS/MS) for the analysis of potential drug targets from complex matrices (plant extracts for example). But this requires some knowledge of the way the target molecules fragment in MS/MS and some understanding of gas-phase fragmentation. Natural products offer some interesting challenges to structural characterisation by MS/MS. With peptides, the cleavage points are well established to be at (or neighbouring) the amide bonds and, although side chain fragmentation can add a layer of complexity, peptide sequencing, as a result, can often be automated. Natural products do not have this regularity of structure and so interpretation has to be manual. Often, complete fragmentation is hampered by simple neutral losses (water, ammonia, methanol etc.) which offer little structural information. The purpose of this mini-review is to present some novel methodologies and new ways of thinking to aid structural studies by electrospray ionisation (ESI) MS/MS using collision induced dissociation (CID) and to increase our understanding of gas-phase fragmentation of complex molecules.

**Aromatic elimination**

The loss of mass 92 (a neutral molecule of toluene) from highly unsaturated natural products has been known since the 1960s. It was observed in electron ionisation (EI) mass spectrometry of β-carotene and also the pyrolysis of polyenes. Over the last 50 years, losses of aromatic molecules from carotenoids have been reported via a number of mass spectrometric methods. Examples are: chemical ionisation (CI) of carotenoids, fast atom bombardment (FAB) of carotenoids, electron capture negative ion CI of β-carotene and matrix-assisted laser desorption / ionisation (MALDI) post source decay of β-carotene. The losses of the appropriate aromatic molecule are regularly used as standard transitions on LC-MS.

What is interesting is that this elimination reaction occurs whether or not the analyte has the unsaturated chain at the terminus – i.e. this loss is often due to an internal rearrangement or ‘aromatic expulsion reaction’. A mechanism for this reaction was proposed in 1965, but it
seems to have been largely overlooked as it was applied to the specific case of the pyrolysis of polyenes in cigarette smoke. A study by Guaratini et al., stimulated by research in our laboratory (and those of our collaborators) on the fragmentation of unsaturated natural product antibiotics, set out to see how universal and useful this aromatic elimination reaction was and to see if there were any rules regarding molecular types that could undergo this reaction.

Table 1 (page 31) shows a summary of some of fragmentations observed in this study. It should be noted that these are all due to internal rearrangement and ejection of a neutral aromatic molecule. In some instances, the fragmentation occurs from the radical molecular ion $M^+$ in others from the protonated molecule $[M+H]^+$ or sodiated molecule $[M+Na]^+$, in one instance, after an initial fragmentation has occurred. It is interesting to note that the reaction is independent of molecular ion type and ionisation method used and therefore most certainly occurs via a ‘charge remote’ mechanism.

The conclusion of the study was that at least four conjugated double bonds were required for this elimination reaction to occur and a mechanism was proposed that would be applicable to almost any molecular type. However, there are a couple of important caveats, one is that loss of highly stabilised neutrals (for example CO$_2$ from acids) might have completely dominated the aromatic elimination reaction, and that in the case of nystatin the reaction was not observed even though it has the require conjugation length. It is believed that this was the result of the lack of flexibility in the structure preventing the require cis/trans isomerism. An important note is that Rapamycin only has three conjugated double bonds, however the transition for the loss of toluene was observed in the $MS^2$ spectrum after loss of methanol had occurred. There is one methoxy substituent one carbon along from the triple conjugation and loss of methanol from here would generate the four bond conjugation required.

Recently, these types of aromatic eliminations have been described in a range of other natural products including polyketides and polyether antibiotics. In some cases it has actually been used to help assign the structure to new natural product for example in a study by Hong et al. In this case, the aromatic losses observed were used to elucidate the methylation pattern of the unsaturated polyketide side chain in a newly discovered natural analogue of the macrolide toxin mycolactone (a potential antibiotic).
Isobaric fragmentation in polyketide antibiotics

A study of the fragmentation of polyketide antibiotics conducted in this laboratory has highlighted the dangers of relying on low-resolution ion-trap type instrumentation to perform MS\textsuperscript{n} analysis. As part of this study, the semi-synthetic macrolide antibiotic roxithromycin has been extensively studied by sequential mass spectrometry (MS\textsuperscript{n}) in an attempt to increase our understanding of the fragmentation of such complex molecules and in this case study, the effect of the N-oxime substituent group on the fragmentation observed.

Figure 1 shows MS\textsuperscript{n} spectra of roxithromycin run on both a low resolution Ion Trap (spectra (a) and (c)) and high resolution Orbitrap (spectra (b) and (d)). This study is part of a much more extensive investigation into the total fragmentation pathways of polyketides and polyethers to probe the structures of biosynthetic co-metabolites and newly synthesised non-natural analogues. MS\textsuperscript{n} is used to ‘drill down’ into the structures to generate as many fragmentation pathways as possible\textsuperscript{12}.\textsuperscript{17}

Formula matching shows that the ion at m/z 522.3256 matches to C\textsubscript{25}H\textsubscript{48}NO\textsubscript{10}\textsuperscript{+} to a mass accuracy of 3.3 ppm, whereas m/z 522.3409 matches to C\textsubscript{29}H\textsubscript{48}NO\textsubscript{7}\textsuperscript{+} to a mass accuracy of 3.1 ppm. It is fairly easy to arrive at structures matching these formulae by a series of simple fragmentations corresponding to losses of combinations of water, the oxime group or the sugar moieties. This is a very good example of isobaric fragmentation.

Unfortunately, the Orbitrap does not allow separation of these two routes in further MS\textsuperscript{n} steps as the isolation window is too wide.

### Table 1: Aromatic elimination reactions observed for a range of natural products in ESI tandem mass spectrometry (ESI-MS/MS). Data obtained by FTICR-MS\textsuperscript{n}.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Precursor Ion</th>
<th>Transition</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lycopene</td>
<td>M\textsuperscript{+}</td>
<td>m/z 536 → 444 (-92)</td>
<td>Loss of toluene</td>
</tr>
<tr>
<td>β-carotene</td>
<td>M\textsuperscript{+}</td>
<td>m/z 536 → 444 (-92)</td>
<td>Loss of toluene</td>
</tr>
<tr>
<td>Retinal</td>
<td>[M+H]\textsuperscript{+}</td>
<td>m/z 285 → 193 (-92)</td>
<td>Loss of toluene</td>
</tr>
<tr>
<td>Bixin</td>
<td>[M+Na]\textsuperscript{+}</td>
<td>m/z 417 → 311 (-106)</td>
<td>Loss of dimethyl benzene</td>
</tr>
<tr>
<td>Amphotericin</td>
<td>[M+Na]\textsuperscript{+}</td>
<td>m/z 393 → 301 (-92)</td>
<td>Loss of toluene</td>
</tr>
<tr>
<td>Nystatin</td>
<td>[M+Na]\textsuperscript{+}</td>
<td>m/z 946 → 868 (-78)</td>
<td>Loss of benzene</td>
</tr>
<tr>
<td>Rapamycin</td>
<td>m/z 564 from MS/MS [M+Na]\textsuperscript{+}</td>
<td>m/z 532 → 440 (-92)</td>
<td>Loss of toluene after methanol loss generates 4 conjugated double bonds</td>
</tr>
</tbody>
</table>
require application of Fourier-transform ion cyclotron resonance mass spectrometry (FTICRMS) where it is possible to separate isobaric species using their different fragmentation thresholds and thus get separated fragmentation spectra for each species.

**Isomeric fragmentation in polyketide antibiotics**

Although isobaric fragmentations can cause confusion when employing low-resolution instrumentation, the precursor ions are easily resolved by application of high-resolution techniques. Isomeric fragmentation, however, is not so easily resolved and care has to be taken not to confuse separate competing fragmentation routes from structural isomers. This is exemplified in the MS² analysis of the polyketide antibiotic erythromycin A. Isomeric fragmentation is observed in the MS² and the MS³ of the m/z 535 ion (Figure 2, page 30) clearly showing that the two routes followed to obtain m/z 535 cause some slight difference in the structure to occur. The expansion in spectrum (c) and (d) clearly show the absence of the peak at m/z 409 in the MS² analysis. Also the MS³ analysis has an extra high intensity peak at m/z 517 – presumably a water loss from the precursor ion and the MS² spectrum has an extra peak at m/z 239. There are also several smaller, less obvious, differences between the two spectra.

These differences in the spectra of the same m/z (535 in this case) during different stages of MS³ clearly show that some kind of isomerism has occurred. It is assumed that this is due to alternative conjugations occurring after one of the water losses. Although losses of simple neutrals might not be considered to be very structurally diagnostic, when they do occur they may generate isomeric species that go on to fragment in different ways. Increasing our understanding of these processes would clearly be of benefit in structural studies and aid the elucidation of novel compounds and co-metabolites.

It is possible to use the recently developed technique of differential ion mobility mass spectrometry (IM-MS) to separate structural isomers and thus get individual fragmentation spectra for each individual isomer. This has been readily demonstrated with a mixture of reverse peptides (peptides with the same amino-acid sequence but with their ‘N’ and ‘C’ termini swapped). Application of similar technology could be used to separate isomeric fragmentation routes in complex polyketide and polyether natural products.

**Conclusions**

This short review aims to highlight some of the complexities involved with natural product structural elucidation studied by ESI-MS² and hopefully also demonstrate some of the new technologies available to help overcome these. The first example clearly shows use of the well-know aromatic expulsion mechanism for polyenes to help elucidate the structure of a new polyketide. In the second example, the pitfalls of low-resolution analysis is demonstrated with a case of isobaric fragmentation which could potentially lead to mistakes in structural assignments. The third example demonstrates confusion that might occur due to competing isomeric fragmentation pathways and how it is essential to fragment every ion and every stage of MSⁿ else important routes could be missed. It is clearly dangerous to assume that just because ions have the same mass (and formula), that they are the same ion.

**Acknowledgements**

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**References**

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Innovation with Integrity
Traditionally, the ‘battle’ between the triple quadrupole mass spectrometer (MS/MS) vendors has been fought in the field of sensitivity. With the introduction of the latest MS/MS models reaching levels of sensitivity previously unheard of, how far do you think the quest for sensitivity can be pushed and what, if any, will eventually put a limit to it?

Richards: Sensitivity is not yet limited by physics. Therefore further improvements are possible. There is always a demand for greater more sensitivity but not at any cost. The MS/MS market is now a relatively high volume, mature market. The limiting factor is likely to be the cost effectiveness of higher sensitivity solutions compared to what already exists.

Moreau: There are two main challenges to address in achieving higher sensitivities in MS/MS detection; increased sampling efficiency at high flow ESI and minimising chemical (neutral) noise. Increasing the sampling efficiency in the ion source remains a considerable challenge, as the physics in this region are highly complex and are dominated by high velocity gas jets, temperature and space charge. Whilst sampling efficiency in nano-ESI is close to the theoretical sampling efficiency limit, high flow ESI sampling efficiency is far from optimal and a significant improvement in sensitivity could be achieved in this region.

If the issue of sensitivity of an MS/MS may gradually reach a plateau as a marketing tool, what other areas (other than lowering the price) do you think the vendors
will move into to attract and retain customers? For example, the instrument coming with dozens of validated methods, related libraries, extended warranties, etc., which are all customised to a buyer’s specific needs. Can you comment?

Moreau: Mass spectrometry development has continually evolved to meet the demands of ever-increasing sample throughput. This evolution adapts to the dynamic changes in science and market needs. Future drivers are likely to see the further development of tailored informatics and application layered software to address specific application sectors in routine analysis (for example, therapeutic drug monitoring in a clinical setting or pesticide screening in EFS) whilst at the same time creating instruments that robustly deliver high data quality for non-experts. Enhancing data quality will be possible by increasing the speed of data acquisition to maximise sample information and by increasing selectivity to avoid uncertainty.

Richards: Hardware is only part of the package that the buyer purchases in order to satisfy their needs and the needs of their customers (be they internal or external). Robustness and running costs are very important considerations but perhaps the greatest consideration is productivity. An instrument which offers increased productivity through software solutions tailored to the buyer’s workflow from sample preparation to report will be increasingly sought after. To satisfy these needs flexible, customisable software will be necessary as will the willingness of the vendor to work with the buyer to carry out the customisation and thereby deliver the desired solution.

Currently in bio-analysis, LC/MS/MS using multiple reaction monitoring (MRM) is the industry standard for quantitation. Do you believe that novel technologies, such as LC/MS/MS/MS, will lead the way and eventually take over MRM, even so adopted by regulatory agencies and incorporated in their guidelines? Can you put a timeline on it?

Richards: LC/MS/MS is the industry standard because it delivers both the specificity and sensitivity necessary to make very low level measurements in complex matrices. LC/MS/MS/MS offers greater specificity but not necessarily an advantage with respect to sensitivity. One could argue that because of the losses encountered in the second collision event MS/MS/MS is inherently less sensitive than MS/MS. Increased selectivity can be generated chromatographically using the LC part of LC/MS/MS during method development on the occasions when it is needed. So there is no compelling driver for an LC/MS/MS/MS solution from either the bioanalytician or the regulators. While LC/MS/MS/MS may be accepted by regulatory agencies it is unlikely to replace LC/MS/MS as the industry standard.

Moreau: The key test in quantitative bio-analysis is the sample cycle time which requires high sensitivity at high scan speeds. Whilst selectivity (and sensitivity) in bio-analysis is classically challenged by ion suppression (phospholipids, drugs, metabolites and proteins are compounds known to cause ion suppression) and/or isobaric interferences, adopting an efficient precursor ion selection strategy will significantly improve MRM detection and reduce both data redundancy and analysis time. For novel technologies such as LC/MS/MS/MS to replace MRM-based assays, the task will be to achieve a highly selective detection without compromising sensitivity and for many groups it must be considered within the appropriate cost model.

In general, the manufacturer of an MS instrument is liable for the results its equipment produces. In North America, in clinical diagnostics, this liability is now transferred from the manufacturer to the laboratory. Can you comment on the reasons which initiated this process, and whether this will affect the use of MS in the clinical practice?

Moreau: The main drive for growth in clinical LC/MS/MS in low molecular weight analytes analysis has been due to several factors which include the recognised strengths and weaknesses of immunoassay and the ability of LC/MS/MS to develop a new assay rapidly in the face of changing therapeutic drug panels. In the future, it is likely that more assays will be approved by regulatory authorities (including CLIA, FDA and CE Mark), enabling an increasing number of analytes to be tested using LC/MS/MS.

With MS manufacturers in close competition, the various MS systems produced have several unique features, which means that the data they generate cannot be translated by other vendors’ systems. Do you think it will be possible in the foreseeable future to have an application which enables the scientists to freely transfer and interpret MS data between systems made by different MS manufacturers?

Richards: Yes, this is currently possible for some applications. One example is Mass-Metasite which allows the use of Waters, Agilent, Thermo, ABSciex and soon Bruker data for DMPK applications. This is, of course, third party software driven by the needs of a consortium of pharmaceutical companies. They have generated a compelling need for instrument manufacturers to supply the necessary information for their data to be available to Mass-MetaSite. Whether manufacturers could ever agree on a generic data format, readable by all is a more complex question. It is not so much the format of these data that is important but the meta-data necessary to translate these data into meaningful spectra and allow further processing. For example, mass calibration is carried out quite differently on different MS systems. Even systems of nominally the same type (e.g. triple quadrupoles) from different manufacturers calibrate in different methods and generate a different type of meta-data.

Moreau: Developing a data standards strategy has been challenging for many differing group initiatives in mass spectrometry, mostly due to the diverse and changing needs in meta data. The development of open XML (extended mark-up language) based data formats has merit and the approach of the Allotrope Foundation is to be applauded. However, XML data formats do not meet all user needs, particularly in high data density file formats (large raw data binary files) resulting in very large text files, and it is more than likely that future data file exchange will involve reading binaries directly.
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Mass spectrometry in the biopharmaceutical industry: from the mundane to the cutting edge

The use of mass spectrometry in the biopharmaceutical industry has increased steadily during the past several decades. Initially applied to small molecule drugs for the confirmation of their structure, the use of the technology quickly expanded to cover the detection, characterisation and quantitation of process impurities and metabolites. Developments in ionisation techniques and mass analysers have enabled the use of mass spectrometry to the analysis of large, labile molecules such as peptides and proteins, initially as a tool for the characterisation of novel therapeutic proteins, subsequently to be used in monitoring and adjusting process development and manufacturing parameters and for characterising final product.

The rapid growth of genomics and the complete sequencing of the human genome as well as the genomes of many other organisms, some of which are used extensively in life sciences research and development, fuelled the growth of proteomics, where mass spectrometry has been the enabling core technology. The constant demand for improved mass accuracy, higher sensitivity, greater dynamic range and higher throughput have resulted in significant developments in mass spectrometric technologies, which in turn have contributed to their widespread use, with novel applications continuously being developed.

Small molecules
This has been the area in which mass spectrometry initially took its foothold in the pharmaceutical industry. It is now used routinely, along with other spectrophotometric techniques (IR, NMR) to confirm
structures of target molecules and to identify and characterise process by-products and impurities as well as metabolites from in vitro and in vivo analyses. Typically, it is electrospray ionisation mass spectrometry (ESI-MS) that is coupled with high performance liquid chromatography (HPLC), the latter used to obtain separation of components in mixtures (Figure 1). On-line liquid chromatography coupled to mass spectrometry (LC-MS) is well suited for non-volatile thermally labile small molecule drugs, which cannot be analysed by gas chromatography coupled to mass spectrometry (GC-MS); GC-MS is quite extensively used in environmental applications.

Measuring molecular weights accurately is quite valuable but has obvious limitations, especially in cases where the measured molecular weight is different from the expected one or in cases of unknowns, such as synthetic or process impurities and by-products and metabolites. Tandem mass spectrometry (usually abbreviated as MS/MS), which involves fragmenting selected precursor ions in the mass spectrometer and measuring the mass of fragment ions, can be used to obtain structural information of molecules of interest. For small molecule analysis, several types of mass spectrometers with MS/MS capabilities are used, such as triple quadrupoles, ion traps, quadrupole-time-of-flight instruments and Fourier transform ion cyclotron resonance mass spectrometers. However, for reasons that are discussed in more detail below, the triple quadrupole mass spectrometer has been the workhorse instrument in drug metabolism and pharmacokinetic (DMPK) analyses of small molecule pharmaceuticals.

Improvements in HPLC (better pumps, detectors and columns) have gone hand-in-hand with improvements in mass spectrometry in the analysis of small molecules and metabolites. Thus, better chromatographic separations and higher mass spectrometer duty cycles (i.e. more high quality data acquired per unit time) along with faster computers have all contributed to the ability of LC-MS systems to acquire a lot of data over a wide dynamic range, spanning five to six orders of magnitude.

For small molecule drugs under development, the accumulated knowledge and expertise of medicinal chemists and toxicologists can be leveraged to predict possible degradation and metabolic pathways that can be further elucidated and confirmed by in vitro (e.g. liver microsomes) and in vivo animal testing. The bigger challenge is to be able to identify and quantitate drugs and their metabolites in biological fluids, like plasma or urine, or in tissues, where these molecules are typically in the presence of much more abundant interfering molecules. For such applications, in addition to sample clean-up methods such as solid phase extraction (SPE) prior to LC-MS analysis, it is typical to employ the use of stable isotopes and mass spectrometric techniques such as multiple reactions monitoring (MRM).

Stable isotopes ($^{13}$C, $^{15}$N, $^{18}$O, $^2$H, hence no radioactivity to contend with) can be used to synthesise analogues of the small molecule drug and its known or suspected metabolites. These can be spiked into the biological sample (plasma, urine), which is then processed by SPE to partially purify and concentrate the molecules of interest. The isotopically-labelled small molecule drug or metabolite behaves identically to the unlabelled compound with regard to its chemical and chromatographic properties and can be used to monitor the efficiency of the SPE process. Furthermore, this method, known as isotopic dilution mass spectrometry, can be used to obtain absolute quantitation information in LC-MS analyses, since the isotopically labelled molecules, which can be spiked in precisely known amounts, have chromatographic and mass spectrometric properties identical to those of the unlabelled analogues but, of course, they have different molecular weights and thus can be distinguished in the mass spectrometer.

Limits of quantitation can be improved with a technique called Multiple Reaction Monitoring (MRM). MRM involves fragmentation of the
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precursor ion of interest but instead of acquiring a full spectrum only a few selected fragment ions are monitored (Figure 2, page 38), thereby significantly increasing the analysis duty cycle compared to full-scan MS/MS. The increase in the sensitivity of MRM analyses can be more than two orders of magnitude compared to the corresponding full-scan MS/MS.

Unlike discovery research and, to a lesser degree, drug development, small molecule analysis of pre-clinical and clinical samples is a highly regulated area, in which validated methods and analytical protocols and the associated instrumentation do not change readily. Typically, significant improvements in throughput or instrument performance and reliability must be demonstrated and methods, instruments and software must be fully validated before GLP/GMP laboratories will undertake changing methods and practices.

Proteins, peptides and other biological molecules: protein pharmaceuticals

The analysis of large biological molecules such as peptides, proteins and carbohydrates, became widespread once ‘soft’ ionisation techniques became available that did not result in the decomposition of such labile molecules during sample ionisation. Initially much of the application of ESI-MS and matrix-assisted laser desorption-ionisation time-of-flight (MALDI-TOF) mass spectrometry was focused on the characterisation of therapeutic proteins. Thus it became possible to characterise peptides, separated by HPLC, that were produced by digestion of a protein of interest (peptide maps), reliably and quickly, especially compared to N-terminal (Edman) sequencing, as a result of extensive work by many research groups that resulted in the formulation of clear and reproducible rules for the fragmentation of peptides in the mass spectrometer. This application of mass spectrometry continues to be a significant component of the characterisation work performed by biotechnology companies that discover and develop therapeutic proteins and therapeutic antibodies; the latter often conjugated to small molecule drugs. The high mass accuracy and resolution available in modern instruments makes it fairly straightforward to confirm the sequence of such large biomolecules and also identify process impurities and degradation products during stability studies.

In addition to the characterisation of peptide maps, the ability to accurately measure the molecular weight (MW) of proteins has proven quite useful as well. Depending on the size of a protein it may be possible to obtain from its accurate MW measurement such information as the presence of heterogeneity due to fermentation or cell culture conditions (e.g. C-terminal truncation), the extent and nature of post-translational modification (e.g. glycosylation), the number of small molecule drugs conjugated to an antibody, and how and to what extend a protein may be modified during stability studies (e.g. deamidation, oxidation). Both ESI and MALDI-TOF mass spectrometers can be used for intact protein work. Certain instruments also have the capability in addition to measuring the MW to also fragment and measure the mass of fragment ions of intact proteins, thus providing amino acid sequence information (‘top down’ analysis) that may be complementary and sometimes replace the sequence information obtained by enzymatic digestion and LC-MS analysis or proteolytic peptides (‘bottom up’ analysis).

Additionally mass spectrometry is being used to characterise the carbohydrates of glycosylated proteins. It is important for reasons of process control related to drug efficacy (e.g. bioavailability) and safety (e.g. antigenicity) to produce recombinant protein pharmaceuticals with not only the correct amino acid sequence but also the correct carbohydrate structure. The latter can be characterised either by measurement of the protein molecular weight, or by the characterisation of the glycosylated peptides in the peptide map, or by removal (chemically or enzymatically) of the carbohydrate and its separate analysis by LC-MS/MS. Similarly, other protein modifications such as small molecule conjugation or conjugation to polyethylene glycol (PEG), which reduces antigenicity and improves bioavailability of PEGylated proteins, can be assessed by MS analysis.

More recently the approach that had been used for some time in small molecule DMPK analyses, namely MRM and isotopic dilution mass spectrometry, has also been applied to the sensitive quantitation in the pharmacokinetic analyses of protein drugs in biological fluids from animal and human test subjects. It is possible to select a few of the peptides known to be generated by enzymatic digestion of a protein of interest and identify a few fragment ions to monitor in an

Figure 3: SILAC labelling of samples. Cell culture is carried out using media with no isotopically labelled amino acids (‘Light sample’) and with one or more amino acids with stable isotope labels (‘Heavy sample’). The samples are then mixed and the proteins from both are fractionated before further processing. In this example, SDS-PAGE is used but other fractionation methods (e.g. chromatography) may be employed. Each protein fraction is then digested with an enzyme, proteins are identified from the peptide MS/MS spectra using proteomics methodologies, and proteins from the ‘heavy’ (isotopically labelled) and ‘light’ (unlabelled) samples can be quantitated relative to each other from the respective peptide pairs. Thus different samples (healthy vs. disease, drug-treated vs. untreated, etc.) can be compared. This approach, just like those involving chemical labelling, can also be multiplexed across more than two samples by employing more than one type of isotopically labelled amino acid.
MRM experiment and also obtain synthetically such peptides with stable isotopes incorporated in their sequence and use these for accurate quantitation.

**Proteomics**

The most dramatic increase in the use of mass spectrometry during the past 15 years, in academia as well as in the biopharmaceutical industry, has been in the field of proteomics and proteomics-based biomarker discovery and validation. This has been facilitated by the advances in genomics and the complete sequencing of the human genome and the genomes of quite a few other species, many of which are either laboratory animals (mouse, rat) or bacterial or viral pathogens. Proteomics has used the techniques and approaches originally applied to individual proteins (enzymatic digestion followed by LC-MS analysis of proteolytic peptides) but with mixtures of hundreds or thousands of proteins obtained from the organism (or specific tissues or biological fluid) under study.

Starting with mixtures containing hundreds or thousands of proteins, it is, of course, impossible to examine and interpret the tens or hundreds of thousands of peptide fragment ion mass spectra generated by proteolysis, so software that uses such data to search protein databases and match them to peptides from protein sequences in the selected databases has been developed; proteomics-related mass informatics has been a continuously evolving area of research and development. In this manner, proteins that are present in the sample under study can be identified from peptide data matched to their sequences through the corresponding MS/MS spectra. The major challenge in this approach, however, is to identify proteins of low abundance, the peptides of which yield weak signals in the mass spectrometer. Since mass spectrometry software typically select peptides to fragment based on signal intensity, abundant proteins, the peptides from which yield strong signals, are typically overrepresented in such LC-MS data. This is a problem, since often proteins of significance (e.g. cytokines) or modified forms of proteins (e.g. phosphorylated) are present at relatively low abundance compared with other proteins that are of no interest. This becomes particularly acute in plasma samples, where a handful of proteins constitute more than 90 per cent of all protein present, and discovering or identifying a low abundance disease marker is tantamount to finding the proverbial ‘needle in the haystack’.

To get around such issues better chromatographic separation methods as well as faster and more sensitive mass spectrometers have been developed. Capillary chromatography, with flow rates at or below 1 μL/min is used to obtain efficient peptide separation, allowing peptides of low abundance to move away from the ‘shadow’ of larger peptides in the chromatographic elution profile. Faster scan rates make it possible for instruments to acquire more peptide MS/MS spectra per unit time, ‘intelligent’ software allows for the exclusion of abundant non-interesting signals, and improvements in dynamic range and speed allow for high quality MS/MS spectra from very weak peptide signals. It is typical to be able to obtain good MS/MS spectra from peptides in the low fmol range or below, which corresponds to picogram amounts of protein.
Proteomics and proteomics-based biomarker discovery have also utilised two techniques that have proved quite useful in small molecule as well as in protein drug analysis, MRM mass spectrometry and isotopic dilution. The former has been used extensively in the validation of research discoveries, by quickly screening samples for the presence of low level peptides (corresponding to proteins of interest). The latter has given rise to a number of innovative approaches involving chemical or biological derivatisation of proteins and peptides with isotopic or isobaric labels made from stable isotopes. Peptides or proteins can be labelled specifically (e.g. at lysine amines or cysteine thiols) with isotopic or isobaric reagents (ICAT, iTRAQ, TMT) and the mixtures of peptides thus generated, which behave the same way chromatographically and ionise equally well, can be used to obtain relative quantitation and, by spiking synthetic analogues, absolute quantitation as well. Biological labelling of proteins in cell culture with stable isotopes (SILAC) also provides similar information and has the advantage that no chemical derivatisation is required (Figure 3, page 40).

Table 1: Mass spectrometers used in pharmaceutical research and development

<table>
<thead>
<tr>
<th>Analysis type</th>
<th>Predominantly used ionisation method</th>
<th>Mass analyser type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small molecule R&amp;D</td>
<td>ESI</td>
<td>Triple quadrupole, ion trap, Q-Tof, FT-MS</td>
</tr>
<tr>
<td>Small molecule DMPK</td>
<td>ESI</td>
<td>Triple quadrupole (MRM)</td>
</tr>
<tr>
<td>Protein pharmaceutical R&amp;D</td>
<td>ESI, MALDI</td>
<td>Ion trap, FT-MS, MALDI-TOF, Q-Tof</td>
</tr>
<tr>
<td>Protein pharmaceutical DMPK</td>
<td>ESI</td>
<td>Triple quadrupole, ion trap</td>
</tr>
<tr>
<td>Proteomics/biomarker discovery</td>
<td>ESI</td>
<td>Ion trap, Q-Tof, FT-MS</td>
</tr>
<tr>
<td>Proteomics/biomarker validation</td>
<td>ESI</td>
<td>Triple quadrupole, FT-MS</td>
</tr>
<tr>
<td>Tissue imaging</td>
<td>MALDI</td>
<td>MALDI-TOF, MALDI-ion trap, MALDI-FT-MS</td>
</tr>
</tbody>
</table>

ESI: Electrospray ionisation
MALDI: Matrix-assisted laser desorption ionisation
Q-Tof: Quadrupole-time-of-flight
FT-MS: Fourier-transform mass spectrometer

Tissue imaging
MALDI-TOF mass spectrometry was initially a parallel and complementary technique to ESI-MS, but it has generally been eclipsed by the latter, primarily due to the difficulty of interfacing MALDI-TOF with on-line HPLC. It became primarily a means of obtaining molecular weight information of intact proteins and certain types of non-biological samples (e.g. polymers) until tissue imaging applications began gaining ground. Tissue imaging with MALDI involves placing a tissue slice (obtained, for example, by microdissection) and scanning the surface in two dimensions with the MALDI instrument laser. As the laser ablates material off the tissue molecules ionise and patterns (fingerprints) are generated across the surface of the tissue. This approach has been used effectively to determine distribution of small molecule drugs and their known metabolites across different parts of tissues. It has also been used, often in conjunction with pathology evaluation, to determine boundaries between healthy and diseased tissues in biopsy samples. Increasing tissue imaging has found its way into the workflows of the biopharmaceutical industry, where it is being integrated into various drug discovery and development programs.

Conclusion
Mass spectrometry, because of its flexibility in terms of its application to a wide range of molecule types and samples, has become an indispensable tool in biopharmaceutical research and development (Table 1). The ease of operation and the robustness of the hardware and software of modern mass spectrometry systems, along with a wide range of instrument capabilities and price tags, have also made it possible for mass spectrometers to be installed and used outside centralised core facilities or laboratories, in the laboratories of individual investigators, where bench scientists use them just like other sophisticated analytical instruments. With these improvements in instrumentation there have also been great advances in the development of reagents and accessories, whether these are chemicals, isotopes, sample handling and purification tools and materials, enzymes, and software. Similar trends are also evident in academic laboratories, where the next generation of scientists who will be discovering and developing the next generation of pharmaceuticals, are receiving hands-on training in the application of this enabling technology to pharmaceutical research and development.

References


Ioannis Papayannopoulos has been working with mass spectrometry of peptides and proteins for a quarter century. He received his undergraduate degree from Bowdoin College and his PhD in Chemistry from the Massachusetts Institute of Technology, under the supervision of Professor Klaus Biemann. He has worked in senior scientific and management positions in the biopharmaceutical industry, at companies such as AstraZeneca, Applied Biosystems, Biogen and EMD Pharmaceuticals, and in academia, most recently as the director of the proteomics facility at the Koch Institute for Integrative Cancer Research at MIT. Since 2013, he has been a Principal Scientist at Targanox, a small research-based biotechnology company in Cambridge, Massachusetts.
ASMS, a non-profit corporation, was formed in 1969 to promote and disseminate knowledge of mass spectrometry and allied topics. Its membership includes over 8,500 scientists involved in research and development who come from academic, industrial and governmental laboratories. Their interests include advancement of techniques and instrumentation in mass spectrometry, as well as fundamental research in chemistry, geology, forensics, biological sciences and physics.

The 62nd ASMS Conference on Mass Spectrometry and Allied Topics’ conference programme is coordinated by Jenny Brodbelt (VP Programs), University of Texas, Austin. The conference will begin at 17:00 on Sunday 15 June with tutorial lectures from Lucinda Cohen, Merck Research Laboratories and Ron M.A. Heeren, FOM-AMOLF. These will be followed by the opening session and plenary lecture entitled ‘The James Webb Space Telescope: From Fight Light to the Search for the Earth 2.0’, which will be taken by Jason Kalirai from the Telescope Science Institute. A Welcome Reception in the Exhibit-Poster Hall will follow.

Monday through to Thursday will consist of full programme days of simultaneous oral sessions, poster sessions (which will allow for four hours of viewing) and workshops. Topics include:

- Nucleic Acids
- Informatics: Protein Identification
- Characterisation of Biologics and Biosimilars
- Quantitative Analysis in Drug Discovery and Development
- Instrumentation: Mini / Portable / Fieldable Mass Spectrometry
- Ion Mobility: Separations
- Imaging: Pharmaceuticals and Metabolomics
- Fundamentals of Peptide Fragmentation
- Imaging: Fundamentals, Instrumentation, and Method Development
- Protein-Protein and Protein-Ligand Interactions
- Nano-Scale and Microfluidic Separations and Mass Spectrometry
- Drug Target Discovery and Validation
- Pharmacoproteomics and Toxicoproteomics for Drug Development
- Proteomics: Infectious Diseases
- FAIMS and DMS: New Developments and Applications
- Biomarkers in Drug Discovery, Development and Diagnosis.

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The programme will conclude on Thursday 19 June with a plenary lecture entitled ‘How the Genome Folds’, taken by Erez Liebermann Aiden of the Baylor College of Medicine and Rice University. This will be followed by a ticketed closing event at the National Aquarium.

Closing Event

At 18:30 on Thursday 19 June, visitors will be able to join their friends and colleagues at the National Aquarium to celebrate another successful ASMS Conference. The aquarium is located less than a mile from the Baltimore Convention Center along the Inner Harbor. The aquarium will be closed to the general public during the ASMS Closing Event, and ticket holders will be able to enjoy all the aquarium has to offer, including an iMax film and a dolphin event. Hors d’oeuvres and drinks will be offered throughout the event.

Tickets for the Closing Event are USD 30, and those wishing to attend can purchase them online during registration or via the printable registration form.

Date: 15 – 19 June 2014
Location: Baltimore, USA
For more information on ASMS, please visit: www.asms.org
In her opening remarks, Christel Fenge, VP Marketing and Product Management, Fermentation Technology, at Sartorius Stedim Biotech, commented that product quality data was a key driver of Quality by Design (QbD), in association with Process Analytical Technology (PAT), and of significant importance to Sartorius as a company. Describing it as a key differentiator, she noted how QbD enables customers to do process development faster with fewer resources, how it helps them to move towards “the fully automated facility of the future” and how it facilitates the more efficient analysis and assessment of existing data to make better use of extant information. A higher level of automation in single-use facilities, she commented, will result in less operator intervention and decreased downtime. Getting safe, high quality, efficacious products to market in a time- and cost-efficient manner, by implementing QbD/PAT, not only benefits drug manufacturers, it also benefits the patient.

This was a sentiment that was reiterated by Professor Dr Jose C. Menezes. A senior researcher at the Institute for Biotechnology and Bioengineering, Technical University of Lisbon, Professor Menezes’ presentation described how different components of PAT, within the QbD context of biopharmaceutical manufacturing, can and should be integrated as an end-to-end process and throughout the entire product lifecycle. He stated that there are huge benefits in having better processes, which essentially means better products and that companies are doing a better job for their patients. “Yet,” he adds, “QbD/PAT is something that should not only be seen as a way to improve current production platforms, it should also be applied to the knowledge and technology platforms that we’re using to develop future products. Patients need products, and we can get these products to market faster by implementing QbD/PAT practices, which will, in turn, benefit those patients.”

Application and Implementation
Professor Anurag Rathore from the Department of Chemical Engineering, Indian Institute of Technology, adds, “If we don’t follow the principles of QbD/PAT, then, essentially, we are manufacturing and selling products that contain gaps in our knowledge base. Although Big Pharma has operated this way for quite some time, it does result in product recalls and, occasionally, drugs that do not have the required efficacy.” Discussing some of the tools that play a critical role in biotech QbD — PAT, multivariate data analysis (MVDA), design of experiments (DoE), high throughput process development (HTPD), mechanistic modelling and computational flow dynamics (CFD) — he noted that most Indian companies, particularly those involved in producing biosimilars, are actually following these principles, and making drugs for emerging economies that are not actually asking for QbD. “They’re doing it themselves because they realise that, in the long-term, quality products mean a good reputation and repeat business.”

Quality by Design started to gain momentum in the biotechnology industry when the US Food and Drug Administration (FDA) published its directive, ‘PAT: A Framework for Innovative Pharmaceutical Manufacturing and Quality Assurance’. Whereas the underlying concepts are now becoming clear and widely accepted, the realisation that technological advancements are necessary to facilitate QbD implementation is also dawning on the industry. However, even though the paradigm of process monitoring intensification, combined with science-based process engineering, has been transforming the way procedural industrialisation and higher product quality consistency is performed, there are still several roadblocks to be overcome.
Professor Reiner Luttmann from the Hamburg University of Applied Sciences explained that PAT and QbD are proven technologies in many other industries, generating a return on investment in a few months or, at most, a year, even in the chemical sector. “QbD requires a thorough understanding of the manufacturing process,” he says, “with an upfront investment in time and resources. It’s surprising that Big Pharma is [once again] a slow adopter; in the food industry, spectroscopic solutions are used as standard to measure 10 million chickpeas per hour. But, it seems the pharmaceutical industry is not able to measure five to ten tablet parameters per hour. I think this is madness; as a customer, I want high levels of quality control and what I don’t want is to pay for product recalls. The food industry is driven by money. So, perhaps, Big Pharma is making too much money. I’m being facetious, of course, cost savings (as well as quality and efficacy) are paramount, so implementing PAT and QbD is an imperative and should be done as soon as possible.”

Quality in, quality out

A basic part of QbD is to create a design space and to define the related process control space wherein process inputs can be changed without any loss of quality. This design space is defined by the key and critical parameters identified during process characterisation studies. These parameters are the primary focus for inline, online or at-line PAT applications. Dr. Fai Poon, Director of Cell Culture at Hisun Pharmaceutical, explains, “When I approach a problem, I don’t instantly think about how I’m going to get a drug approved, I think that by doing the next experiment, I’m going to learn something. And what I learn will help me when I do the next experiment. Also, I’m thinking how do I record this experiment or benefit from the experience. My take home message is that PAT and QbD are not just there to help you get a drug approved, but also to better understand your system and your science.”

Andreas Schneider, VP at Roche Diagnostics International Ltd, agrees, “QbD, by definition, means we should build the quality into our processes and products. It’s about ensuring that the drugs we produce are safe and efficacious. What we gain from QbD is a better understanding of our system variables and, right now, it’s about getting better control of those variables and driving quality to the desired levels. Are we there yet? As an industry overall, no. But in some cases, yes. We’re on the right track.”

Professor Rudolph Kessler of Reutlingen University added that spectroscopy will play an important role in the transition of a reactive industrial production philosophy into a proactive system. “QbD and PAT are paving the way for the manufacturers of measurement equipment to integrate online control and monitoring into bioreactors, for example, and downstream processing, which is a great advantage,” he says. To an increasing extent, spectroscopic techniques are being integrated into real-life production and extending the information that can be derived from conventional sensors (or replacing them). As spectroscopic techniques can simultaneously detect all the morphological and chemical features of a chemical component, the fundamental functionality of the compound is inherent in every spectrum. However, redundant and unnecessary information must be excluded from the spectral features by using complex data analysis.

Barriers to adoption

To date, only one drug has been produced / approved that has been developed using a full QbD approach. “One difficulty we have, focusing on orphan drug approvals, is that we’re not using a platform technology to produce our therapeutics; we’re going after molecules that are hard to make,” says Timothy Taylor, Senior Scientist at BioMarin Pharmaceutical Inc. And each individual molecule has its own set of attributes that need to be monitored and complex assays that need to be done: I can’t see the agencies letting us get away with doing a full QbD approach; they’re still going to want to see a Certificate of Analysis; it’s hard to go down that road when everything you make is different. If you have a generic platform, such as for mAbs, for example, it might be easier, but that’s the hurdle that we struggle to overcome.”

Considering that QbD has only really been around since 2007, it’s still a relatively new biotech concept, and agencies are wary of giving any company carte blanche to do whatever they want in the design space; and there’s a good reason for that. “If you look at how we develop these processes, we don’t begin with commercial-scale production,” says Anurag Rathore. “All the work is done with scale-down models and, however good they may be, it’s not true manufacturing and you’re not verifying the production parameters at full-scale. Things will change with time; but, that’s why the regulatory bodies are hesitant to grant approval. We’ve already come a long way but I don’t think we’ll ever see 100 per cent of filings using QbD principles because it doesn’t make sense for multi-product manufacturers,” he said.

Summary

QbD-enabled process optimisation is now much faster, it was agreed, which results in more reliable procedures, but further steps still need to be taken – in terms of equipment, analytical and measurement tools, services, etc. – to facilitate the industry adoption of PAT/QbD. “As an industry, we need to control and provide reproducible quality because, essentially, process development, scale-up, etc., is already extremely complex and we don’t fully understand every aspect of every variable, so if you add another level of variability that you don’t fully understand — by measuring and recording new parameters — it becomes even more difficult to make sense of the data, irrespective of whether the variations are large or small,” Christel Fenge summarises.
Recent years have witnessed an expansion in the disciplines encompassing drug discovery outside the pharmaceutical industry. This is most notable with a significant number of universities worldwide now that host infrastructure such as compound libraries and automated screening centres. An archetypal small molecule drug discovery project will aim to identify chemical starting points that modify the functions of genes, cells, or biochemical pathways. In some but not all instances, these functions may be linked to disease processes, and an opportunity will exist to further develop the chemical starting points into novel therapeutic agents. In small molecule drug discovery, the ultimate aim is to identify new therapeutics, an activity that for reasons of high risk and cost has historically been conducted within the commercial sectors.

The first practical steps in drug discovery include the selection of a target (followed by its cloning, expression and purification), development of an assay to monitor the activity of the target, and the synthesis and management of molecular libraries. The second practical steps include the use of the above in screening campaigns to identify primary hits, followed with their validation. In the context of drug discovery projects that make use of biochemical assays with purified targets, the activities of selected primary hits would typically be further evaluated in biophysical assays such as surface plasmon resonance and isothermal titration calorimetry. This effort would be expected to lead to the identification of validated hits with some of these selected for optimisation using multiple criteria including structure activity relationships, selectivity, physicochemical properties and liability.

The practical aspects of this workshop will examine microtitre plate biochemical assays for kinase, histone deacetylase and protease enzymes.

The Practical workshop – biochemical assays for screening is designed for scientists at all levels (undergraduates, postgraduates and laboratory based scientists within academic and industrial research organisations) engaged in early stage drug discovery and have an interest in the development, validation and utilisation of biochemical assays for screening against small molecule libraries. The Practical workshop – biochemical assays for screening is equally well suited to technically focused staff from core facilities or contract research organisations who may wish to extend their expertise. The evening dinner on the first day will offer the opportunity for the participants to network and establish relationships that would be mutually beneficial.

The main learning objectives of the Practical workshop – biochemical assays for screening will be to examine by way of practical sessions and lectures, the design and application of biochemical assays for small molecule screening campaigns in drug discovery. All participants will take part in the practical sessions and these will involve the development of screening compatible biochemical assays, primary screening using a small molecule library, and profiling of compounds in dose-response experiments. Participants in this workshop will discuss and demonstrate practically: (1) the appropriate steps in selecting suitable assays in light of the fact that a multitude of assay technologies are currently available for a given target; (2) how to select an appropriate technology; which criteria should be examined during the early stage drug discovery process; (3) whether a generic, flexible set of assay methodologies or customised solutions should be applied to the targets being investigated; (4) annotation of hits using cell health assays (e.g. cell viability, proliferation, apoptosis, mitochondrial toxicity).

It is envisaged that upon completion of the course, attendees will have gained an insight into the key parameters to be considered when developing biochemical assays and performing small molecule screening campaigns, associated data analysis, validation of hits and their annotation using a variety of cell health/toxicity/liability assays.

**Workshop instructor**

Sheraz Gul is Head of Biology at European ScreeningPort, Hamburg, Germany where he manages the assay development and screening of academic targets. Prior to this, he worked for GlaxoSmithKline for seven years where he developed biochemical and cellular assays for high throughput screening as well as hit characterisation. In addition, he has worked in academia for five years on proteases and kinases. He is the co-author of the Enzyme Assays: Essential Data Handbook.

E-mail: sheraz.gul@screeningport.com

**Continuing Professional Development (CPD)**

Approved by the Society of Biology for purposes of Continuing Professional Development (CPD), the Practical workshop – biochemical assays for screening workshop may be counted as 72 CPD credits.

Please note that these credits are only valid if attendees are registered on the Society of Biology CPD Scheme.

For more information on this Workshop, please visit: [www.europeanpharmaceuticalreview.com/july-workshop](http://www.europeanpharmaceuticalreview.com/july-workshop)
The use of handheld Raman spectroscopy has quickly emerged as a standard for quick, non-destructive identification (ID) testing of materials for diverse reasons including specificity, through-liner scanning capabilities, portability, etc.¹ The measurement principle of Raman spectroscopy relies on inelastic scattering of monochromatic radiation¹. Basically, a sample of interest is exposed to a monochromatic wavelength source and the scattered light is analysed to obtain information on material properties.

For sites with both manufacturing and packaging operations, the
use of this application for testing of polymeric packaging materials may be seen as a natural expansion of the technology, following successful use for rapid ID testing of manufacturing raw materials. However, from experiential learning, ID testing of packaging components / bottles can develop into a PAT application of its own merit, the reason being mainly related to both material volumes and the relatively extensive sample preparation required to perform conventional laboratory ID tests.

For example, with previous implementation of the portable Raman instrument for ID testing of high density polyethylene (HDPE) bottles at a Pfizer manufacturing facility, significant testing time reductions were accomplished compared to the conventional test, contributing to an overall leaner process. In addition, the application provided safety benefits as material cutting technologies were used during sample preparation to conduct the conventional ID test. Using Raman for non-destructive HDPE bottle scanning / testing requires no sample preparation, thus helping to lower potential laboratory safety risks.

From a practical standpoint, the capabilities and portability of commercially-available Raman instruments provide the flexibility to maximise use of the instruments. This inherent flexibility – coupled with the relative lower cost when compared to some of the bench top units – also helps ease project justification. From a business standpoint, there is specific published guidance to estimate potential savings from implementation of portable Raman instruments. Previous implementation experience has shown payback periods of a year or less (depending on project scope) for investments relating to portable Raman ID testing applications.

**Vision-based system for blister integrity testing**

Blister integrity is a regulatory requirement to check for proper sealing and the absence of pinholes in blister packed pharmaceuticals. The blue dye test is currently the industry standard for testing blister integrity. This test involves the submersion of blister packs into a methylene blue dye solution for a period of time and then performing visual inspection of the individual pockets for dye ingress. Some of the inherent disadvantages with the blue dye test are that it is a destructive and thus potentially costly test (pharmaceutical product and packaging materials are destroyed during this test), sampling frequencies are low, and the test is laborious and time consuming. The blue-dye test also requires trained personnel to run the test and relies on subjective analysis of dye ingress into the cavities to identify blister leaks.

It is highly desirable to use an empirical technology solution that can eliminate some of the known disadvantages of the blue dye test whilst increasing limit-of-detection capabilities in terms of detectable pinhole size. One of these options is a vision-based system, which is capable of non-destructively assessing blister integrity for multiple sizes, shapes, cavity configurations and materials of construction. The test is relatively fast (about one minute per test cycle after method development), empirical, and provides pass / fail results eliminating any need for human-visual analysis of samples and associated subjectivity. These vision-based systems also allow for tool-less analysis of blister packs which allows rapid development and deployment of methods for almost all blister products manufactured at a site.

In terms of practical aspects, these systems are relatively simple to install as well as operate, and they are mostly intended as bench top units for off-line or at-line testing. The instrument applies cycles of both positive pressure and vacuum to a blister-holding chamber while an embedded camera captures images of the blisters at the different pressure / vacuum stages. It then utilises image analysis algorithms to compare images and identify subtle elastic deformations in individual blister cavities due to pressure changes. Sealed blister cavities will show deformation with pressure changes, and leaking blister cavities will show little or no deformation due to free passage of air (i.e. pressure equilibrium between the inside and outside of the leaking cavity).

Considering some of the financial statistics (return-on-investment,
payback period, etc.), these systems can achieve favourable and appealing numbers, especially if matched for use with blister products exhibiting relatively high volume and / or high manufacturing costs per unit, when taking into account savings from the non-destructive nature of the test. In addition, due to the seemingly low complexity of the system hardware, and the fact that some systems do not require product-specific tooling, expected post-purchase cost of ownership is low.

**X-ray for inline blister inspection**

Inline systems using X-ray technology for blister inspection – mostly for empty blister cavities detection – have been commercially available for a while. However, given the capabilities this technology brings, and the reenergised industry efforts placed on reducing the costs of poor quality in support of leaner operations, it is worth considering these systems in greater depth. In general, the use of X-ray inspection in the pharmaceutical industry provides an alternate means of inspection for operations in which other inspection technologies such as vision based systems may not be suitable, for example, foil packaging.

In principle, X-rays have the ability to penetrate solids and ionise gases. The electromagnetic radiation of X-rays has a wavelength range of 3x10¹⁶ Hz to 3x10¹⁹ Hz (0.01 to 10 nanometres). As reference, they are shorter in wavelength than UV rays (and visible light) and longer than gamma rays. X-rays can penetrate objects to varying depths depending on intensity. Typical industrial systems utilise low-energy radiation to penetrate the samples of interest, and software analyses data enabling reject of out-of-specification blisters.

At the operational level, the system is relatively easy to set-up. Among the most important parameters are the configuration of the area of scrutiny for each blister cavity, and the selection of the ‘golden’ (reference) blister samples for initial set-up. The threshold limits and all X-ray data generated will be relative to these ‘golden’ blister samples. The defects that X-ray systems are able to detect typically include missing product in blister cavities, missing blister(s) in box packages (potentially eliminating the use of a check-weigher), partial and/or damaged product in blister cavities, and some types of damaged blisters.

From a business standpoint, adoption of this technology can help support cost avoidances, especially if any of the issues that the technology detects have a history of triggering customer complaints. In addition, adoption of the application supports elimination of off- / at-line testing for missing blister(s) in box packages and therefore cost improvements may be possible to calculate. This application also enables 100 per cent inline verification and automatic reject of defective / damaged packaged product, a level of quality assurance that, if required, would otherwise be considerably time and resource consuming. On the down side, depending on the product and the application of the X-ray technology, data may be required to
demonstrate that there is no impact to a product’s efficacy and stability as a result of the application of X-ray radiation.

**Thermal imaging for inline bottle foil seal integrity verification**

The increasing availability and diversification in industrial use of thermal imaging technology, which was originally developed for defence applications (a typical example being night vision), has reached the pharmaceutical industry. Technology offerings are now commercially available, enabling first-ever inline inspection capabilities such as bottle foil seal integrity verification.

Warm bodies emit electromagnetic radiation due to thermal motion of their molecules. In principle, thermal imaging cameras detect the radiation emitted by objects above absolute zero. Thermographs capture information on temperature variations given that the amount of radiation emitted by an object in the infrared spectrum increases with temperature. Wavelengths emitted by objects are able to penetrate most plastics, and can thus provide images of covered foil that are not possible with visible light-based imaging systems.

In the case of a bottle packaging / foil seal verification application, the thermal imaging camera is positioned after the induction sealing step. The system is able to capture patterns of temperature that are then analysed with image processing software. Pre-determined thresholds for thermal pattern variability allow detection of a range of defects associated to bottle foil seal integrity. Based on previous empirical experience, defects that can typically be detected using inline inspection with thermal imaging technology include:

- Bottles without caps
- Bottles with caps but without seal
- Bottles with skewed caps
- Bottles with high cap / unengaged cap

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**Figure 3:** Left: Set-up of Safeline X-ray inspection system for blister inspection at a Pfizer manufacturing facility in Puerto Rico. Right: Partial view of system display showing image of scanned blister sample

**Figure 4:** Left: Temporary installation of a DIR Technologies thermal imaging camera during pilot study at a Pfizer facility in Puerto Rico. Right: Visuals of selected “recreated” defects detected – at typical process conveyor speeds and with caps in place – and corresponding thermal images obtained with camera system
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From a practical standpoint, the system is relatively easy to adapt as an additional inline standalone unit to an existing bottle packaging line, assuming the additional required space is available. The full system incorporates a thermal imaging camera, a computer for data acquisition / image analysis / data logging, position sensors, and a reject module. Given that just recently this system became commercially available, it is worth noting some of the system benefits as demonstrated during a recent plant trial.

- No radiation is emitted by the system.
- The camera captures the infrared radiation emitted by the foil seal exiting the induction sealing machine; therefore, there is no risk of impacting product stability
- No interference with process speeds. In the case of our particular application, the system was capable of inline inspection at typical conveyor speeds
- Ability to collect thermal imaging data relating to foil seal integrity through the caps
- System software was developed targeting the pharmaceutical industry (21 CFR Part 11 Compliance)
- Aside from rejecting bottles with bad seals, the system is practically non-invasive – the camera captures thermal information as bottles pass through a conventional conveyor.

For more information on Eyecon™, please contact: Ciarán O’Connell, Sales & Marketing Specialist email: oconnellc@innopharmalabs.com

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We have seen progress in the number and type of PAT applications that can support packaging operations

Concluding remarks

This article is aimed at providing an overview of some of the packaging PAT applications that are emerging and enabling expeditious, non-destructive quality testing. Four applications were reviewed that included: 1) Handheld Raman for packaging raw material identification testing, 2) Vision-based system for blister integrity testing, 3) X-ray monitoring for inline blister inspection, and 4) Thermal imaging for inline bottle foil seal integrity verification. The technology behind each PAT application was briefly explained, an example provided through visuals, and some of the practical and financial considerations mentioned.

In general, we have seen progress in the number and type of PAT applications that can support packaging operations. This shows not only the diversification of PAT to expand the impact of...
technologies across the different pharmaceutical operations, but also the continued commitment on innovation by vendors and manufacturers in search of alternatives to reduce the costs of quality while maintaining or increasing the levels of technology assurance. While cost reduction and economic pressures continue to play a key role in the pharmaceutical industry, we cannot deny the fact that technology with a purpose – that is, to improve quality, reduce rejected product, lower manufacturing costs, etc. – can help create a competitive advantage. Once suitable technological applications are identified and business cases are clearly developed, the sum of investment decisions is likely to play a role in cost-effectiveness and thus, long-term strategy and sustainability of existing manufacturing operations.

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55 **Mesenchymal stem cells in cancer therapy: our chance to take charge**
Sarah Baird, Department of Pharmacology and Toxicology, University of Otago

60 **Ovarian cancer stem cells – an essential target for durable remission**
Graham Kelly, Chief Executive Officer, Novogen & Gil Mor, Department of Obstetrics Gynecology and Reproductive Sciences, Yale University School of Medicine

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Mesenchymal stem cells in cancer therapy: our chance to take charge

A tumour is made up of more than the mutated cancer cells themselves. All other contributing cells, collectively known as the ‘tumour stroma’, are now widely acknowledged to take a driving role both in the early development and metastasis of the tumour. One such tumour stromal cell type, the mesenchymal stem cell (MSC), which homes to the tumour from the bone marrow, is playing an increasingly important role in our understanding of the formation of the tumour, our ability to manipulate it and in the provision of promising therapeutic strategies.

MSCs are historically distinguished using cell culture conditions as the plastic-adherent cells isolated from bone marrow. They resemble fibroblasts in terms of morphology. Their identity is now defined in terms of a set of generally agreed upon cell surface markers: they should lack hematopoietic markers such as CD45, CD34 and CD14 and display CD105, CD73 and CD90. Some authors prefer to use larger groups of markers and additional markers, such as Nestin, are expressed in MSC-like cells under specific conditions.

MSCs are therefore likely to be a heterogeneous population made up of multi-potent non-haematopoietic cells. Their remaining, defining characteristics are the stem cell’s capacity for self-renewal and the ability to differentiate into a diverse range of specialised cell types, classically osteoblasts, chondrocytes and adipocytes, but also myocytes, pericytes and myofibroblasts, depending upon the requirements of the site to which they are recruited.

MSCs have been found at sites of inflammation and tissue injury as...
IN-DEPTH FOCUS: STEM CELLS

well as in tumours. One study found that half of ovarian tumour stromal cells were derived from the bone marrow. Their fate, once absorbed into a tumour, is incompletely determined but includes formation of fibroblasts, pericytes, adipocytes, osteoblasts and myofibroblasts, also known as tumour-associated or activated fibroblasts, which are abundant in the stromal compartment. Studies in mouse models have found that between 20 – 90 per cent of myofibroblasts are derived from MSCs**. An interesting human study of female allogenic bone marrow transplant patients who had received bone marrow from male donors identified myofibroblasts with Y chromosomes in rectal and gastric neoplasia**.

The importance of tumour stroma

Tumours require stromal cells in order to be able to continue to grow beyond 1-2 mm³ and to metastasise. The tumour stroma consists of a mixture of cell types and extracellular matrix proteins, all with cancer-specific functions. Under normal physiological conditions, when cancer is not present, the stroma serves as an important barrier to epithelial cell transformation, maintaining epithelial cell polarity and modulating growth inhibition**. However, in cancer, stromal cells have decisive roles in cancer progression, which include recruitment of immune and endothelial cells, provision of growth factors and promotion of angiogenesis, which together promote growth and metastasis**. Gene expression signatures from within stroma, built from stromal cells harvested from breast cancer samples, have been found to more accurately predict cancer progression and outcome than those derived from whole tissue**. The prognostic power of the stroma emphasises the importance of targeting and interrupting stromal development, and offers a potential therapeutic avenue for intervention.

The role of MSCs in cancer

What are the effects of having MSCs as part of the tumour stroma? There has been a wide range of approaches to this issue. Some studies have used co-culture or other in vitro strategies, while other studies have attempted to tease out the effects of MSCs in vivo, often in mouse models. Many in vivo studies use fluorescent imaging approaches, for example a mouse bone marrow transplant model, in which wild type mice are given a transplant of fluorescent bone marrow cells from, as an example, a green fluorescent protein-expressing mouse. The movement of the fluorescent cells can easily be followed and cells can even be isolated from the tumour for further in vitro study**. MSCs have been shown to contribute to pro-tumorigenic stromal function. They increase initiation and growth of a variety of tumour types, generally through the secretion of anti-apoptotic and pro-proliferative cytokines***, for example via production of CCL5 (RANTES)**. MSCs also enhance invasion, motility and metastasis of human breast carcinoma cell lines and can promote angiogenesis via pericyte formation and secretion of VEGF***. MSC presence in the tumour stroma may also regulate immune system function, although the overall effect is difficult to determine. For example, MSCs inhibit the proliferation of leukocytes in solid tumours** but decrease proliferation of B cells and the activation of helper T cells**.

A number of conflicting results showing MSCs as anti-tumorigenic have also been presented, for example Kaposi sarcoma tumours in mice are growth inhibited in the presence of MSCs** as are murine hepatoma and lymphoma cell lines both in vitro and in vivo**. MSCs have also been shown to produce the anti-migratory molecule TIMP**. Therefore the effect of the presence of MSCs in tumour stroma seems to be context dependent. From the currently available data, it appears that the specific set of consequences of MSCs may depend on tumour type and stage, as well as the genetics of the tumour cells and the particular mixture of stromal cells. An improved approach may be required to bring this picture into a cohesive focus.

Some interesting insights are provided by a study that examines the role of MSCs in a non-solid tumour type. MSCs were shown to increase the adhesion of multiple myeloma cells to the bone marrow, promoting their proliferation and providing protection from chemotherapy**. The work highlighted the differences between MSCs from healthy donors and those from patients, a difference which could also be generated by co-culture of MSCs with cancerous cells*.

Further research in this area, comparing MSCs from healthy donors and those with cancer, should be undertaken.

Assumptions made by most researchers regarding the older definition of MSCs as the plastic adherent cells, which also provides a very convenient means of cell collection, should also be questioned. A related set of experiments showed that isolation by plastic adherence results in a varied population including myeloid-like cells. Separation of the myeloid MSCs from the rest of the population, followed by co-implantation of each population into mice along with lung cancer cells demonstrated that only the population containing the myeloid-like cells promoted tumour growth and metastasis, calling into question the conclusions of some of the studies mentioned above which relied upon this method of MSC collection.

The cells that make up the tumour stroma provide the tumour with a complex but delicate balance of pro- and anti-tumorigenic signals

The cells that make up the tumour stroma provide the tumour with a complex but delicate balance of pro- and anti-tumorigenic signals. MSCs appear to contribute to both of these, although the current evidence comes down strongly on the pro-tumorigenic side. Disrupting stromal function and the myriad of signals which support the tumour may provide a promising approach to cancer treatment, upsetting tumour advancement. The cytokine and signalling molecules involved are many and are often redundant. It may be more feasible to instead unbalance the contribution of a particular cell type, such as the MSCs.

How are MSCs attracted to the tumour site?

During the normal homing process, common to both hematopoietic and mesenchymal stem cells, cells detach from the bone marrow via proteolysis, move passively throughout the body via the bloodstream and are directed to a particular site by chemotraction. It is hypothesised that MSCs would migrate towards a tumour due
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The promise of MSCs in cancer therapy

Use of MSCs in therapy has so far been concentrated on exploiting MSC tropism for tumour sites to deliver gene therapy, drugs or viruses to the tumour in a targeted way.

Intravenous injection of interleukin-2 or chemokine (C-X3-C-motif) ligand 1 expressing MSCs reduced metastasis in mouse models. MSCs engineered to secrete interferon-γ suppressed growth of breast, prostate, pancreatic and melanoma cancers in animal models, as did interleukin-12-expressing MSCs in renal cell cancer. MSCs have also been transduced with interferon-γ, tumour necrosis factor-related apoptosis-inducing ligand (TRAIL), and soluble decoy receptors such as type-I insulin-like growth factor receptor.

There are a number of instances of MSCs used in prodrug strategies. For example, the expression of cytotoxic deaminase converted 5-fluorocytosine to 5-fluorouracil in colon and melanoma cancers. In another study, MSCs expressing rabbit carboxylesterase converted a camptothecin derivative to the active drug SN-38 in glioma. MSCs have also been used as a vehicle for tumour-specific delivery of viruses, including a therapeutically successful conditionally replicating adenovirus in renal cell carcinoma.

Other possible therapeutic approaches using MSCs, such as depletion of MSCs in the tumour and the blocking of MSC homing to tumours, are more speculative. Studies in pancreatic cancer have found that the proportion of MSCs in a tumour increases as time goes on, suggesting blocking the MSC homing process towards a tumour might alter the normal tumour progression. In another study, using...
MANIPULATING THE BEHAVIOUR OF THEstromal compartment is likely to become a more and more important strategy in cancer therapy

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do actually home to tumours and comparing MSCs derived from tumours and from healthy patients would aid greatly in the possibilities of bringing MSC-based cancer therapies into the clinic.

Also to be considered are the potential regenerative effects on the tumour of a large influx of MSCs appearing at the cancer site, as would occur in cell-based cancer therapy. And since the role of MSCs in the bone marrow is immunosuppression, the effect on the immune system at the tumour site of a large influx of MSCs should also be examined.

Maximising the potential of MSCs in cancer therapy

Manipulating the behaviour of the stromal compartment is likely to become a more and more important strategy in cancer therapy. Stromal cells make up a greater volume of the tumour than the mutated cancer cells and do not mutate to develop chemoresistance. Since MSCs originate outside the tumour, altering their behaviour, especially their homing patterns, may be an especially promising focus for disrupting the supportive stromal microenvironment and could be a powerful avenue for controlling tumour development and reducing tumour advancement, including metastasis. This would, however, require an increased understanding of MSC biology, in terms of homing mechanisms and influence on tumour behaviour. The goal would be to harness the specificity and tropism of MSCs for the tumour and direct it in a way that is beneficial for the patient, through cancer therapy, rather than harmful, as in tumour progression.

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At one end of the spectrum are poorly differentiated, long-lived, slow-growing, self-renewal tumour cells comprising the minority of cells within a tumour. At the other end are better differentiated, rapidly-dividing, shorter-lived, non-self-renewing tumour cells comprising the majority of cells within a tumour. In the middle are cells that morphologically and functionally sit somewhere between the previous two groups of cells, and which undergo limited rounds of cell division prior to terminal differentiation. It has become accepted practice to refer to the first group of cells as CSC (or tumour-initiating cells) because of their stem cell-like features; the second group as somatic (or daughter) cancer cells; and the group of cells in the middle as progenitor cancer cells.

The two main ongoing points of debate regarding CSCs are (i) whether they represent aberrant normal stem cells, and (ii) whether they play a pivotal role in tumour initiation, spread and recurrence. Their source remains undetermined. The two leading options appear to be that a CSC is a normal stem cell that, through an accumulation of mutations, plays a primary role in tumour initiation, or that it is a post-initiation event, simply highlighting the plasticity of cancer cells, with mutated progenitor or somatic cancer cells de-differentiating in a process of invoking their embryonic origins.

Their role in the cancer process is becoming better understood, with growing evidence to support the hypothesis that the inherent radio-resistance and chemo-resistance of CSC allows them to survive the therapeutic onslaught and to repopulate the tumour in the form of recurrent disease.

Leaving aside any debate about their origin and role in the tumorigenic process, there is little doubt that many, if not most, tumour phenotypes are heterogeneous tissues comprised of a spectrum of cells of variable phenotype, genotype and behaviour.

A consensus panel convened by the American Association of Cancer Research in 2006 defined a cancer stem cell (CSC) as “a cell within a tumour that possesses the capacity to self-renew and to cause the heterogeneous lineages of cancer cells that comprise the tumour.” Eight years on, the role of the CSC in cancer biology remains only marginally better defined. There is little argument that many, if not most, tumour phenotypes are heterogeneous tissues comprised of a spectrum of cells of variable phenotype, genotype and behaviour.
possessing the potential to form a clone of cells with a competitive growth advantage that eventually forms a tumour.

The CSC model is less random and holds that tumours are organised into an orderly hierarchy with CSC at the apex and that they are the source of tumorigenesis because they are the only cells within the tumour with the capacity of infinite self-renewal and thereby with the capacity to sustain tumour growth.7-9 However, neither model is mutually exclusive, with the growing awareness of the plasticity of cells endowing any tumour cell with the potential to acquire the machinery of stem cells. Whether the origin of a tumour is a random and self-selecting event, or a more organised and hierarchical event, the presence in a tumour of cells with an infinite capacity of self-renewal would seem to point to an important contributory role.

One of the key features of stemness is the ability to self-renew. In nature, self-renewal occurs in two ways – symmetrically or asymmetrically. In symmetric cell division, the stem cell produces two identical daughter cells of equivalent stemness; in asymmetric cell division, one daughter cell retains its parental stem cell identity, with the other daughter cell displaying a more differentiated phenotype.10 The normal homeostatic balance between the two self-renewal mechanisms is believed to be disturbed in cancer to the extent that asymmetric cell division becomes more pronounced, thereby endowing the CSC with the capacity to provide a continuous supply of cancerous daughter cells, as well as the capacity to maintain and to even expand its own population of CSC.11

Like normal stem cells, CSCs are long-lived. Both normal stem cells and CSC (along with somatic cancer cells) maintain their telomeres, although whereas normal stem cells do age and senesce, CSC appear to do so at a much slower rate, if at all.12 Arguably, the outstandingly clinical relevant characteristic of CSC lies in their sensitivity to conventional therapies. Relative to somatic cancer cells, CSC are considerably more resistant to standard chemotherapy and radiotherapy, leading to them being regarded as the putative mediators of chemotherapy resistance and tumour progression.13-14 That resistance is thought to endow CSC with the ability to survive conventional treatments that usually target fast-dividing cells, and to give rise to recurrent tumours that are more chemo-resistant and more aggressive.15,16

This inherent radio- and chemo-insensitivity could be due to a number of factors. One is better developed efflux mechanisms including ATP binding cassette and multi-drug resistance transporter that remove cytotoxic chemicals from the cells.17,18 Another is more active DNA-repair mechanisms.19 A more practical explanation

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perhaps lies in their slow growing characteristic that allows them to avoid many of the effects of conventional chemotherapy.

**Ovarian CSC**

CSC originally were identified in leukemia\(^{20-22}\), and more recently in a range of solid tumours including breast, prostate, pancreatic, cervical, hepatic and colorectal carcinomas\(^{23-33}\).

CSC were first isolated from ovarian cancer by Bapat et al\(^{34}\) as a single clone isolated from malignant ovarian cancer ascites. Since then, CSC have been isolated from ovarian cancer tissue on the basis of CD44, CD133, CD24 and ALDH1 cell surface markers\(^ {35-41}\), although to what extent this diversity of genotype represents a hierarchy of CSC within any ovarian cancer or simply mirrors the histological diversity of ovarian cancer is not clear. Ovarian cancer is classified histologically into serous, endometrioid, clear cell and mucinous, with each type having distinctive clinico-pathological properties, making histologic factors a potential cause of ovarian CSC diversity.

Recent studies by our group have established that epithelial ovarian cancer (EOC) tissue (ascites and solid tumour) contains two predominant cell types with distinct genotype and biological characteristics: CD44+/MyD88+ EOC cells and CD44-/MyD88- EOC cells. CD44+/MyD88+ EOC cells give rise to MyD88-/CD44- ovarian cancer cells (OCC) and have tumour-initiating capacities; we refer to these cells as ovarian cancer stem cells (OCSC)\(^{35}\).

The summary of the characteristics of this cell is:

- OCSC are CD44+/MyD88+, undergo differentiation into OCC that are CD44-/MyD88- cells. OCSC cells are undifferentiated, morphologically larger, and slow-growing; OCC are better differentiated, smaller, and fast-growing\(^\star\).
- OCSC cells have tumour-initiating capacity. When isolated and cloned and injected into athymic mice, they have the capacity both in vitro and in vivo to rebuild the original tumour expressing the full hierarchy of the original tumour (OCSC and OCC)\(^\star\).
- Pure populations of OCSC have the capacity to differentiate in vitro and in vivo into OCC cells\(^\star\).
- OCSC cells can differentiate into endothelial cells and therefore serve as tumour vascular progenitors\(^\star\).
- OCSC cells express markers of pluripotency (CD44, MyD88, b-catenin, Oct-4 and SSEA-4)\(^{43}\).
- OCSC cells have the capacity to undergo epithelial-mesenchymal transition, forming mesenchymal cells with migratory and tumourigenic capacity\(^{44}\).
- OCSC cells generate mesenchymal spheroid-forming cells with metastatic potential\(^{44}\).
- OCSC cells are chemoresistant and their levels in tumour samples inversely correlate with shorter progression-free survival\(^\star\).
- OCC have lost the capacity to self-renew, are more sensitive to chemotherapy, and are terminally differentiated\(^\star\).
- OCC cells lack the capacity to be tumorigenic in mice.

**Primary and recurrent ovarian cancer: different diseases**

EOC remains the leading cause of gynaecologic cancer deaths\(^{47,48}\).
Approximately 20 per cent of newly diagnosed cases are classified as Stage I (cancer confined to the ovaries) and these patients have a 10 year survival rate of about 73 per cent. The remaining 80 per cent of patients are diagnosed with Stages II–IV disease and in these patients the 10 year survival rate drops to 5–20 per cent and mortality is almost always associated with recurrent disease.

Diagnosis of EOC normally requires surgery for confirmation of diagnosis, disease staging and debulking. That normally is followed by chemotherapy consisting of a taxane (Paclitaxel or docetaxol) and/or carboplatin. Approximately 25 per cent of cases exhibit either no clinical response to first-line therapy or show an initial response but recur within six months. Such cases are regarded as chemoresistant.

Although 75 – 80 per cent of cases respond to first line chemotherapy, unfortunately, 60 – 80 per cent of these patients will reoccur within six months to five years. Recurrent disease is characterised by chemoresistance and carcinomatosis; the main causes for the high mortality associated with ovarian cancer. Our studies suggest that the cells responsible for recurrence and resistance to therapy are the cancer stem cells. Furthermore, recent data obtained with samples from patients with recurrent disease or from our animal model indicate that the surviving cancer stem cells present further modifications associated with mesenchymal characteristics that allow them to spread through the peritoneal cavity and enhance their resistance to chemotherapy. Consequently, in order to prevent recurrence and improve survival there is a need to develop new therapies that would target the ovarian cancer stem cells.

**Future strategies**

In a recent study that is strongly suggestive of the clinical situation,
OCSC cells were injected into teratocarcinoma and into athymic nude mice and allowed to establish an intra-abdominal carcinomatosis containing both OCSC and OEC. Paclitaxel treatment of these mice resulted in the elimination of the OEC but enrichment of the OCSC that then went on to demonstrate a more aggressive and more migratory phenotype compared to untreated OCSC cells.

Current management of EOC fails to take into account the change in the molecular and cellular natures of primary disease and recurrent disease. Use of chemotherapy, at best, buys a degree of time for most patients, but comes at the cost of selecting for survival a sub-population of EOC that are more chemoresistant, more aggressive, and more likely to spread than the original tumour population.

Our studies suggest that more durable remission will require eradication of the full hierarchy of cells within a tumour, but particularly the OCSC that have the capacity to withstand current chemical onslacht and to respond to that onslaught with even greater aggression.

A number of experimental drugs (monoclonal antibodies, small molecule inhibitors) are under development that seek to target the CSC preferentially, principally through interaction with cell markers (CD44) and signaling pathways (focal adhesion kinases) that are believed to be over-expressed in CSC. It remains to be seen whether this highly-specific targeting approach proves any more beneficial against CSC than it has against somatic cancer cells in tumours with broad mutagenic damage, where any anti-cancer effect has been modest at best.

Our programme has focused on the screening and identification of drugs with potential efficacy on OCSC. We have cloned and established OCSC cell lines, which maintain the characteristics described above. Using these cells, lines we have identified a novel compound, Trx-1, which is effective on inducing cell death on ovarian cancer stem cells. The identification of this compound is encouraging for two reasons: 1) demonstrate that it is possible to target specifically the OCSCs, and 2) provides further evidences for the heterogeneity of ovarian cancer and the need to design combinational therapies that target the different cell types present within the tumour. Through the implementation of this approach we might reach the objective of improving the survival of ovarian cancer patients.

Dr. Graham Kelly has a BVSc (Hons) degree from The University of Sydney. He then obtained a PhD from the Faculty of Medicine of The University of Sydney. He spent 25 years studying the biology of cancer development in organ transplant recipients before founding the public biotechnology company, Novogen Ltd. He currently is CEO of Novogen Ltd and of CanTx Inc, a joint venture company between Novogen and Yale University focused on the development of new therapies for ovarian cancer.

Gill Mor is a Professor of Obstetrics and Gynecology and Reproductive Science at Yale University School of Medicine. In his research, he examines topics related to the immunology of pregnancy and the role of inflammation in cancer formation and progression. Dr. Mor’s laboratory isolated and cloned the ovarian cancer stem cells, a source of recurrence and chemo-resistance. He is the Division Director of the Reproductive Science Division at the Department of Obstetrics and Gynecology. He directs the Reproductive Immunology Unit and the Translational Research Program ‘Discovery To Cure’ at Yale University. Dr. Mor has been the Editor in Chief of the American Journal of Reproductive Immunology since September 2009. Dr. Mor is funded by grants from National Institute on Child Health and Human Development and National Cancer Institute as well as several pharmaceutical companies and is widely published in the area of immunology and reproduction with more than 230 publications and is the editor of a two books on ‘Immunology of pregnancy’ and ‘Apoptosis and Cancer’. Dr. Mor is the recipient of several national and international prizes, including the J. Christian Herr Award- and the ARI Award from the Society for Reproductive Immunology.

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The meeting will open with the Presidential Symposium on 18 June from 13:00 – 15:20 local time. The symposium is the stage for the formal recognition of the 2014 recipients of the McEwen Award for Innovation and the ISSCR Public Service Award. Another prestigious award, the ISSCR-BD Biosciences Outstanding Young Investigator Award, will be presented during Plenary V and will be followed by an award lecture.

**Presidential Symposium speakers will include:**
- Brigid Hogan, Duke University Medical Center, US
- Gordon Keller, McEwen Centre for Regenerative Medicine Ontario Cancer Institute, Canada
- Olivier Pourquie, Strasbourg University Medical School, France
- Lorenz Studer, Sloan–Kettering Institute for Cancer Research, US

**The McEwen Award for Innovation (Presidential Symposium)**

- Azim Surani, Wellcome Trust/Cancer Research UK Gurdon Institute, UK

**The ISSCR Public Service Award (Presidential Symposium)**

- Paolo Bianco, Sapienza University of Rome, Italy
- Elena Cattaneo, University of Milan, Italy
- Michele De Luca, University of Modena and Reggio Emilia, Italy

**The ISSCR-BD Biosciences Outstanding Young Investigator Award (Plenary V)**

- Valentina Greco, Yale University, Department of Genetics, Yale Stem Cell Center, US

The 2014 Annual Meeting Program Committee is chaired by Fiona Watt from the King’s College London Centre for Stem Cells and Regenerative Medicine. Watt and the committee have worked together over the last year to assemble a diverse program and an international contingent of stem cell researchers, clinicians and industry professionals to share the newest research, technologies and clinical advancements. Speakers will continue to be selected from submitted abstracts to add further depth to the planned programme.

“Each year, the ISSCR endeavors to bring together established, emerging and future leaders in stem cell research to explore a broad range of important topics in our field,” Watt said. “This year is no exception, and we look forward to hearing from the recipient of the ISSCR-BD Biosciences Outstanding Young Investigator Award, Valentina Greco, who has made significant early-career contributions to stem cell biology and regenerative medicine.”

This year’s ‘Therapies in the Clinic’ plenary session will feature cutting edge gene and cell therapy approaches to blood and neurodegenerative disorders. Speakers include James Shapiro from the University of Alberta, who led the clinical team that developed the Edmonton Protocol, the first trial to achieve consistent 100 per cent insulin independence in a series of islet-alone transplant recipients with Type 1 diabetes.

In the ‘Road to the Clinic: Challenges Ahead’ concurrent session, invited speaker Christopher Breuer, of Nationwide Children’s Hospital, will present his experience with driving a treatment into a clinical trial and explore the challenges he sees ahead. He developed a tissue-engineered vascular graft for pediatric congenital heart surgery and will take his findings back to the laboratory to better understand what is observed clinically.

The ‘Sensory Systems Repair’ concurrent session will feature a presentation from Albert Edge of Harvard Medical School, senior author of a recent Cell Press paper on the regeneration of sound-sensing cells in the ears of mice with hearing damage.

The 2014 Annual Meeting will feature over 1,600 posters from meeting participants all over the world. Selected posters, highlighting exceptional abstracts, will be briefly presented as part of each concurrent session. These and other posters offer a first look at new work from both emerging and established professionals, which may be further explored during evening receptions on Wednesday, Thursday and Friday.

“The poster sessions are where a lot of the great scientific exchange between meeting attendees takes place,” Watt said. “Networking is one of the most valuable components of the ISSCR Annual Meeting, and attendees frequently cite the poster sessions as the genesis of great ideas and collaborations.”

ISSCR Annual Meeting registration dates and costs are as follows:

- Advance registration deadline: June 12 (USD815 member / USD1,115 nonmember)
- Regular registration deadline: June 21 (USD940 member / USD1,240 nonmember)

Additional registration information and instructions are available on the meeting website along with the meeting schedule at www.isscr.org/2014.
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Excipients in medicines for children: scientific and regulatory paradigms

There is an ongoing debate over the use of pharmaceutical excipients in medicines for children, triggered by the increased number of formulations suitable for this target patient population. Pharmaceutical excipients can be regarded as essential / necessary enablers in formulation development. These are materials other than the ‘active pharmaceutical ingredient’ which are added to the formulation to achieve a specific function. This may include aiding in the processing or manufacture of the drug delivery system such as lubricants or flow aids, controlling the release of the active ingredient to achieve modified release, enhance patient acceptability by improving taste of medicines or to develop easily swallowed dosage forms.

Bearing in mind the current regulatory framework for excipient development and approval, it is becoming increasingly challenging to introduce new excipients to the market. This comes in an era when there is a real need for research on excipients that facilitate the formulation development of novel drug delivery systems, or formulations with a focus on personalised treatments for specific patient populations.

Consequently, a combination of technical, safety and regulatory challenges for the use of excipients in paediatric preparations should be investigated and addressed.

Technical considerations for excipient development in paediatric formulations

Excipients are subdivided into multiple functional classes dependant on their composition and the role they undertake in the final dosage form. The new excipients developed for paediatric formulations sometimes belong to more than one functional group (multifunctional) and hence, pose additional burden on the manufacturer to demonstrate their precise role during regulatory submissions.

There has been a considerable amount of mixed or co-processed excipients (not new chemical entities) introduced to the market for the
development of fast disintegrating or dissolving dosage forms for children. Some examples of these are Ludiflash, Pharmaburst and F-Melt, which are usually a mixture of two or more excipients with contrasting functionalities to achieve desirable ready-to-use excipient blends. These products could simplify the formulation development process and reduce the associated technical challenges as the mixtures contain optimised amounts of diluent, disintegrant and binder. This in turn can provide the desirable target product profile of the orally disintegrating tablet (ODT) or the child-friendly dosage form.

Essentially, most of the ready-to-use excipients contain at least one polyol (sugar alcohol) such as mannitol or sorbitol alongside excipients which enhance swelling and disintegration of the tablet in few seconds. Other additional materials may include flavours or colourants to enhance the aesthetic properties of the dosage form.

Accordingly, some of these excipients require further characterisation of their composition to fully understand the functionality and physico-chemical/mechanical properties to support quality by design (QbD) to formulation development. It is also critical that analytical testing of co-processed excipients is performed to confirm no chemical change during processing.

Research groups have already started compiling information on excipient functionality in an electronic database. In our laboratory, functionality of excipients used in solid dosage forms for children were studied using novel methodologies that enhance our understanding of the composition of excipients on the nano and micro-scale. In addition, excipient-excipient and excipient-drug interactions were studied on the nano-scale to unveil scientific information on materials behaviour in the solid state to reduce the risk of interactions during downstream operations. Mannitol and MCC were some of the excipients investigated to elucidate the pros and cons of their use in children solid formulations.

In liquid oral formulation, the use of excipients including propylene glycol, benzyl alcohol, surfactants and ethanol is undesirable for children of certain ages due to inherent toxicities and incomplete maturation of metabolic function especially in neonates. Nevertheless, the substitute for these excipients is still largely missing and manufacturers are experiencing difficulties with finding appropriate paediatric excipients.

Despite that, research into novel excipients derived from natural chemical entities continued to overcome some of the solubility and palatability issues of medicines for children. There is a growing trend for the use of cyclodextrins as excipients for the delivery of poorly soluble drugs and for taste masking of paediatric oral liquid formulations. Cyclodextrins are ‘cup shaped’ molecules (Figure 1) composed of cyclic oligosaccharides that were discovered in bacterial digest isolated from starch in 1891.

The cup shaped molecule inner cavity is hydrophobic while the outer surface is hydrophilic, hence poorly soluble or unpalatable drugs can be included inside the cup to prevent immiscibility or contact with the outside aqueous environment. In one study, midazolam, a preoperative anaesthetic commonly given to children, was successfully incorporated into γ-CD in order to mask its bitter acidic taste.

Parenteral products also contain excipients such as solubilisers, buffering agents, stabilisers and preservatives. These excipients present technical challenges such as the need for sterility besides the ability to withstand terminal sterilisation of aseptic processing. This in turn limits the choice of available excipients.

While the technical challenges in excipient research and development for paediatric formulations continue to appear especially with the future development of more complex entities from biotechnological sources, nevertheless, important questions have to be raised questioning the safety of these novel and multifunctional excipients.

In liquid oral formulation, the use of excipients including propylene glycol, benzyl alcohol, surfactants and ethanol is undesirable for children of certain ages due to inherent toxicities and incomplete maturation of metabolic function especially in neonates. Nevertheless, the substitute for these excipients is still largely missing and manufacturers are experiencing difficulties with finding appropriate paediatric excipients.

**Figure 1:** An image showing Cyclodextrin ‘cup shaped’ structure and a drug molecule incorporated in the inner cavity of the cup.
– is there enough data to justify their use and will there be methods to predict their toxicological profile in paediatrics?

**Perspectives on excipient use in children and associated regulations**

A major hurdle in paediatric formulation development is the lack of safety and toxicity data on some of the commonly used excipients. The first documented major case of excipient toxicity was in children. In 1937, 107 people died, which included a large group of children due to the untested use of diethylene glycol (DEG) in an elixir of Sulfanilamide (Figure 2). Consequently in 1938, the Food, Drug and Cosmetic Act (FD&C Act) was passed in the United States to place safety burden on the drug manufacturers. The previous example of cyclodextrins holds a potential for use in paediatric formulations because of their low toxicity profile. They tend to exhibit good oral safety profiles as the intestinal absorption is less than 0.5 per cent. This may result from the hydrophilic outer surface of the oligosaccharide limiting the absorption of intact Cyclodextrins. The use in children formulations was also demonstrated by a study of Cyclodextrin Itraconazole in 26 HIV-infected children (age 5 – 18 years old) with oropharyngeal candidiasis (OPC). The study showed that the combination CD-ITRA was well tolerated and efficacious for the treatment of OPC in children ≥ 5 years old. On the other hand, the use of Beta-Cyclodextrin in parenteral formulations is inappropriate as it leads to necrosis of the proximal kidney tubules when administered via intravenous or subcutaneous routes. This signifies the dependence of excipient toxicity on the particular route of administration which is of prime importance.

Unlike the above example, many excipients have no available safety data to support their use in children and usually an assessment of the risk / benefit obtained from using a specific excipient is applied to justify their use during regulatory approval.

While the maximum oral safe dose of mannitol is known in adults (20 grams / day) which if exceeded will have a laxative effect, the dose in children is not established yet due to the lack of evidence-based data. However, it is a fact that mannitol is poorly absorbed by the GI tract which enhances its safety when taken orally.

In addition, medicines containing mannitol or other polyols such as maltitol, sorbitol or xylitol are regarded as ‘Sugar Free’ due to their extremely low caloric content. Hence, according to the British National Formulary for children (BNFC), it is advisable to use ‘Sugar Free’ preparations whenever possible to reduce the development of dental carries especially during chronic treatment.

Other diluents such as lactose were reported to cause issues in children with severe lactose intolerance including diarrhoea, dehydration and metabolic acidosis. Moreover, BNF for children states that “The lactose content in most medicines is too small to cause problems in most lactose-intolerant children. However in severe lactose intolerance, the lactose content should be determined before prescribing.”

Some enteric coating polymers used to protect acid-labile medicines were reported (methacrylic acid copolymer Eudragit L30 D-55) to cause fibrosing colonopathy in children treated with high strength pancreatic enzyme formulation. Despite that, the conclusions...
were not firmly established and various researchers posted conflicting reports to support or oppose the association between the coating polymer and the fibrosis.

Likewise, the use of parabens (propyl and methyl) as preservatives in formulations for children has been debated; the negative impact on sperm counts discussed by Oishi and conducted in a juvenile rat model could not be replicated in a recent toxicological study in juvenile rats of the same age. However, according to the European Medicines Agency (EMA), the use of preservatives should be avoided particularly in the case of paediatric formulations and where appropriate the concentration should be at the lowest feasible level.

The EMA reflection paper on paediatric formulations states that “Products containing high levels of propylene glycol should not be administered to paediatric patients below the age of four years” which indicates that the excipient still retains a feasible use in paediatric patients of school age and adolescents. In neonates, it was reported that propylene glycol half-life is more than three times that in adults; hence it is a toxicity concern in this age group.

The conflicting nature of the reports mentioned above possibly reflects the variations in the procedures used to assess the toxicology and safety profiles of excipients in children. Furthermore, it indicates the lack of solid evidence to support decisions for use / avoidance of excipients in children formulations.

Our perspective
It is suggested that systematic reviews for adverse reactions caused by excipients of concern to be conducted, in a similar way to what has been done to APIs with conflicting reports of adverse events, to reach to a conclusive evidence-based use in children. It is also important to emphasise that toxicity issues are mostly prominent in neonates due to incomplete maturation of metabolic functions / physiological barriers.

The innovation in excipients research has been struggling as a result of the gap in toxicity / safety data of materials in the paediatric population and because of the consequent regulatory obstacles. Regardless, some excipient manufacturers have shown willingness to fund toxicology studies for novel excipients to facilitate future drug development.

Given that new excipients use is only allowed as part of a new approved drug product, it is imperative that risk assessment is carried out on new excipients following the right regulatory procedure that ensures patient safety. Accordingly, efforts have been made both in America and Europe to establish an excipient safety database (STEP database) to facilitate excipients use in paediatric medicines.

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Ali Al-khattawi is a postdoctoral research associate at Aston University in the UK. He carried out his PhD in drug delivery at Aston University and MSc in pharmaceutical sciences with distinction at Kingston University. Ali’s research interests include the development of age-appropriate formulations for children and elderly patients and research on excipient/material functionality assessment.

Azfar R. Mohammed is a Senior Lecturer in Pharmaceutics at Aston University. His research is focused on studying various dimensions associated with paediatric formulation development and delivery. His research encompasses material processing and characterisation to formulate orally disintegrating tablets, taste masking and taste assessment.

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Detection, quantification and visualisation of in situ protein interactions for hit profiling by high content screening

Speakers:

Dr. Bernhard Ellinger
Principal Scientist, European ScreeningPort

Dr. Thomas Juehne
Principal Scientist, Sigma-Aldrich

The webinar "Detection, quantification and visualisation of protein interactions by HCS" given by Dr. Bernhard Ellinger (Principal Scientist, European ScreeningPort GmbH) and Thomas Juehne (Principal Scientist, Sigma-Aldrich), on 8th April 2014, discussed how Duolink® (based on the in situ PLA technology) can be used to monitor individual proteins, their interactions and post-translational modifications, providing single molecule resolution and objective and unbiased quantification in cells and tissues on endogenous expression levels.

Bernhard Ellinger presented a case study where Duolink® was used to further profile compounds that had been identified as actives from an High Throughput Screen by automating the approach into a 384-well format and implementing automated image analysis including spot detection, localisation and quantification. The target mechanism involved the disruption of the interaction between amyloid β oligomers and RAGE (Receptor for Advanced Glycation Endproducts). Special emphasis was given to the critical steps in the assay development process.

Thomas Juehne followed with a presentation on the Duolink® technology and applications. He presented research clearly showing advances over conventional fluorescence or bulk measurements. These examples demonstrated the advantage of single cell and localisation specific analysis.

The webinar was followed by a lively Q&A. People were especially interested in the fluorophores and antibodies that are compatible with Duolink®. The applicability of the technology to other fields, such as in vivo or homodimer formation, was also covered.

Here is a selection of questions asked by our audience:

- What is the minimum and maximum number of cells / tissue needed?
- Can I combine a directly conjugated primary antibody and a PLA probe?
- Is there a maximum distance that has to be respected in order to have the interaction of the probe plus and minus?

To discover the answer to these questions, watch the on-demand version of this webinar.

www.europeanpharmaceuticalreview.com/hts-webinar
In recent years, pharmaceutical manufacturers have faced many challenges that will impact the overall drug development and production workflow in years to come. As costs and regulations increase, the focus on lean manufacturing to produce products that meet stringent guidelines in a timely manner is critical to the success of a drug company. Without quality management systems (QMS) to ensure compliant products are produced using the most cost-effective method, drug production growth will not be sustainable.

A core part of the manufacturing process includes the analysis of raw materials, so it’s vital for quality managers to obtain the most efficient QMS. Bree Allen, Vice President and General Manager at Rigaku Raman Technologies, elaborates: “The traditional QA/QC process involves sending an incoming shipment of material to quarantine while a sample is sent to an offsite laboratory for identification analysis before it can be released to manufacturing. This can be costly and time-consuming. By implementing an identification method at the point of receipt that involves a tool that can be used by any warehouse employee, companies will benefit from a process that is much more efficient to get to the same release stage.”

With the Progeny™, Rigaku Raman Technologies has revolutionised raw material identification (RMID) by taking an accepted lab-based technology – Raman spectroscopy – and developing a customisable, handheld Raman analyser. “Progeny™ is designed for seamless integration into an existing workflow,” says Allen. “It delivers what truly matters to a customer – error-free operation, repeatability, ease of use, and the widest range of sampling capabilities – and it’s all achievable in handheld form.”

Implementing a new method involves changing standard operating procedures (SOPs), which can be a lengthy process for a company, but Progeny™ combats these challenges, as Allen explains: “Until now, the software available on handheld devices has not allowed great flexibility in the customisation of SOPs and analyses of report formats. This implies that companies have been adapting their workflow to the instruments’ limited flexibility. Progeny™’s software offers complete customisation, while keeping it simple.” Progeny™ offers the ability to mimic existing SPOs by following a few logical steps similar to a smartphone set-up procedure. Companies can also take advantage of Progeny™’s powerful report generator and write entirely new SOPs. The integrated barcode reader with camera improves data tracking. In addition, Progeny™’s digital signature capability completes its 21 CFR Part 11 compliance requirement.

As the system is portable and handheld, you may ask how results are shared. “Progeny™ has been engineered to serve multiple markets, and since each has very different needs, the instrument incorporates multiple ways to achieve this task,” explains Allen. “Where allowed, Wi-Fi and Bluetooth would represent the most modern and convenient way to send data to a server for remote archival purposes. However, in regulated environments where data security is mandatory, Progeny™ ensures typical wired connectivity and compatibility with LIMS systems. It’s the customer’s choice, but Progeny™ is ready for any implementation.”

There are indirect advantages to implementing this technology for RMID at the point of receipt, as Allen reveals: “We live in a ‘do more with less’ environment where productivity must be optimised with fewer resources without compromising quality. Handheld RMID improves the percentage of material testing while lowering the cost of laboratory analysis for such a task. Once RMID is deployed, laboratory resources can be shifted to focus on higher value tasks, providing an additional layer of safety, productivity, and quality.

Implementing change only makes sense when the benefits exceed cost and there could be multiple reasons why pharmaceutical companies have not yet adopted a handheld analysis technique. “Until now, handheld Raman has had the ability to identify a wide range of materials, with the exception of problematic compounds such as coloured substances and others that naturally fluoresce. Progeny™ expands material analysis capabilities and simplifies workflow integration, which may result in a highly profitable change,” Allen concludes.
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