

Drug

TARGET REVIEW



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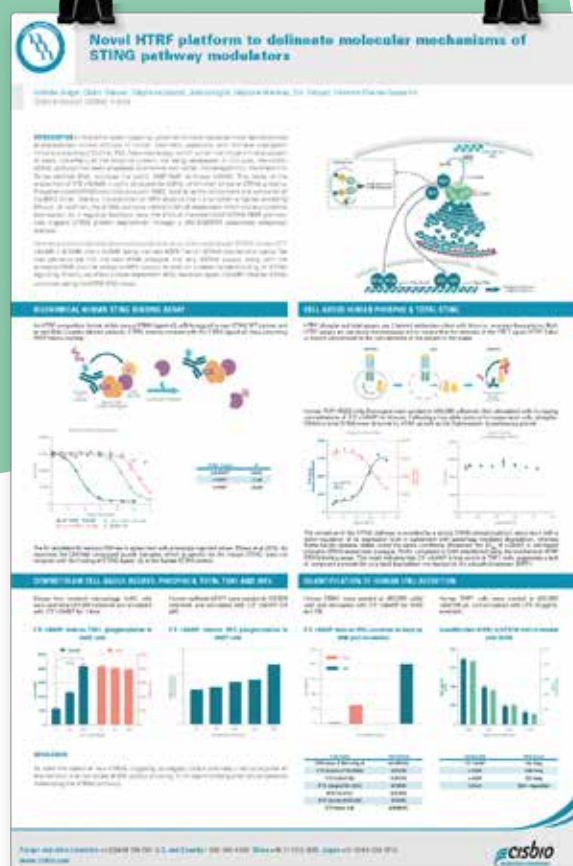
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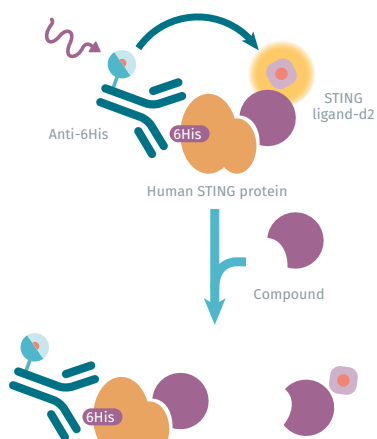
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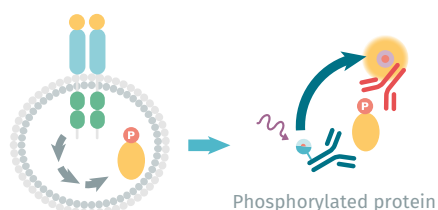
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Published June 2019

A promising future

Monoclonal antibodies: firmly in the driving seat of drug discovery and development.



NIKKI WITHERS
EDITOR
nwithers@russellpublishing.com

THE GLOBAL monoclonal antibody (mAb) market has seen significant expansion over recent years, with seven of the 'top 10 blockbuster drugs' classified as biologics in 2018 and most based on mAbs. As of April 2019, there are 182 mAb drugs undergoing Phase III clinical trials worldwide, with the expectation that many mAbs will be approved and rolled out into the market in the forthcoming years. It is therefore no surprise that mAbs feature heavily in this issue – from novel approaches to produce radiolabelled antibodies to their use in cancer therapeutics.

Indeed, scientists from independent medical research charity LifeArc are currently using imaging technologies to identify the most promising candidates for antibody-based cancer therapies. Their article on page 43 discusses

how routine use of imaging technologies in primary screening campaigns will lead to better characterised biotherapeutics being selected as lead candidates for cancer therapy.

Also focusing on cancer, Elizabeth Turner Gillies and Fang Tian from the American Type Culture Collection (ATCC) discuss how they are using CRISPR/Cas9-engineered cancer model systems to accelerate the development of targeted therapies. "By using CRISPR-based genome engineering to make precise changes to the genome of a target cell line, we can now alter those lines in a way that closely mimics the spontaneous development of cancer in a healthy tissue," they say. Turn to page 15 to read more.

Drug companies are increasingly adopting stem cells for research; specifically, for identification of potential new therapies. Interestingly, we are starting to see new applications and the amalgamation of stem-cell technology with an immunotherapy approach is one such exciting development in cancer therapy. In our stem cell In-Depth Focus, Aparajita Dubey discusses how this combination of immunotherapy and stem-cell technology has the potential to revolutionise cancer treatment regimes, while Trisha Rao and Thomas Drucan expand on how they are utilising stem cells to study the effects of cannabis on neuronal development.

This issue also features a round-up of the latest industry news, a screening In-Depth Focus and an article detailing ways that native analysis is advancing the field of mass spectrometry.

I hope that you enjoy the issue. We are always looking for interesting contributions, so please get in touch if you have research to share. ☺

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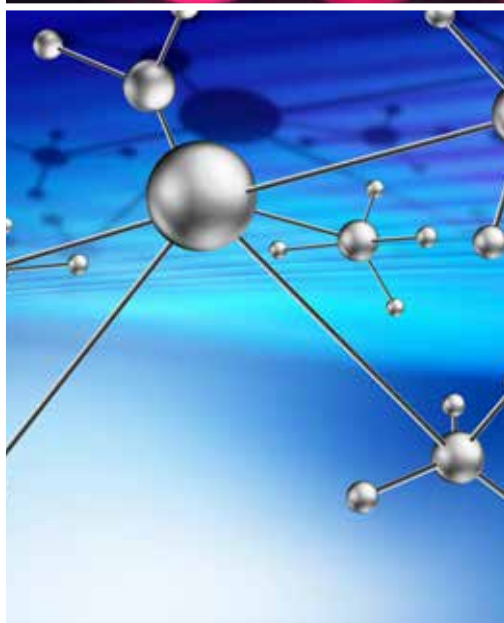
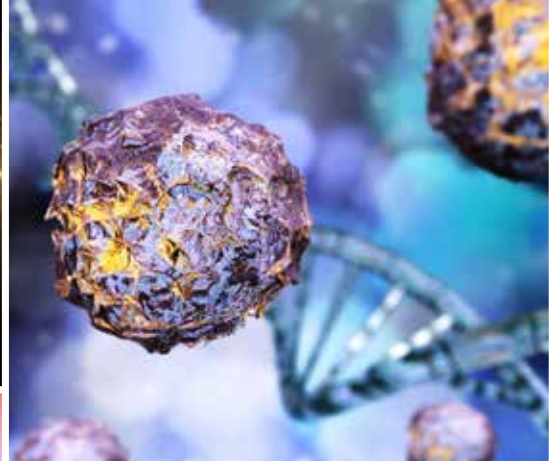
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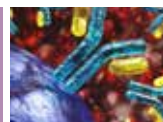
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Drug Target Review is looking for scientists, researchers and pharmaceutical professionals to contribute articles on a number of fascinating drug discovery topics in 2019, including assays, antibodies, genomics, NGS and upstream bioprocessing. If either of these or any other areas of the industry appeal to you, please get in touch with the Editor.

Contact Nikki Withers, Editor, via email at nwithers@russellpublishing.com or visit drugtargetreview.com



9-12 September 2019

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Big data in molecular medicine: from hype to mainstream

AS WE HURTLE towards the “\$100 genome” on the back of rapid advances in DNA sequencing technology, the world’s genomic databases are expanding exponentially with primary sequence data from increasing numbers of people every day. In parallel, we are accumulating a wealth of complementary data from diverse sources including population-wide genomic studies, digital health records and clinical trials.

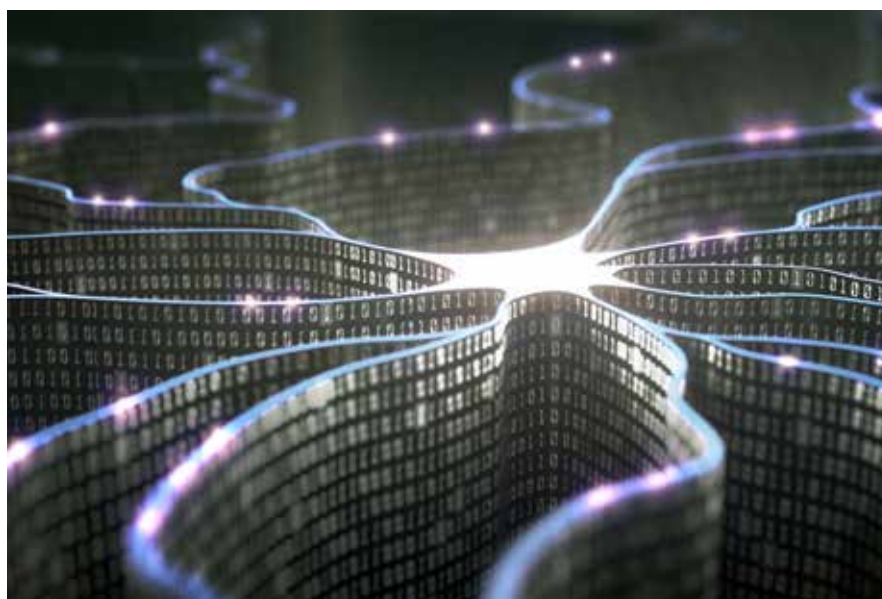
Buried in these terabytes – soon to be petabytes – of data are untold insights into the biological mechanisms of human health, ageing and disease. The big question is, how can we realistically compile, integrate and mine this vast goldmine of ‘big data’ in order to build more accurate individual health profiles and create more predictive disease models?

Questions such as these will be a key focus at one of Europe’s premier bioinformatics conferences, [BC]², which will take place at BASEL LIFE 2019 from 9-12 September 2019. This year [BC]² is also being held in conjunction with EMBO at BASEL LIFE, the BASEL LIFE Networking Village 2019 including MipTec, the Innovation Forum, and the BASEL LIFE Job Fair.

This unique constellation of events addresses the raw interface where life science research meets bioinformatics, bringing together top academic and commercial researchers to discuss the latest findings in such diverse but interdependent topics as:

- Ageing and disease
- Biotherapeutics and drug discovery
- Computational biology and bioinformatics.

So what is the current role of big data in molecular medicine? How can we find effective ways to put all this good data to work to improve human health? It is obvious that there are no simplistic



solutions to solve global healthcare problems. However, with interdisciplinary global cooperation, we can improve our infrastructure for compiling, annotating, integrating and managing data in a way that enables researchers and clinicians to tap into the information they need to better model disease, inform drug discovery workflows and improve clinical decisions. Once the basic architecture is in place, we will be well placed, particularly in oncology, to take advantage of ongoing advances in computational biology and AI.

There are many examples where the vision of data-driven precision medicine is already becoming a reality, but by all accounts, we have barely begun to scratch the surface of what is possible. How can we encourage the cross-disciplinary connections and education that is necessary to leverage big data and accelerate progress?

BASEL LIFE addresses this need through a series of workshops and

plenary sessions as part of [BC]².

Practical sessions include a workshop in deep learning, which will demonstrate how to build and extract knowledge using interpretable approaches in the functional analysis of raw DNA sequencing data and in drug sensitivity prediction models. Other workshops explore how to collect, manage, cluster and analyse data into domain-specific databases. Ultimately, if the clinician can intervene sooner in the course of a patient’s declining health, it will significantly reduce global healthcare spend.

BASEL LIFE will demonstrate with decisive clarity that big data not only has a role in molecular medicine, but has become mainstream – and neither the academic nor the commercial researcher can afford to ignore it. ■

Check out the full scientific programme and register at: www.baselife.org/2019



For more information, visit: www.baselife.org/2019

Personal genomics: the fast track to "precision medicine"

Pushpanathan Muthuirulan

Harvard University, Cambridge, US



The way that we treat diseases has changed considerably since the completion of the human genome project. Pushpanathan Muthuirulan discusses how personal genomics is gaining traction in treating diseases.

SOON AFTER its conception, the human genome project (HGP) was viewed with skepticism by both scientists and non-scientists. They raised the question of whether spending a large quantity of money on sequencing the human genome would outweigh its potential benefits. Today, however, the project has overwhelming successes, delivering a completed version of high-quality human genome sequencing. The completion of HGP in 2003 is a major scientific breakthrough and has led to significant advances in genomic technologies used to decipher DNA sequences today. HGP has inspired many scientists and clinicians around the world to utilise high-throughput genomic technologies to understand our shared molecular heritage, ancestry tracing, forensics and to examine the constellation of genomic changes in an individual to guide therapeutic decisions and offer personalisation in healthcare.

Precision medicine is an emerging approach that considers the genetic and environmental basis of disease to personalise prevention, diagnosis and treatment. This is in contrast to a one-size-fits-all approach, in which disease treatments and management strategies are designed for the average person, with little consideration for the differences existing between individuals. With aggressive technological advancements in the field of genomics (eg, next-generation sequencing) and intense competition in the area of targeted molecular therapy, precision medicine renders itself a sophisticated healthcare delivery model while simultaneously providing new vision to the future of medicine. However, this model is still in its infancy and needs to overcome many barriers and challenges.

Personal genomics is an emerging area of scientific activity that plays a pivotal role in the evolving model of precision medicine. This approach strengthens the fight against diseases by unlocking the actionable information

from the genome, helping to accelerate the development of novel therapeutics. The personal genomics approach is the result of increased knowledge of the human genome and the ways that a patient's genotype can be correlated with disease susceptibility, tailoring treatments and prevention strategies based on each patient's unique genetic makeup. Personal genomics considers the genomic aspects of medicine including mutations, single nucleotide polymorphisms, variant segregation, gene-environment interaction and so on. Oncogenomics and pharmacogenomics make use of the personal genomics approach. Oncogenomics aims to unravel specific cancer-related genes in a patient, which could be used to create a personalised diagnosis and treatment plan. Pharmacogenomics aims to understand how genes affect a patient's response to drugs and indicates which drug is most appropriate for patients.

During recent years, biotech companies and genetic testing laboratories have begun developing technologies for large-scale genetic screening of patients to evaluate genomic errors associated with genetic disorders. For instance, Baylor College of Medicine offers a clinical non-invasive prenatal multigene sequencing screen that provides a more complete picture of the risk of genetic disorders. It works by sequencing cell-free foetal DNA for disease-causing pathogenic variants associated with single-gene disorders affecting skeletal, cardiac and neurological systems. Successful implementation of personal genomics into clinical healthcare will require clear standards and careful consideration of accompanying ethical pitfalls, such as social and clinical implications, but the potential clinical applications of genomic information are indispensable for understanding disease pathogenesis. Thus, the precision medicine revolution is finally happening, and it will change the way we think about treating diseases. 📷

“ *The personal genomics approach is the result of increased knowledge of the human genome and the ways that a patient's genotype can be correlated with disease susceptibility* **”**

BIOGRAPHY

PUSHPANATHAN MUTHUIRULAN is currently a Research Associate at Harvard University studying the developmental and genetic basis of human height variations using functional genomics approaches. Previously, he worked as a Postdoctoral Researcher at the National Institutes of Health, where his research focused on developing state-of-the-art technologies using CRISPR-Cas9 and super-resolution microscopy to map neural circuits that involves visual motion information processing in *Drosophila*. His expertise lies in omics technologies, drug discovery, neuroscience and developmental and evolutionary genetics.

ROUND UP

The editor's pick of the most interesting developments within the drug discovery industry

Colon cancer proteins and gene analysis uncovers potential new treatments

CANCER

THE STUDY, led by researchers at Baylor College of Medicine and published in the journal *Cell*, supports the comprehensive characterisation of tumour tissues as a means to guide further research leading to early diagnostic strategies and new treatments.

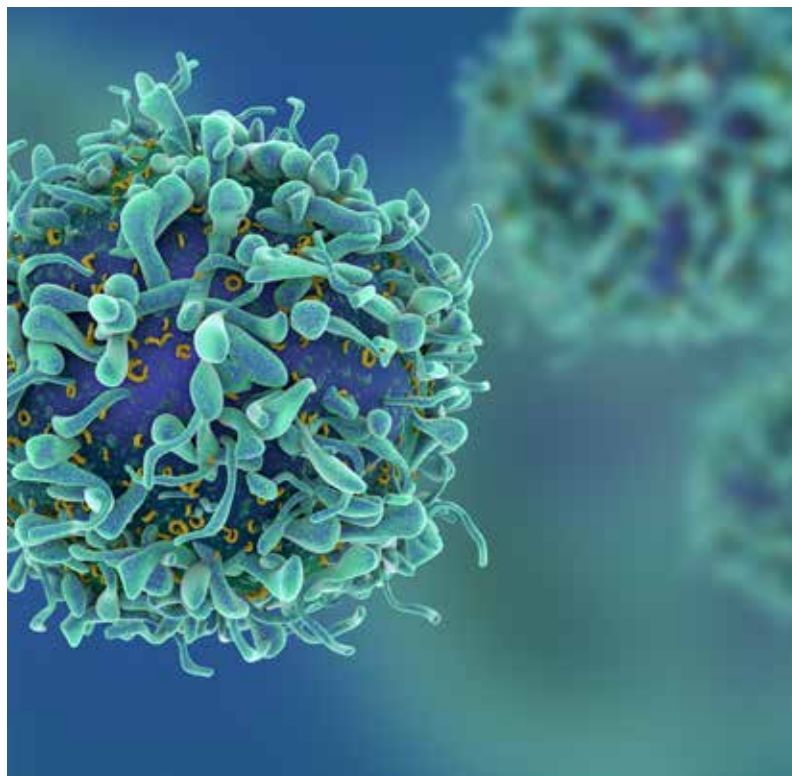
The research team, as part of the National Cancer Institute Clinical Proteomic Tumour Analysis Consortium, generated the genomic and proteomic data and applied bioinformatics analyses to the data. The result is the first systematic catalogue of the different proteins produced by colon cancer tumours and adjacent normal tissues.

"We were able to not only confirm previously described colon cancer molecular markers but also to uncover new differences between proteins produced by tumours and normal tissue that may be worth further study," said corresponding author of the study, Dr Bing Zhang, Department of Molecular and Human Genetics at the Baylor College of Medicine and Lester and Sue Smith Breast Centre at Baylor.

Furthermore, the researchers learned that genomic and proteomic data complement each other in ways that provide researchers with a better understanding of what goes on inside colon cancer cells.

"One example is SOX9," Dr Zhang continued. "Our genomic dataset indicated that SOX9 is a tumour suppressor gene because it is frequently mutated in colon cancer in ways that suggested that the function of the protein coded by the gene would be destroyed and that the protein would not be produced or produced in lesser amount. But when we looked at the proteomic data – at the actual protein in cancer tissue – we observed quite the opposite; SOX9 protein was very abundant in these tumours, more than normal. The proteomic data thus challenged the indication that SOX9 was a tumour suppressor."

Through bioinformatics analyses, Zhang and his colleagues identified new clues regarding why immunotherapy does not work for all mismatch repair-deficient colon cancers that may lead to new therapeutic approaches.



Genomic analysis identifies potential prostate cancer targets

TARGETS

THE STUDY outlines findings from the largest-ever prospective genomic analysis of advanced prostate cancer tumours. Using comprehensive genomic profiling (CGP) to analyse thousands of tumour samples from men with advanced prostate cancers, the researchers identified that 57 percent of the samples evaluated had genomic characteristics that suggested the tumours were candidates for targeted therapies.

The purpose of this study, co-led by Foundation Medicine and Huntsman Cancer Institute (HCI) at the University of Utah, was to analyse prostate tumours using CGP to identify genomic changes to inform potential targeted treatment strategies.

CGP analyses tumour samples to identify genomic changes so that, where possible, a patient can be matched to available targeted treatments.

"This study demonstrates that routine clinical use of comprehensive genomic profiling frequently identifies genomic alterations that can inform targeted therapy options, as well as potential therapy development targets, for patients with advanced or metastatic prostate cancer," said Jon Chung, associate director of clinical development at Foundation Medicine.

Researchers in the study analysed nearly 3,500 unique tumour samples, including 1,660 primary site tumours and 1,816 metastatic site tumours from unmatched patients.

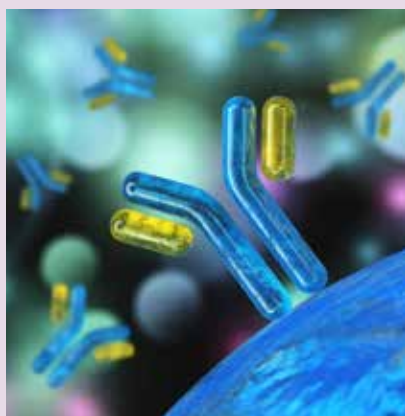
The researchers utilised the FoundationOne test developed by Foundation Medicine for CGP.

The team anticipates these findings may be used to accelerate the development of new drugs and treatment approaches, including immunotherapies, for men with advanced prostate cancers.

Method for radiolabelling antibodies against breast cancer developed

ANTIBODIES

RADIOACTIVE antibodies are used in nuclear medicine as imaging agents for positron emission tomography (PET) – an imaging technique that improves cancer diagnosis and monitors chemotherapy. Radioactive drugs can also be designed to kill tumours by delivering a radioactive payload specifically to the cancer cells. This treatment is called targeted radioimmunotherapy.

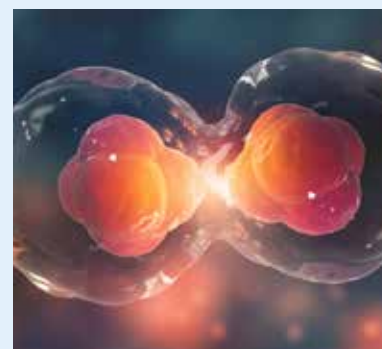


This new method, developed by Jason P Holland, professor in the Department of Chemistry at the University of Zurich, uses UV light to synthesise radioactive drugs and diagnostic agents. "By combining photochemistry with radiochemistry, we are now able to make radiolabelled proteins much more quickly and easily," Holland said.

The team produced a series of novel chemical compounds (chelates) that have two distinctive properties: first, they are able to bind radioactive metal ions like gallium, copper and zirconium. Second, the molecules have a special chemical group that becomes activated by shining UV light on the sample.

With mice bearing human cancer cells that are targeted by trastuzumab, they showed that for PET imaging the zirconium-labelled antibody worked as well as those produced via established methods.

Holland has submitted a patent application for the new procedure and aims to develop the technology along commercial lines.



New study into unlocking the brain's regenerative potential

NEUROLOGICAL DISORDERS

RESEARCHERS at Kyoto University studying brain chemistry in mice have revealed the ebb and flow of gene expression that may wake neural stem cells.

"No one before us has directly compared active stem cells in embryos with inactive, 'quiescent' adult stem cells," said group leader Ryoichiro Kageyama of Kyoto University's Institute for Frontier Life and Medical Sciences, who points out that at least two genes and their associated proteins regulating activation had already been identified.

The team focused their attention on the protein 'Hes1', which is strongly expressed in the adult cells. This normally suppresses the production of other proteins such as Ascl1, small amounts of which are periodically produced by active stem cells.

Monitoring the production of the two proteins over time, the team pinpointed a wave-like pattern that leads to stem cells waking up and turning into neurons in the brain.

When they knocked out the genetic code needed to make Hes1, the cells started to make more Ascl1, which then activated almost all the neural stem cells.

"It is key that the same genes are responsible for both the active and quiescent states of these stem cells," Kageyama added. "Only the expression dynamics differ between the two. A better understanding of the regulatory mechanisms of these different expression dynamics could allow us to switch the dormant cells on as part of a treatment for a range of neurological disorders."

These findings, which may also apply to stem cells elsewhere in the body, were published in the journal *Genes & Development*.

Pigment-producing stem cells can regenerate vital part of nervous system

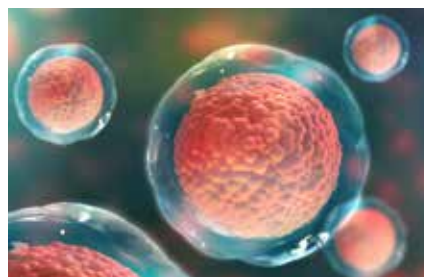
STEM CELLS

RESEARCHERS at the University of Maryland School of Medicine have discovered that a type of skin-related stem cell could be used to help regenerate myelin sheaths, a vital part of the nervous system linked to neurodegenerative disorders.

The discovery into these types of stem cells is significant as they could offer a simpler and less invasive alternative to using embryonic stem cells.

"This research enhances the possibility of identifying human skin stem cells that can be isolated, expanded, and used therapeutically. In the future, we plan to continue our research in this area by determining whether these cells can enhance functional recovery from neuronal injury," said Thomas J Hornyak, Associate Professor and Chairman of the Department of Dermatology, and principal investigator in this research.

Using a mouse model, the team discovered a way to identify a specific version of a cell known as a melanocyte stem cell, which has the ability



to continue to divide without limit. Additionally, the researchers discovered that these stem cells can make different types of cells depending on the type of signals they receive.

Importantly, unlike the embryonic stem cell, which must be harvested from an embryo, melanocyte stem cells can be harvested in a minimally-invasive manner from skin. The team found a new way to not only identify the right kind of melanocyte stem cells, but also the potential applications for those suffering from neurodegenerative disorders.

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SCREENING

Sean Lin, Ina Rothenaigner and Kamyar Hadian from the Helmholtz Zentrum München German Research Center for Environmental Health (HMGU) in Germany discuss the use of phenotypic profiling in drug discovery and how machine-learning algorithms could help improve objectivity, processing throughput, accuracy and mechanisms of action prediction of small compounds.

The advent of CRISPR/Cas9 gene editing, together with the plummeting cost of whole-genome sequencing, has cleared a path for the development of customised cancer cell models. Elizabeth Turner Gillies and Fang Tian from the American Type Culture Collection (ATCC) in Gaithersburg, US, discuss recent developments in the field and challenges associated with targeted-therapy resistance.

Progressing drug discovery projects to a stage that attracts commercial interest is a challenging endeavour. A major cause of this is the lack of comprehensive profiling of compounds prior to initiating translational *in vivo* work. Sheraz Gul, Head of Drug Discovery at the Fraunhofer Institute for Molecular Biology and Applied Ecology describes the minimal information that should be acquired as early as possible for a bioactive compound in the drug discovery value chain.

Native mass spectrometry has the potential to be a fast, simple, highly sensitive and automatable technique for compound screening in drug design and discovery. In this article, Rod Chalk and Nicola Burgess-Brown discuss the key attributes of native mass spectrometry and reasons why it is not yet widely employed.

**SPONSORS:**

Phenotypic profiling in drug discovery

Sean Lin, Ina Rothenaigner and Kamyar Hadian

Institute of Molecular Toxicology and Pharmacology,
Helmholtz Zentrum München German Research Center for Environmental Health (HMGU), Germany

Image-based phenotypic profiling of small molecules has been used for identification and characterisation of small molecules in drug discovery and can provide important insights into their mechanisms of action (MOA). This article discusses the use of phenotypic profiling in drug discovery and how machine-learning algorithms could help improve objectivity, processing throughput, accuracy and MOA prediction of small compounds.

BIOGRAPHY



INA ROTHENAIGNER

is a postdoc in the Assay Development and Screening Platform at the Helmholtz Zentrum München in Munich.

She studied Biology at the Ludwig Maximilians University and gained her PhD at the Helmholtz Zentrum München in the field of virology (HIV). After a postdoc period in the field of zebrafish neurogenetics at the Helmholtz Zentrum and the Institute for Neuroscience at the CNRS, Gif-sur-Yvette in France, she joined the team at the Helmholtz Zentrum München. She has experience in cell-based assays and is responsible for analysis of HTS and high-content screening.

WHILE target-based drug discovery strategies rely on precise knowledge of the targets' identity and MOA, phenotypic drug discovery (PDD) approaches allow the identification of novel drugs without understanding pathogenesis. Image-based phenotypic screening is a potent high-throughput screening (HTS) strategy for PDD that enables large-scale studies of biological systems using genetic and chemical perturbations. It also facilitates characterisation of small molecule actions through the quantification of morphological changes among cell populations. However, phenotypic profiling often generates large and highly complex datasets, making it challenging for lead optimisation and target deconvolution.

Technological advancements in image acquisition and processing, and the implementation of machine and deep learning for analysing large high-dimensional datasets have made phenotypic profiling a viable tool in studying small molecules in drug discovery. Here, we discuss current workflows and challenges of image-based phenotypic profiling.

Phenotypic profiling in drug discovery

Many drug discovery efforts have focused on target-based screens in which disease modelling and pathway analysis generate candidate proteins, which generally leads to high-throughput biochemical screening. Phenotypic high-content assays are screening systems that focus on the modulation of a disease-linked phenotype and are therefore less biased.^{1,2} They can identify compounds acting through either unknown targets or unprecedented MOA for known targets. In contrast to target-based assays, phenotypic screens are physiologically more relevant, since phenotypic studies not only monitor its MOA, but also the toxicity of small compounds.

Despite the ability of high-content screening (HCS) to generate and recognise numerous multiple cellular features, many phenotypic imaged-based hit identification screens have been based on few project-specific and predefined readouts.³ In addition, average readout values are used disregarding the variance of cellular responses in a well. Recent advances in automated imaging and image analysis provide new opportunities and have put yet more content in these high-content analyses.

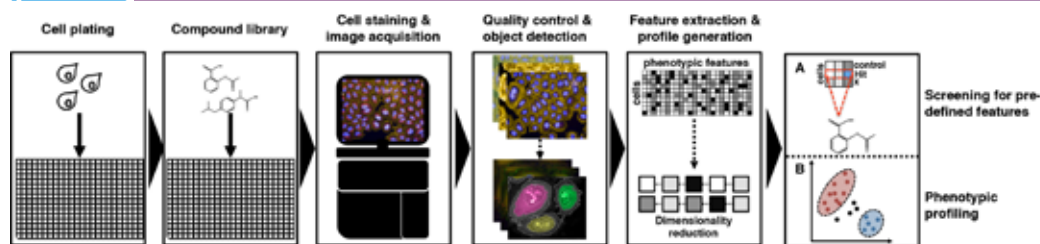
Image-based phenotypic profiling is an HTS strategy for the quantification of phenotypic differences.⁴ Phenotypic profiling analyses as many features as possible in a single cell in order to select for robust, meaningful features. These functional and morphometric data are extracted to generate an individual cell profile. The advantage of this multiparametric assay is reduced human bias. In addition, the computational analysis ensures objectivity and consistency. Various treatment conditions (chemical or genetic perturbations) can be compared to identify biologically-relevant similarities/clusters in order to group compounds and/or genes into functional pathways.⁵

Since image-based screening methods generate huge amounts of data, employing suitable data management and analysis software is crucial in order to extract interesting and novel phenotypes. Here, we discuss the steps taken in image processing for phenotypic profiling and gain insight into the application of machine and deep learning for analysis and classification of cellular phenotypes.

Workflow for image-based profiling

Though the assay design for each image-based cell-profiling screen depends on the biological question at hand, certain steps and equipment are required for all cell-profiling screens.⁶ Image-based

FIGURE 1



LEFT: Workflow of image-based small molecule screening experiments. High-throughput imaging screening experiments usually start with the seeding and incubation of cells in microtiter plates, after which cells are treated with compounds using an automatic liquid handling station. Cells are then fixed, stained and imaged using automated high-content microscopy. Acquired images undergo quality control, whereupon cellular features are detected and measured using automated analysis software. These extracted cellular features are then either filtered **A)** for their pre-defined features or **B)** subjugated further for phenotypic profiling analysis.

screens are usually performed in multi-well plates (eg, 384-well plates). After seeding an appropriate number of cells into each well, they are treated with small molecules, environmental stressors or siRNAs. Following an incubation time, the cells are fixed and stained with multicolour fluorescent probes that label relevant cellular features/compartments. The images are subsequently captured by automated high-content microscopy and passed on to the image-analysis pipeline (**Figure 1**).

Assay reproducibility is crucial when optimising the assay design. Technical variations need to be reduced and variations between reagents and consumables must be avoided. Ideally, positive and negative controls should be added to each multi-well plate to assess assay reproducibility and signal windows. To visualise phenotypes, most HCS uses fluorescent probes to stain specific cellular compartments such as the nucleus, cell membrane, Golgi apparatus, ER or cytoskeleton. A new high-content image-based assay – cell painting – utilises the simultaneous staining of eight broadly relevant cellular components or organelles, enabling multiple morphological perturbations to be monitored in a single cell.⁷

Image-analysis pipeline

After image acquisition, an automated image analysis workflow is required for feature extraction. Several open-source and commercial bioimaging software solutions exist for morphological feature extraction (Open-source: CellProfiler,⁸ Advanced CellClassifier,⁹ PhenoRipper,¹⁰ Fiji/ImageJ,¹¹ Commercial: HighContentProfiler (Perkin Elmer)). The workflow is divided into the following tasks that are important to obtain high-quality cell profiling results (**Figure 1**):

- Illumination correction** – serves to correct each image for spatial illumination heterogeneities introduced by the microscope optics, which can bias intensity-based measurements and impair cellular feature identification.
- Quality control** – aims to identify and remove any problematic images that might impair profiling quality.¹² Illumination problems may lead to over-saturated pixels or improper autofocus. Artefacts caused by dust or other particles should be avoided so that particles

aren't falsely identified and classified as a cellular feature during image analysis. Moreover, edge- and gradient-effects in culture plates might influence data analysis and consequently lead to false biological conclusions. It is therefore crucial to place the controls and samples at appropriate places in order to minimise false positives and maximise genuine hits.

c) Segmentation

d) Morphological feature extraction.⁵

Segmentation and morphological feature extraction entail when objects can be detected by setting intensity thresholds, which delimits objects of interest from the background. Object segmentation often starts with identification of the nucleus, which forms the foundation for identification of secondary regions or objects. Each step needs to be proofread by the user and improved in object detection if necessary. Machine-learning algorithms can also be utilised during object detection to improve feature detection. The classifier is trained to find the desired features in a training dataset until it can accurately generalise towards the dataset while avoiding overfitting. Multi-dimensional features such as fluorescence intensities (eg, mean and maximum intensity), fluorescence distributions, morphology, shape features (eg, area roundness) and texture features (quantify regularity of intensities in images) can be extracted from acquired microscope images. These results are then exported to downstream analysis algorithms, which screen for predefined features (**Figure 1A**) or generate a feature profile for each cell population allowing creation of phenotypic profiles within cell populations (**Figure 1B**).

Machine-learning strategies for phenotypic profiling

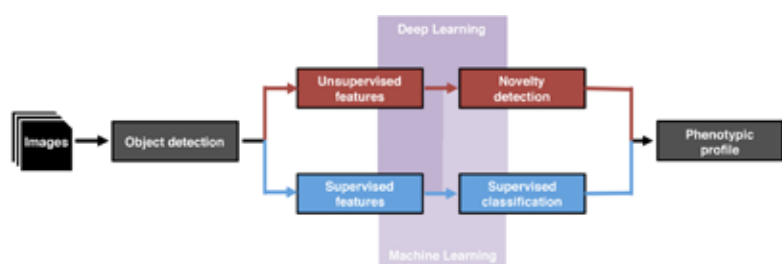
HCS microscopes can generate huge quantities of image data, making analysis procedures challenging.¹³ Common image analysis programmes are either unsuitable or require adaptation for phenotypic profiling. However, manual software adaptations are tedious and potentially perturb the concept of an unbiased approach. The implementation of automated analysis methods

BIOGRAPHY



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FIGURE 1



ABOVE: Data analysis workflows for phenotypic profiling. After image acquisition and object detection, supervised features (blue boxes) or unsupervised features (red boxes) are identified and classified either by supervised/unsupervised machine-learning algorithms (pale purple polyomino) or deep-learning algorithms (purple polyomino).

“In contrast to target-based assays, phenotypic screens are physiologically more relevant, since phenotypic studies not only monitor its MOA, but also the toxicity of small compounds”

such as machine learning and deep learning greatly reduce the experimentalists' workload, while ensuring objectivity and consistency throughout the data analysis. They are also able to identify and assess perturbations between wild type and novel phenotypes.¹⁴ In general, machine learning generalises a training dataset in order to predict an unknown dataset accurately.¹⁵⁻¹⁷ During the learning phase, a dataset is provided for training purposes to develop or optimise the data analysis algorithm, which in turn attempts to recognise cellular features within the data samples. In the second phase, the fine-tuned algorithm is given new data samples in order to predict properties that have previously been trained during the learning phase.

Supervised machine learning

Machine-learning strategies can be subdivided into supervised and unsupervised machine learning (**Figure 2**). Supervised machine-learning algorithms are applied for the identification and classification of phenotypic features and rely on representative datasets of sufficient size and quality, whose data points exemplify the predefined classes. The algorithm infers to the set rules and can subsequently discriminate phenotypes between cell populations based on their predefined features. This approach has been successfully utilised for classification of complex phenotypes and generation of training datasets using genetic perturbation, extracellular stimuli and small molecule compounds.¹⁸⁻²⁴

Supervised machine learning is based on subjective decisions for generating the training datasets and feature detection and so proper generation of datasets and correct implementation of the most appropriate machine-learning algorithm is crucial for successful feature extraction and classification. To evaluate the accuracy of the applied machine-learning algorithm, it is crucial to withhold a portion of the training data to test how well the algorithm has been trained to generalise from the training dataset. In addition,

training datasets may need to be re-evaluated and even re-generated, especially if experimental parameters have been changed.^{17,25-26} Important requirements for a suitable supervised classification algorithm are, therefore, the classification accuracy and versatility in answering diverse cell biological questions without the need to tweak the software.

To date, no universal machine-learning algorithm exists that can be applied to all cell biological questions, but a collection of supervised classification algorithms that are based on either linear or nonlinear classifier models are available. Linear classifier models define a decision boundary (discriminating line, plane or hyperplane) between input features to discriminate predefined classes in the dataset. Supervised classification algorithms operating on linear models have been successfully used for the analysis of image-based studies.²⁷⁻³¹ Although algorithms based on linear models are able to discriminate clear-cut decision boundaries and are robust against noise within the datasets, they can't accurately classify more complex distributions of data points such as discriminating changes in cell morphology within cell populations. In contrast, nonlinear classification algorithms are fully capable of dealing with highly complex decision boundaries. These algorithms require more training data to fit and are generally more complex than linear algorithms. They were effectively used *inter alia* in subcellular localisation, cell cycle arrest phenotypes and cell morphology perturbations.^{22-23,32-34}

Unsupervised machine learning

Although supervised machine learning has been successfully utilised for the classification of complex phenotypes, classification accuracy and data overfitting are obstacles that can be bypassed by unsupervised machine learning.^{16-17,35} In some biological questions it is not possible to generate a suitable training dataset: if either there is no *a priori* knowledge of the outcome phenotypes or if the experimenter seeks to identify novel phenotypes. These limitations could potentially be overcome by implementation of unsupervised machine-learning algorithms that are capable of recognising the above-mentioned phenotypes through clustering or detection of outliers in the data points.

Clustering and dimensionality reduction are the main strategies in unsupervised machine learning. Clustering methods, such as hierarchical clustering, κ -means clustering and Gaussian mixture model, aim to maximise the distance between different cluster centres, while simultaneously minimising the distance between the objects within each cluster.¹⁵ Hierarchical clustering has been applied to studies on cell perturbations, morphological, gene-expression and cytological profiling.³⁶⁻⁴⁰

Dimensionality reduction is a second method used in unsupervised machine-learning

BIOGRAPHY



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algorithms. It facilitates visual presentation of high-dimensionality datasets by transforming the initial feature space into lower dimensions – either using linear methods such as principle component analysis (PCA) or nonlinear methods such as independent component analysis (ICA) – while retaining as much information from the original high-dimensional feature space.^{15-16,41}

Despite the advantages of unsupervised machine learning, the algorithm's output is unpredictable and its performance suffers from experimental noise. These limitations in detecting unsupervised phenotypes can be bypassed by incorporating a cell population context or temporal constraints in the machine-learning algorithm, but such knowledge is not always given in every biological application.^{10,42-45}

Deep learning

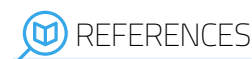
Deep-learning algorithms have recently been implemented to address biological classification problems directly from raw image data or implemented immediately after object detection. Like the human brain, deep-learning algorithms use artificial neural networks consisting of multilayered, interconnected nodes and thus learn during multiple sessions through back-propagation.⁴¹ Studies have

demonstrated successful implementation of deep learning in answering various biological questions, but also as a potent tool in the detection of novel phenotypes (CellCognition Explorer), predicting the biological activity of compounds and as a method for label-free profiling.⁴⁶⁻⁵⁰

Conclusion

Studies have demonstrated that image-based phenotypic profiling is a potent tool for addressing diverse biological questions and for identification and characterisation of small molecules in drug discovery. Every step in phenotypic profiling should be executed attentively to decrease experimental noise and increase accuracy in feature extraction. In order to improve and expand further discoveries using phenotypic profiling, future improvements must focus on developing methods and algorithms that streamline the handling and analysis of generated datasets by implementing more user-friendly machine-learning algorithms. Consequently, deep learning may completely substitute or work in conjunction with manually-curated analysis pipelines, thus improving objectivity, processing throughput, accuracy and MOA prediction of small compounds. ☺

“The implementation of automated analysis methods such as machine learning and deep learning greatly reduce the experimentalists' workload, while ensuring objectivity and consistency throughout the data analysis”



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EXPERTVIEW



Hongshan Li

Marketing Applications
Manager of FortéBio –
Biologics by Molecular Devices

“Bispecific antibodies contain two different antigen-binding sites within one molecule, which offers a clinically validated platform for drug discovery”

Analytical power tool opens upstream bioprocessing bottlenecks for better antibody screening

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Bio-layer interferometry (BLI) is a practical and effective technique for monitoring antibody-antigen interactions with binding events monitored directly in real time and label-free. The adoption of this microfluidic-free technology has increased rapidly in the past decade. This trend is predicted to continue as the technology continues to gain widespread acceptance and diversifies its application base. One such application is the characterisation of bispecific antibodies.

Discovered in the 1960s, the first article describing the therapeutic use of bispecific antibodies was published in 1992, with numerous publications since. Bispecific antibodies contain two different antigen-binding sites within one molecule, which offers

a clinically validated platform for drug discovery. However, there are some challenges in developing them, which could include significant immunogenicity caused by novel epitopes. In April 2019, the FDA published a guidance document for 'Bispecific Antibody Development Programs'. It suggested that “quality attributes such as antigen specificity; affinity and on- and off-rates; avidity; potency; process-related impurities; stability; and half-life may affect pharmacology and should be studied.” Octet platforms provide broad and flexible applications to support these requirements.

Molecular interaction information, such as kinetic rate constants, affinity binding constants and specific molecule quantitation is some of the main characterisation information

that can be monitored with BLI.

For example, one arm of the bispecific antibody can be captured by an antigen on the biosensor. The second arm binds to the second antigen. This way, the bispecific antibody has augmented neutralising abilities because it recognises two different sites of the target. Such information is necessary for biophysical characterisation of bispecific or multi-specific antibodies developed for enhancement of dual or multi-targeting specificity. Automated high-throughput parallel screening of up to 96 samples has been successfully used in early selection and optimisation of hybridoma cell lines. The potential for simultaneous detection of several antigens in crude samples makes it a powerful tool for bispecific antibody research. ■

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CRISPR/Cas9-engineered cancer models: the next step forward for targeted cancer therapy

Elizabeth Turner Gillies, PhD and Fang Tian, PhD

American Type Culture Collection (ATCC), Cell Biology R&D, Gaithersburg, US

The advent of CRISPR/Cas9 gene editing, together with the plummeting cost of whole-genome sequencing, has cleared a path for the development of customised cancer cell models. Here, we discuss recent developments in the field and challenges associated with targeted-therapy resistance.



THE SHIFT towards targeted therapies in cancer treatment, which began with the development of tamoxifen and accelerated following the success of such drugs as rituximab and imatinib (Gleevec), has altered our perceptions of cancer treatment and cancer prognosis. By targeting the molecular pathways that drive tumour proliferation, rather than killing all rapidly dividing cells as in traditional chemotherapy, targeted therapies have the potential to turn a dire prognosis into a manageable condition.¹

However, despite encouraging progress in this area, there remain significant challenges regarding relevant cancer biomarker identification and testing, low clinical trial success rates for new therapies and rapid development of resistance to certain targeted therapies.² Discovery and development of new therapies for current and emerging cancer targets relies heavily on high-throughput screening and iterative refinement of lead compounds and biologics.³ Simple cell systems and historical cancer lines are frequently used for these screenings because they are easy to ▶

“ *Successive gene edits can be made in engineered cancer model lines to mimic the accumulation of oncogenic mutations in physiological tumour samples* ”

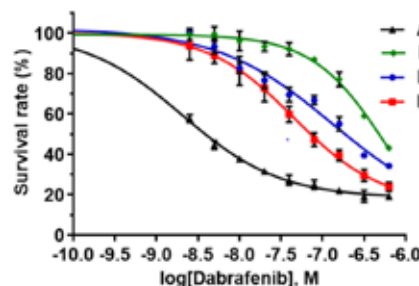
RIGHT: Drug resistance of CRISPR/Cas9-engineered isogenic melanoma models.



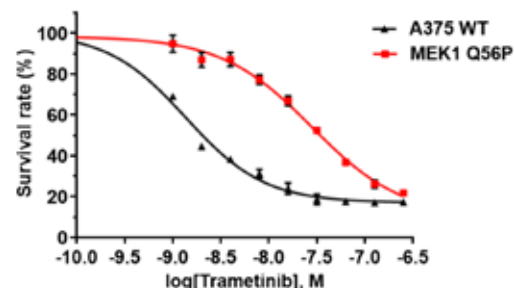
(A) The BRAF inhibitor resistance of engineered RAS and MEK1 melanoma models.
(B) MEK inhibitor resistance of engineered MEK1 melanoma model.

FIGURE 1

A. BRAF inhibitor resistance in isogenic melanoma models



B. MEK inhibitor resistance in MEK1 melanoma model



“Acquired resistance to targeted therapies in cancer is reminiscent of the rise of antibiotic resistance in bacterial infections; patients who initially respond well to a targeted cancer therapy later develop tumours that are resistant to that therapy”

BIOGRAPHY



DR ELIZABETH TURNER GILLIES is a

scientist in Cell Biology Research and Development at ATCC. She received her PhD in Molecular Biophysics and Biochemistry from Yale in 2015 and conducted her post-doctoral research at ATCC. Elizabeth currently works on CRISPR/Cas9 engineered advanced cell models, 3D tissue culture models, and enhanced viral production cell lines for vaccine manufacturing.

work with, relatively inexpensive and scale easily.⁴ However, the success rates of lead compounds and biologics in subsequent animal studies and clinical trials could be increased by using more biologically-relevant and mechanism-specific tumour model systems.

Currently available cancer model systems for targeted drug screening are typically derived from either commonly used tumour cell lines or from established cell lines that have been modified to overexpress specific drug targets. Both model types have their respective pros and cons. Primary or spontaneously immortalised tumour cells more closely model the genetic and phenotypic heterogeneity found in clinical tumour samples, but this heterogeneity is rarely fully characterised or defined, so results can be difficult to interpret. Even when well-characterised cancer lines are used as screening models, the screening of therapies that target a specific oncogenic mechanism is confounded by the complex web of mechanisms that promote tumour growth. Established cancer cell lines rarely come with appropriate control lines that would help to tease apart these complex interactions. By contrast, models derived from cell lines modified to overexpress a particular cancer biomarker have a clear oncogenic mechanism. However, expression of an oncogene at artificially high, non-physiological levels in a cell that would otherwise be non-cancerous doesn't adequately model the complex cascade of events that results in the formation of a tumour *in vivo*.

Accelerating the progression of therapeutics

The advent of CRISPR/Cas9 gene editing, together with the plummeting cost of whole-genome sequencing, has cleared a path for the development of customised cancer cell models. These engineered models have the potential to dramatically accelerate the development of targeted cancer therapeutics. CRISPR/Cas9 has enabled us to reliably and cost-effectively edit the genome of any target organism or cell line,⁵⁻⁷ while whole-genome sequencing has enabled us

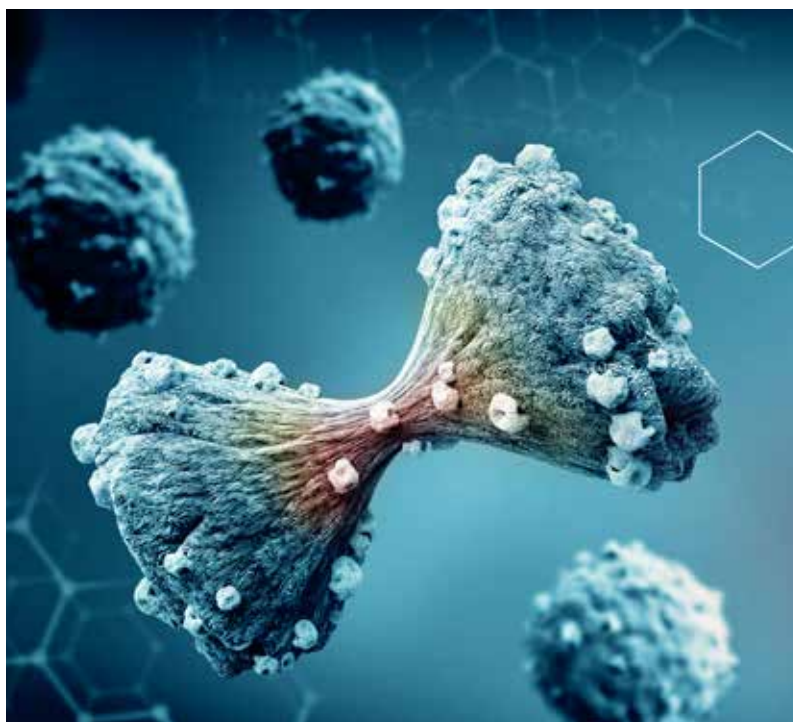
to assemble massive sequence libraries for both healthy and tumour tissue, revealing the diverse genetic changes found in tumour cell populations. With these two technologies combined, we can now better understand cancer genomes and create disease models driven by genetic abnormality.

The effects of this new approach to biological sciences are only now beginning to trickle down to the clinical setting. Use of CRISPR/Cas9-engineered cancer model systems to accelerate the development of targeted therapies is one example of this effect. By using CRISPR-based genome engineering to make precise changes to the genome of a target cell line, we can now alter those lines in a way that closely mimics the spontaneous development of cancer in a healthy tissue. This new, more physiologically-relevant type of cancer model system can be as easy to work with as a historical cancer line or overexpression model for drug discovery and development. Moreover, CRISPR/Cas9-engineered cancer models can be designed to mimic specific cancer genotypes found in clinical patient samples.⁸ When used together with the unedited parental cell line, engineered cancer cells come with a built-in isogenic control with an identical genetic background. Engineered cancer models can then be assembled into isogenic cancer panels by introducing sets of genetic variants in parallel. These panels can in turn be assembled into large libraries of engineered cancer cell lines cross-referenced by tissue type. Successive gene edits can be made in engineered cancer model lines to mimic the accumulation of oncogenic mutations in physiological tumour samples. This precisely defined and well-controlled generation of cancer models provides a new tool to enable accelerated development of novel therapeutics, thereby unlocking the potential of targeted therapies for the treatment of cancer.

Recent developments

ATCC has been steadily building and developing an array of CRISPR/Cas9 genome-editing

capabilities and leveraging its extensive library of human cell lines for the development of more physiologically-relevant human cancer models. These new cancer models are specifically designed to address the lack of suitable model systems needed to develop drugs for newly identified molecular targets. The first new cancer model line to emerge from this gene-editing pipeline was an EML4/ALK oncogenic fusion that was engineered in a lung adenocarcinoma model.⁹ By demonstrating that this engineered gene fusion functions in the same way as spontaneous EML4/ALK rearrangements isolated from clinical tumour samples, this cancer model paved way for the development of more engineered cancer models for other well-known and emerging drug targets. For example, a model glioma line was engineered with an IDH1 R132H allele to recapitulate the way cellular metabolism is altered in IDH1 mutant gliomas.¹⁰ Because the R132H mutation was engineered directly into the endogenous IDH1 gene locus of this model line, rather than overexpressed from a third IDH1 gene inserted elsewhere in the genome, the model shows levels of D-2-hydroxyglutamate accumulation in line with clinical IDH1 mutant glioma samples. This added degree of physiological relevance makes the new glioma model both a useful tool for IDH1-targeted drug screening and an accurate IDH1 biomarker reference model. Similarly, an



engineered IDH2 R140Q mutant cell model for acute myeloid leukaemia was developed that mimics the genetic alternation and metabolic changes that drive progression of AML.¹⁰ Again, this engineered cancer model shows physiologically-relevant ▶

EXPERT VIEW



Tobias Pusterla, PhD
International Marketing
Manager, BMG LABTECH

***"Pharma research
requires precise
and reliable data
to be obtained with
minimal costs"***

Get more out of your samples: microplate readers and multiplexing

Analysing multiple biological targets within the same experiment is one way to get more out of your samples. Here, we discuss the benefits of multiplexing in pharma research.

In drug screening, assay miniaturisation and speed of detection have always been considered the path to follow to reduce costs on reagents and time. Multiplexing allows detection of multiple biological parameters in a single sample and is a possible alternative solution.

Pharma research requires precise and reliable data to be obtained with minimal costs. Traditionally, this meant reducing sample volume and increasing speed of detection. By reducing volumes and time, however, one is faced with additional infrastructural costs.

To analyse several biological parameters, multiple single analyses have, in the past, been performed

sequentially. Although optimised and well-established, these techniques are costly, time-consuming, and sometimes misleading.

Multiplexing is an efficient alternative. In fact, in preclinical small molecule drug discovery, microplate-based assays are increasingly becoming multiplexed, especially in the ADMET area. Multiplexing is defined as the analysis of multiple biological targets within the same experiment. In microplate-based screening it identifies the measurement of multiple parameters from a single sample in a single well during one measurement run on a plate reader.

Biologically speaking, the main advantage of analysing how different parameters are affected

by compounds in the same microplate is that the compound activities are determined under identical experimental conditions. No variables are introduced through consecutive experiments. This helps gain a better understanding of interrelated responses and minimises incorrect interpretation or data ambiguity. Moreover, multiplexing provides a way to normalise data to specific parameters.

Additional benefits include cutback in sample volumes required, in time of analysis, labour and cost.

The majority of today's multi-mode plate readers are capable of multiplexing, with some being able to combine up to five different parameters in a single run. ■

BIOGRAPHY



DR FANG TIAN is the head of the Cell Biology R&D group at ATCC. She is responsible for overseeing over 4,000 human, animal cell lines and hybridomas in the Cell Biology General Collection. Her R&D team has been generating scientific high-value cell lines from various species, as well as developing advanced cell models focusing on diseases, molecular signatures, cell signalling pathways, and genetic alterations.

levels of 2-D-hydroxyglutarate accumulation and responds to IDH2-targeted inhibitors in the same manner as clinical AML isolates. When used as an isogenic pair with parental control cells, engineered cancer models like these IDH1 and IDH2 mutant glioma and AML lines represent an important step forward for the development and use of targeted cancer therapies.

Complexities of resistance

The clinical outcomes of targeted therapy are often compromised by patients developing resistance to therapies. Acquired resistance to targeted therapies in cancer is reminiscent of the rise of antibiotic resistance in bacterial infections; patients who initially respond well to a targeted cancer therapy later develop tumours that are resistant to that therapy. The most notable example of this phenomenon is the development of resistance to BRAF inhibitor therapy in melanoma patients.¹¹ Initially, BRAF inhibitors such as dabrafenib and vemurafenib are highly effective for the treatment of metastatic melanomas with BRAF V600E mutation:¹² tumours shrink and disappear, but can aggressively reappear six months later resistant to BRAF inhibitors. Development of new therapies to treat these resistant tumours is therefore essential. Screening of these new drugs relies on drug-resistant cancer models that carry the oncogenes that resulted in the initial onset of cancer as well as additional mutations that render them resistant to targeted therapy. Most traditional cancer models do not accurately represent this complexity.

We have developed a series of CRISPR/Cas9-engineered melanoma model cell lines designed to meet this specific need. Starting with a BRAF V600E melanoma model line, a series of point mutations associated with acquired BRAF inhibitor resistance were introduced into genes that act either upstream or downstream of BRAF in the RAS/Raf/MEK/ERK kinase signalling pathway.¹³⁻¹⁴ Two of these point mutations, NRAS Q61K and KRAS G13D, confer levels of resistance to BRAF inhibitor consistent with the resistance seen in



clinical tumour samples (**Figure 1A**). Because these engineered NRAS and KRAS mutant melanoma lines can be paired with the parental melanoma cells they were derived from, they provide a more advanced and biologically relevant cancer model system for screening new targeted therapies. Another CRISPR/Cas9-engineered BRAF V600E melanoma model line has been designed that carries a Q56P mutation in MEK1 that renders these cells resistant to both BRAF and MEK-targeted inhibitors (**Figure 1B**). This isogenic melanoma model line is more sensitive to the commonly used BRAF/MEK inhibitor combination than to any equivalent dose of either inhibitor alone – a clear demonstration of the power of gene-engineered cell lines for accurately representing specific cancer disease states. With engineered cancer models like these, we can more confidently anticipate faster development cycles, lower failure rates, and exciting new directions for targeted cancer therapy. 



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Screening and beyond in drug discovery

Sheraz Gul

Head of Drug Discovery at the Fraunhofer Institute for Molecular Biology and Applied Ecology, Germany

Progressing drug discovery projects to a stage that attracts commercial interest is a challenging endeavour, evidenced by the fact that only a small proportion of drug discovery projects are successfully progressed to such a stage. A major cause of this is the lack of comprehensive profiling of compounds prior to initiating translational *in-vivo* work. This article describes the minimal information that should be acquired as early as possible for a bioactive compound in the drug discovery value chain.

BIOGRAPHY



SHERAZ GUL is Head of Drug Discovery at the Fraunhofer Institute for Molecular Biology and Applied Ecology in Germany. He is responsible for the management and development of assay development and medium- and high-throughput screening activities for partners across the world. He has 23 years' experience in both academia (University of London) and industry (GlaxoSmithKline Pharmaceuticals). This has ranged from the detailed study of catalysis by biological catalysts (enzymes and catalytic antibodies) to the design and development of assays for high-throughput screening for the major drug target classes. He is co-author of numerous papers, chapters and the 'Enzyme Assays: Essential Data' handbook.



OVER THE past few decades the reductionist approach has been extensively employed to feed the industry's small molecule drug pipelines.¹ The adoption of this approach was logical at its inception as it enabled the identification of hit compounds that specifically bind and modulate the activity of their respective drug targets. However, this approach yields compounds that are weakly active,

lack selectivity and possess sub-optimal properties. These hit compounds therefore require significant optimisation in order to yield a lead compound² that is potent with optimised selectivity, physicochemical and ADMET properties.^{3,4}

Hit-to-lead

In order to successfully progress compounds to a lead compound, numerous properties must

be determined – including adequate biological, chemical, pharmacological and pharmacokinetic characterisation – and each are summarised in **Table 1**. Most of these studies are *in vitro* and involve complex processes including cell viability assays. Cell viability relies upon the integrity of the cell membrane, key cellular biochemical reactions and specific cellular markers.⁵ Knowledge regarding the extent of cytotoxicity that is induced by a compound is a parameter that must be determined prior to progressing it in the drug discovery value chain. A variety of microtitre plate-based cytotoxicity assays in colourimetric, fluorometric and luminescence detection technologies are available. The colourimetric-based methods have historically been the most widely employed and are well validated. These include quantification of the following:

- 1) Mitochondrial succinate dehydrogenase activity of cells using the tetrazoles XTT and MTT⁶
- 2) Extracellular lactate dehydrogenase activity by measuring NADH consumption⁷
- 3) Acid phosphatase activity as a marker of lysosomal activity⁸
- 4) Remaining glucose in cell culture medium using a glucose oxidase-peroxidase assay⁹

“Cell viability relies upon the integrity of the cell membrane, key cellular biochemical reactions and specific cellular markers”

- 5) Cell proliferation using crystal violet dye accumulation in the nucleus¹⁰
- 6) Lysosomal accumulation of a cationic dye neutral red¹¹
- 7) Protein synthesis using sulforhodamine B binding to proteins.¹²

Fluorometric-based cytotoxicity assays are also available and include those that quantify the following:

- 1) Lactate dehydrogenase activity using a coupled reaction that results in the conversion of resazurin into resorufin (CytoTox-ONE Assay)¹³
- 2) Protease activity that is released from cells with a compromised cell membrane and a non-cell permeable fluorogenic peptide substrate (CytoTox-Fluor Cytotoxicity Assay)¹²
- 3) The activity of two proteases using cell ▶

TABLE 1 Properties of a typical Lead compound

Property	Attribute	Annotation
Biological and chemical properties	Hit validation	Confirmed activity
	Resynthesis and screening of hit	Confirmed activity
	Structure identity and purity	>95 percent purity
	Synthesis of analogue compounds	Confirmed
	Synthetically accessible and tractable	Confirmed
	Measurement of physicochemical	Confirmed
	Potential for patent protection	Assessed
	Confirmed structure (crystal structure)	Confirmed
Pharmacological properties	SAR suggestive that optimisation is possible	Confirmed activity
	Suitable selective profile	Positive results
Pharmacokinetics	Bioavailability for route of administration	Positive results
	Half-life and bio-distribution for route of administration	Positive results
Safety and toxicity	<i>In vitro</i> ADMET studies (CYP450 inhibition and induction and hERG inhibition)	Positive results
	Absence of cytotoxicity in human cell lines	Positive results

“The prospect of discovering a new drug is highly rewarding, as it offers the potential for mankind to live healthier and longer lives”



permeable (giving the live cell measurement) and non-cell permeable (giving the dead cell measurement) substrates (MultiTox-Fluor Multiplex Cytotoxicity Assay).¹⁴

Luminescence-based assays are also available and include those that quantify the following:

- 1) The activity of a protease that is released from cells that no longer retain an intact

cell membrane using a luminogenic peptide substrate (CytoTox-Glo Cytotoxicity Assay).¹⁵

- 2) Glutathione-5-transferase activity using a luciferin-derived substrate, the product of the reaction being a substrate of firefly luciferase¹⁶
- 3) Intracellular ATP using a luciferase reaction (CellTiter-Glo Luminescent Cell Viability Assay).¹⁷

There is also a multiplex assay that uses a fluorescence and luminescence readout essentially the same as the MultiTox-Fluor Multiplex Cytotoxicity Assay mentioned above, except the substrate for dead cells is luminogenic rather than being fluorogenic.¹⁸

Lead-to-candidate

The optimisation of a lead molecule into a candidate is a very challenging, multiparameter process.¹⁹⁻²⁰ It should lead to identification of a molecule that satisfies pre-defined criteria with regard to *in vitro* and *in vivo* activity, pharmacokinetics, toxicological and pharmaceutical properties, and complexity of chemical synthesis (Table 2). It will typically have nano-molar potency against its primary biological target and will have undergone significant medicinal chemistry optimisation, comprehensive *in vitro* selectivity screening, determination of physicochemical and pharmaceutical properties

EXPERT VIEW



David Ferrick
Senior Director,
Agilent Technologies

"Immune cells must persist in hostile, immunosuppressive microenvironments throughout tumour elimination and surveillance"

Immunotherapy raises the bar for cell analysis in drug discovery

With the recent successes of immunotherapies, a fresh breeze of optimism in the fight against cancer has reinvigorated the industry. Here, we discuss advances in immunotherapy platforms for target discovery.

Whispers of cancer cures have prompted genuine expectations of long-term survival, with perhaps the greatest legacy being the pivot from targeting the 'bad guy' to supporting the 'good guy'.

Targeting the immune system to reinforce, restart and/or recalibrate immunosurveillance has laid down the gauntlet for cell-based measurements in terms of live-cell relevancy, real-time kinetics and label-free detection. Targeting cytotoxicity isn't enough to achieve long-term survival; immune cells must persist in hostile, immunosuppressive microenvironments throughout tumour elimination and surveillance. With the emergence of quantitative, live-cell analysis

platforms researchers can make sensitive time-resolved measurements to monitor the temporal components of an ever-evolving immune response.

A pivotal study using the Agilent Seahorse XF platform validated new targets for directing cell fate and function by driving different metabolic programmes. CAR T-cells containing the 4-1BB signalling domain elicit an aerobic programme that biases central memory formation and fitness. By contrast, CD28 reprogrammes metabolism towards glycolysis, resulting in enhanced effector memory cell fate. This revelation offers a novel strategy: establishing the right balance of effector and memory function to optimise tumour

elimination, persistence in the microenvironment and memory.

Another sensitive, time-resolved platform gaining use in development is the xCELLigence real-time impedance-based system. In a recent study, researchers were able to identify optimal target combinations based on a sensitive quantification of killing efficacy using low effector-to-target ratios (1:10). Simultaneously, escape phenotypes were revealed due to the time resolution of the platform. This would go undetected in end-point assays.

Immunotherapy is thus ushering in a new era of cell-centric target discovery that is focused not on eliminating pathogenic cells but bringing the natural defense system back online. ■

and initial formulation studies, detailed *in vitro* ADMET studies, mutagenicity testing, detailed secondary pharmacology studies, detailed *in vivo* pharmacodynamics and pharmacokinetic studies and comprehensive toxicological profiling.

Although drug discovery is fraught with challenges, as highlighted above, the prospect of discovering a new drug is highly rewarding, as it offers the potential for mankind to live healthier and longer lives. 

TABLE 2 Summary of the attributes of a typical candidate

Property	Attribute	Annotation
Chemical properties	- Physicochemical properties - Chemistry and patents	- MW - CLogP and cLogD - tPSA - H-bond donors and H-bond acceptors - Physiochemical properties determined and no major issues identified - Aqueous solubility pH 7.4 - Synthetic route appropriate for progression to development stage - Scalable (50g batch prepared) - Purity level > 95 percent - Acceptable stability and formulation - Appropriate crystalline (salt) form identified and single enantiomer - Back-ups identified - Clinical development plan - Patent life target 15 years or greater
Mechanism of action	- Biological activity and mode of action studies - Cytotoxicity (against mammalian)	- Mode of action understood - Selective potent <i>in vitro</i> activity - Dose response relationship defined - Activity in a relevant animal model - Pharmacodynamics determined - Oral bioavailability in rat, dog or marmoset - No major effects on human CYP450 metabolism - Metabolism in human liver S9 fractions determined
ADME	<i>In vitro</i> and <i>in vivo</i> DMPK - Bioanalytical methodology <i>In vitro</i> metabolism - Plasma protein binding - Pharmacokinetic studies	- Intrinsic clearance in hepatic microsomes - Intrinsic clearance in hepatocytes - Preferred human plasma protein binding <95 percent - Preferred bioavailability: F >20 percent - Appropriate permeability (PAMPA) - Appropriate pK_a - Appropriate drug efflux pump profile - Stability in simulated gastric fluid stability, plasma and blood stability (mouse/human), media, milli-Q water, FeSSIF and FaSSIF
Toxicology	<i>In vitro</i> cell toxicity - Selectivity index and safety profile - Cardiac safety - Gene toxicity - CYP450 inhibition <i>In vivo</i> rat safety studies	- No issues identified in secondary pharmacology assays - No hERG inhibition - No effect in zebrafish and Ames test - Minimum lethal dose (rat) suggestive of an acceptable therapeutic ratio - No deaths or major organ damage following 7-day dosing at 100mg/kg in rat

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Compound and fragment screening by native mass spectrometry

Rod ChalkHead of Mass Spectrometry,
Structural Genomics Consortium (SGC)**Nicola Burgess-Brown**Principal Investigator of the Biotechnology Group,
Structural Genomics Consortium (SGC)

Native mass spectrometry has the potential to be a fast, simple, highly sensitive and automatable technique for compound screening in drug design and discovery. In this article, Rod Chalk and Nicola Burgess-Brown discuss the key attributes of native mass spectrometry and reasons why it is not yet widely employed.

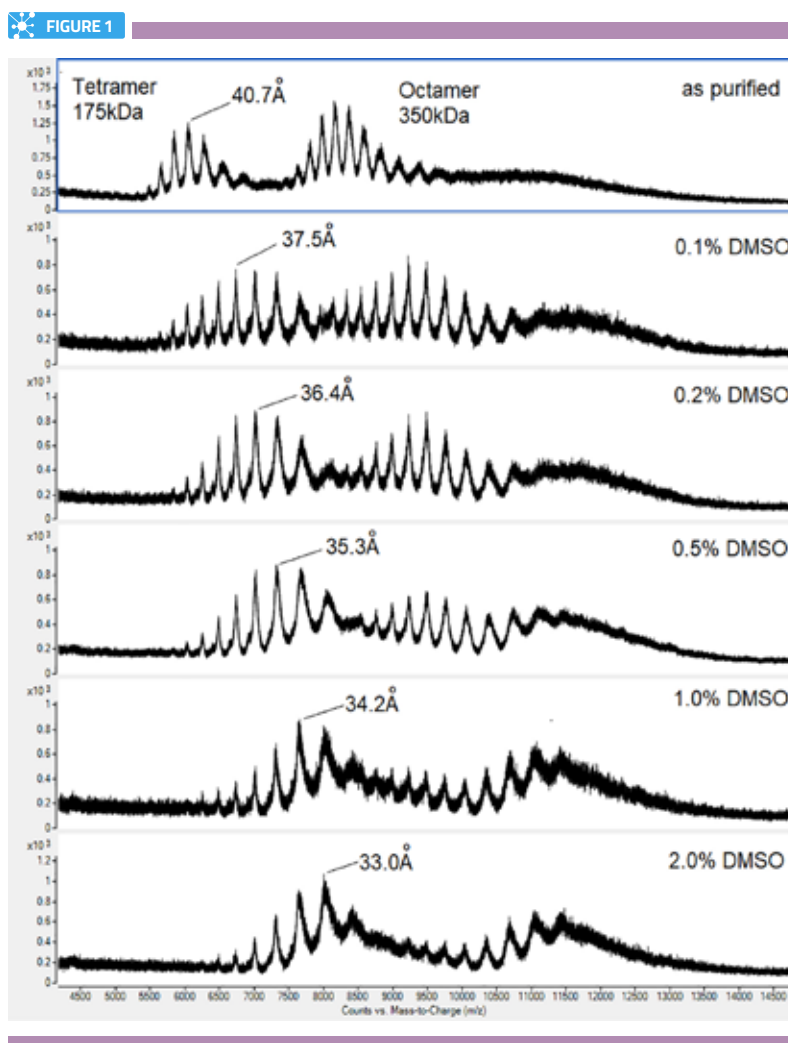


EFFECTIVE and efficient drug discovery is essential if we are to sustain a viable pharmaceuticals industry and meet the medical challenges of the 21st century.

Strategies include *in silico* drug design, discovery from natural products, high-throughput screening of large compound libraries or screening of smaller drug fragment libraries. A variety of screening methods are employed, each with a different propensity to produce unwanted false positive or false negative results.¹ Frequently, two or more orthogonal methods are used generating only partially overlapping results, and there is no generally agreed strategy for primary screening and secondary/tertiary validation.

Advantages of native MS

In principal, native mass spectrometry (MS) is an attractive screening option. Primarily, it is a generic tool that relies on a measurable shift in mass or charge to indicate a binding event, and can thus be applied to any target protein. Contrary to popular opinion, and unlike ion mobility, assignment of charge in electrospray occurs exclusively in the liquid phase,² and native MS is thus a better reflection of the cellular drug-target interaction than, for example, crystal soaking – a solid phase interaction; or SPR – an interaction at the solid-liquid interface. Since immobilisation is not required, screening artefacts resulting from fusion proteins or chemical conjugates can be avoided. Similarly, artefacts resulting from (expensive) fluorophores are also avoided. Whilst the false negative rate cannot be readily determined, false positives should not theoretically occur. Observation of one mass shift, indicative of a single binding event, is assumed to be specific, whereas multiple binding events indicate lack of specificity. Weak mM interactions are maintained, making the technique suitable for fragment screening, and titration of the compound allows the



binding affinity to be determined.³ Protein-protein interactions can be readily measured, as well as the formation or disruption of homomultimeric complexes – an effect that is difficult to detect by other means. Changes in conformation or unfolding resulting from drug binding may also be detected. ▶

↑ ABOVE: Native m/z spectra for a tetramer-octamer homomultimeric protein complex illustrating the conformational changes induced by different concentrations of DMSO



BIOGRAPHY

**NICOLA BURGESS-BROWN**

is the Principal Investigator of the Biotechnology Group at the SGC, responsible for managing all biotech research for the Oxford site. Nicola's team develops high-throughput screening processes from cloning to expression, purification and mass spectrometry analysis of human proteins for structural and functional studies. Nicola obtained a first class degree in Applied Biochemical Sciences from the University of Ulster in 1997, then worked as a molecular biologist for SmithKline Beecham. She received her PhD in Molecular Microbiology at the University of Nottingham in 2001 and then moved back to industry to work on high-throughput cloning and validation of therapeutic cancer antigens for Oxford Glycosciences and subsequently Celltech R&D.



Robust methods exist for native MS compound screening in the presence of detergents,⁴ which are essential for drug discovery related to membrane proteins such as GPCRs, representing a significant proportion of all drug targets. Finally, and uniquely, native MS has the capacity for multiplex compound screening. The resolution of the technique is such that mass differences smaller than 1Da can be resolved from a protein-ligand complex. In a thoughtfully designed chemical screen in which isobaric compounds are excluded, multiple interactions can be measured simultaneously, where the identity of each compound is encoded by its mass. Hundreds, and potentially thousands, of compounds can be screened in a single binding experiment, each functioning as an internal control for the others, drastically reducing the time, cost and protein consumption of a screening campaign.

Challenges of native MS

If the advantages of native MS as a screening tool are so profound, one is forced to question why it is not more widely employed. There are several reasons and understanding them is important in order to make an informed decision as to whether to utilise the technique. Firstly, native MS, like all MS, works only in volatile salt-free buffer. In drug screening, buffer conditions are perceived to be of paramount importance and a typical "physiological" AlphaScreen assay buffer containing 50mM Tris (pH 7.4), 150mM NaCl, 0.05 percent BSA, 0.02 percent Tween, and 1mM DTT is essentially incompatible with the technique. Exchange into a MS-compatible volatile buffer, such as 50mM ammonium acetate pH 6.5, is often assumed to be non-physiological and thus invalid for screening purposes. Our experience, and that of others, indicates this is not generally true. One advantage of the technique is that natively folded

protein is readily distinguishable from protein beginning to unfold. Deviations of 0.5pH unit either side of the pH optimum and transfer to salt-free buffer have no discernible effect on the stability or conformation of most proteins. Secondly, instrument vendors have been slow to appreciate the importance of native MS for drug screening. Consequently, although excellent equipment exists, data analysis is comparatively slow and users have been obliged to develop their own software – though recently, commercial third-party software has become available.⁵ Thirdly, protein consumption in a native MS experiment may be as high as 50µg, making it unsuitable as a primary high-throughput screen where the supply of target protein is limited. Fourthly, while the general level of MS expertise within the pharmaceutical industry is very high, there is far less experience and understanding when applied to native MS. This ranges from misconceptions regarding the equipment required, to disbelief that native MS has any validity at all.

Native MS and DMSO

When native MS screening has been applied, results do not always confirm those detected using orthogonal methods.⁶ Native MS is not unique in this respect, but one aspect of the method, the use of DMSO, may be responsible. Since many compounds are both soluble and stable in DMSO, and it is relatively non-toxic, it is frequently used in high-throughput compound screening. With a 189°C boiling point, it is effectively non-volatile, but small amounts of less than 5 percent are tolerated in conventional electrospray MS. If compound libraries are stored in DMSO, small amounts of the compound will remain in a drug-binding experiment, but providing the concentration is low enough, one might reasonably expect any effect to

“ If the advantages of native MS as a screening tool are so profound, one is forced to question why it is not more widely employed ”



be minimal. Our experience with DMSO is that its effects on protein structure are both measurable and significant, and it is inconceivable that this would not occasionally alter the outcome of a compound-binding experiment. Worryingly, we find that these conformational alterations occur at any DMSO concentration.

It is not difficult to understand how this can happen. In electrospray ionisation (ESI), charged solvent droplets containing the protein of interest are emitted from a spray tip and enter a stream of hot, dry nitrogen gas where desolvation

occurs. As the droplet shrinks and surface charge accumulates, a series of Coulombic explosions and further desolvation ultimately leads to ionisation by transfer of the surface charges on to the solvent-free protein. For ESI to work properly, all components in the droplet other than the protein must be volatile. In the case of DMSO, water in the ESI droplet will evaporate at a far greater rate, causing the DMSO to concentrate; so whatever the initial concentration, the final DMSO concentration at the desolvation/ionisation point will always be significantly higher. The effects of this are dramatic. We tested different

BIOGRAPHY



ROD CHALK gained his PhD in 1992 at The Liverpool School of Tropical Medicine, working on the innate immune peptides from the mosquito *Aedes aegypti*. He began using mass spectrometry for bioactive peptide natural product drug discovery at Queens University Belfast in 1996, subsequently working on SNP genotyping and cryodetector mass spectrometry. More recently, Rod has worked in proteomics and biopharmaceuticals using a variety of techniques and instruments. Rod joined the SGC in 2008. His current interests include high-throughput protein characterisation, membrane proteins, quantitative mass spectrometry and the application of native mass spectrometry to structural biology and drug discovery.

EXPERTVIEW



John O'Rourke
Head of Product Development,
Cell Analytics at Sartorius

"With the need to discover new therapeutic targets against challenging antigens, innovation in antibody screening technologies is paramount"

Innovations in antibody screening

Continued development of new screening technologies and tools that can analyse large numbers of samples and datasets will be critical for development of the next generation of antibody drugs.

With breakthroughs in molecular engineering and antibody humanisation, monoclonal antibodies (mAb) are one of the fastest-growing classes of biopharmaceuticals. mAbs are the backbone of many treatment modalities including checkpoint inhibitors and CAR T-cell therapy. With the need to discover new therapeutic targets against challenging antigens, innovation in antibody screening technologies is paramount.

Innovation in antibody library construction has yielded fully synthetic human combinatorial libraries – an immune system in a tube. By circumventing immune tolerance, these libraries may contain antibodies with high affinity

to therapeutic self-antigens that the natural immune system would have edited. With libraries containing up to 1,011 individual members, antibodies with unique properties that aren't found in nature are sure to be discovered.

Besides innovation in library construction, new mammalian cell surface display technology that selects antibodies based on a phenotypic output rather than binding is poised to revolutionise antibody selection. These 'autocrine' display technologies have identified rare antibodies that convert stem cells to dendritic cells, induce leukaemic cells into normal cells, and agonist antibodies that bind to surface receptors inducing alternative

signalling pathways compared to normal cellular ligands. This unbiased, target agnostic screening method may lead to the identification of novel targets and a deeper understanding of cell signalling plasticity.

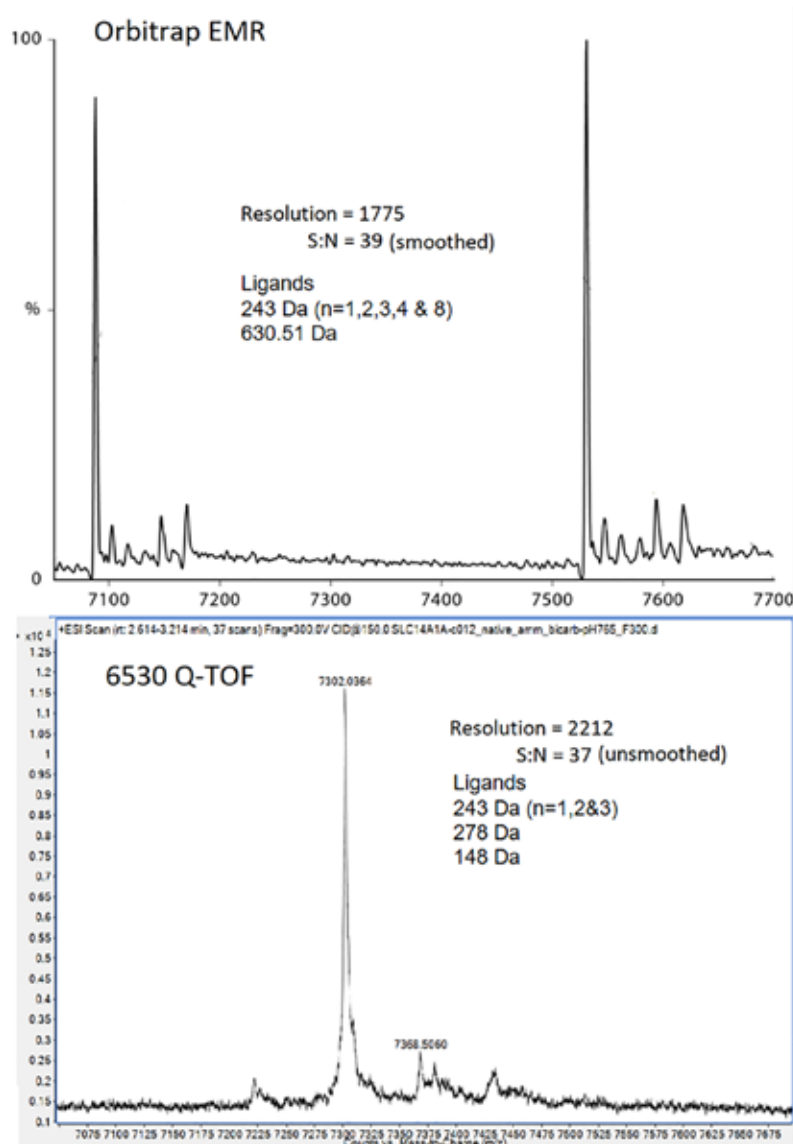
High-throughput screening tools are required to identify these rare antibodies. Advancements in flow cytometry such as the Intellicyt iQue Screener PLUS are enabling antibody discovery scientists to take advantage of these innovations. The combination of multiplex cell-based screening assays, rapid sampling speeds and real-time analysis and visualisation of results provides actionable answers in minutes per plate, not hours or days. ■



BELOW: Deconvoluted spectra for integral membrane protein SLC14A1 from Agilent 6530 Q-TOF (top) and Orbitrap EMR (bottom).



FIGURE 2



concentrations of DMSO on a protein that exists in nature as a 175kDa tetramer with a mean charge radius of 40.7Å and a 350kDa octamer with a mean charge radius of 56.1Å. Even at the lowest concentration, conformational changes equivalent to a 3.2Å reduction in charge radius, or a 19 percent reduction in solvent accessible surface area for the tetramer were observed (**Figure 1**). Similar DMSO effects have been observed by others.^{3,7} We do not believe that incorporation of DMSO invalidates native MS screening results, but rather our data suggest that exchanging DMSO for a more volatile solvent such as methanol will drastically improve the results of a native MS compound screen.

Practicalities

Sample preparation is critically important in any native MS experiment. This involves buffer exchange, usually into a volatile ammonium salt at low molarity pH 6.5 – pH 7.5. This can be carried out offline using three passes through a pre-equilibrated size exclusion spin column; offline by overnight dialysis; or, increasingly, inline and automated by size exclusion LC-MS. Offline automation can be achieved using a Nanomate robot with a microfluidic chip.³ This enables up to 400 MS analyses in a single pass, each acquisition taking just five seconds. A single batch of protein prepared by spin column or dialysis is aliquoted into 96- or 384-well microtitre plates with individual test compounds applied to individual wells. Single samples are usually applied via a static needle drawn by hand and gold-plated for electrical conductivity. Approximately one microlitre of concentrated sample is either drawn into the ESI source by the first stage vacuum or is driven by positive gas pressure. However, in our hands, more dilute sample applied via

a syringe pump to a standard nebuliser tip produces equally good spectra.⁸ Specialised mass spectrometers are not required. All electrospray instruments can operate up to m/z 3,000 and analyse protein-ligand complexes up to 30,000Da. Ion traps have double this m/z range, whereas modern TOFs have m/z ranges of 20,000 or 40,000 and are able to analyse complexes well over 1,000,000Da. High resolution is essential if the bound test compound is to be identified by mass, rather than by a mass-shift associated to its position in the sampling array. Although Orbitrap instruments offer good mass resolution at low mass, this must be somewhat compromised even in

instruments specifically designed to operate at high m/z . We have found that equal or better resolution can be achieved using a TOF (**Figure 2**). MS is an inherently fast technique and recent advances in sample introduction, such as ultrasonic sampling, are expected to establish native MS as a truly high-throughput technique. 

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FURTHER INFORMATION

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“MS is an inherently fast technique and recent advances in sample introduction, such as ultrasonic sampling, are expected to establish native MS as a truly high-throughput technique”



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A high-throughput approach to developing T-cell immunotherapies

This webinar, supported by Sartorius, will discuss the promise and pitfalls associated with developing individualised antigen-specific T-cell therapy products for clinical use.



IMMUNOTHERAPY for cancers involves artificially prompting the immune system to treat cancer. Many cancer cells have surface tumour antigen molecules, which can be detected by the immune system's antibody proteins, which bind to the antigen.

T-cell immunotherapy and CAR T-cell therapies use different approaches to turn our immune system's cancer-fighting cells into better fighters by either increasing their fighting ability or numbers.

T-cell immunotherapy is a rapidly growing personalised

treatment, which expands unmodified cellular products *ex vivo* for re-infusion into patients.

Key learning points

- Methods for designing multi-colour assays specific to T-cell products using advanced flow cytometry
- Identifying unique cytokine combinations that improve T-cell growth and reactivity
- Assays for multiplexed evaluation of cell health, phenotype and cytokine profiling. 

KEYNOTE SPEAKER:



Christopher Lazarski, Ph.D

Director, Flow Core Staff Scientist II, Center for Cancer and Immunology Research, Children's National Medical Center

Dr Lazarski is an immunologist with extensive training in T-cell responses in animal models, vaccines and cell therapy products. His experience in academia and industry involved investigating the signals required for CD8+ and CD4+ effector function in models of infection and immunity, along with the pathways for processing and presentation of vaccine antigens to T cells. He has also developed novel measures for flow cytometric-based analysis of the frequency of lymphocyte subsets and activation. As a Research Scientist at GenVec, he established improved methods for immune cell characterisation including protocols for multi-parameter analysis of T cell, NK-cell and dendritic cell subsets and activation in the spleen, regional lymph nodes, mucosa and within solid tumours. He is currently a Staff Scientist III at Children's National Medical Center (CNMC) where he manages the operations of the Flow Cytometry Core and works within the Immune Testing and Characterisation Lab as part of the Children's Cell Therapy GMP facility.



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Radiochemistry and drug synthesis 'in a flash'

Professor Dr Jason P. Holland

University of Zurich, Switzerland

Conventional methods for radiolabelling proteins are time consuming and difficult to automate. In this article, Jason P. Holland discusses how combining photochemistry with radiochemistry enables development of radiolabelled proteins far more quickly and easily – literally in a flash of light.



BIOGRAPHY



JASON P. HOLLAND is an SNSF Professor of Radiochemistry and Imaging Science at the University of Zurich, Switzerland. Key research areas in the Holland group are the development of novel methods for introducing radioactive nuclides to small-molecules, peptides, proteins and nanoparticles. Novel radiosynthesis methodology is combined with targeted positron emission tomography (PET) imaging of cancer biomarkers to measure alterations in cellular signals that occur during cancer progression and treatment. Recently, the team in Zurich has introduced novel photochemically active tools that allow radiopharmaceuticals to be produced in just a few minutes by simply shining light on the mixtures.

Abstract

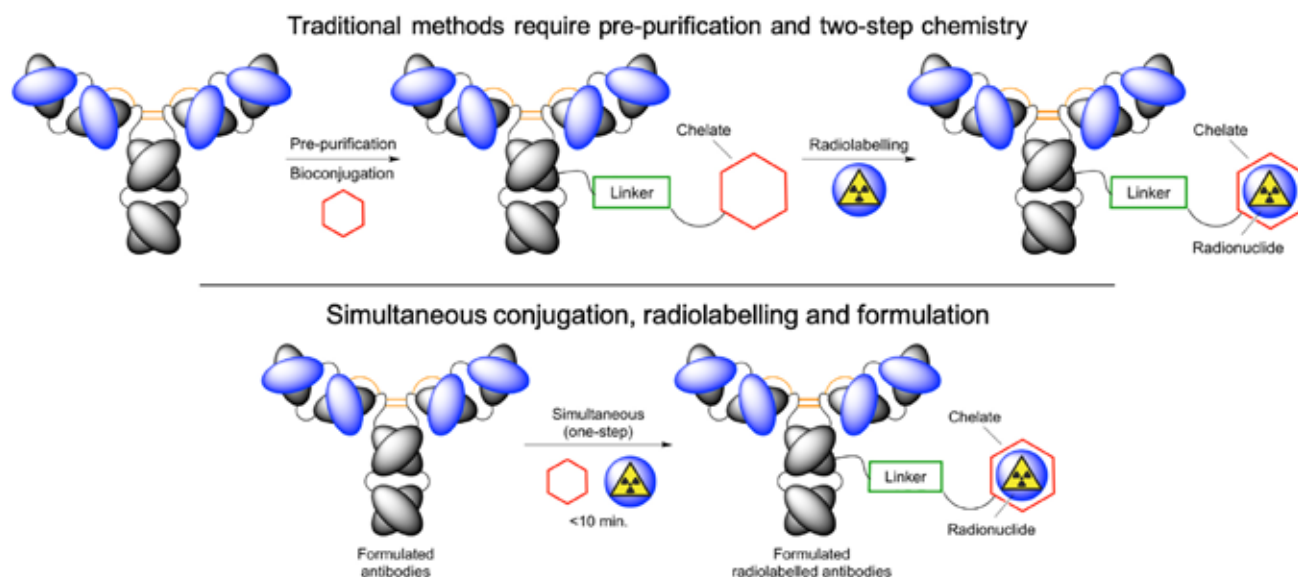
Radiopharmaceuticals are a cornerstone of modern nuclear medicine, but the constraints of working with radioactivity mean that these advanced technologies are often limited to places that can produce radioactive drugs in-house. The exquisite affinity, selectivity and pharmacokinetic properties of monoclonal antibodies (mAbs) and related biologically-active fragments or biosimilars make them attractive starting points for developing targeting agents for immuno-positron emission tomography (immuno-PET) and radioimmunotherapy (RIT). However, for many

small-to-medium sized medical centres, options for radiopharmaceutical production are limited due to the complexity of production and associated high costs. Alternative chemical routes that simplify the production of radiolabelled antibodies are the key to improving access.

Fast, precise, efficient

Chemistry lies at the core of drug discovery and radiotracer development. When it comes to working with radioactivity, time is one of the most crucial parameters.¹ The chemical reactions used to incorporate a radioactive atom into a

FIGURE 1



ABOVE: Established two-step (top) and recent one-step simultaneous (bottom) strategies used to functionalise and radiolabel antibodies and other proteins for immuno-PET



small-drug molecule or an antibody must be fast, efficient, high-yielding and reliable. For production of radiolabelled antibodies (and other proteins) using nuclides such as ^{64}Cu , ^{68}Ga , ^{90}Y or ^{89}Zr , the process typically involves two distinct steps (Figure 1). First, the protein is pre-purified from a stabilised formulated source, followed by functionalisation to add a chelate that binds radioactive metal ions, and then the intermediate construct (the functionalised-mAb) is re-purified. Prior to translation, the chemical, physical and biological properties of the intermediate must be characterised, before being stored until further use. In the second step, a radioactive metal ion is introduced.

Classical manual batch processes dominate the production of radiolabelled antibodies for preclinical and clinical applications but recent advances from a team in Amsterdam have also shown that the radiolabelling step and purification can be automated.² With current clinical solutions, one of the main impediments to the more widespread use of radiolabelled antibodies is the requirement to functionalise, isolate, characterise and store the intermediate protein. In accordance with the regulations from most major authorities, including the United States Federal Drug Administration, the European Medicines Agency and Swiss Medic, the functionalised intermediate is often classified as a new molecular entity (NME) and is therefore subject to stringent adsorption, distribution, metabolism, excretion and toxicological (ADME-tox) testing before it can be used in human trials. Providing full ADME-tox data is time consuming,

expensive and often beyond the reach of most research groups or small medical facilities. Furthermore, limits on the physical quantities of conjugated antibodies that can be produced in the two-step process also present challenges to fulfilling the regulatory requirements.

From thermochemistry to photochemistry

A potential solution to the financial, legislative and practical problems of making radiolabelled antibodies is to seek alternative technologies. Traditional methods for adding the chelate to the antibody use chemical reactions that are stimulated by heat. Many options exist, but in general, the most widely used conjugation reactions are dominated by modification of the mAb at either cysteine (thiolate) residues using maleimido-based reagents, or lysine residues using chelates bearing *N*-hydroxysuccinimide (NHS) or isothiocyanate (NCS) groups.^{3,4} The trouble is that these existing thermochemical methods cannot be readily combined with the radiolabelling step and the reaction conditions are not suited for developing simplified one-pot procedures.

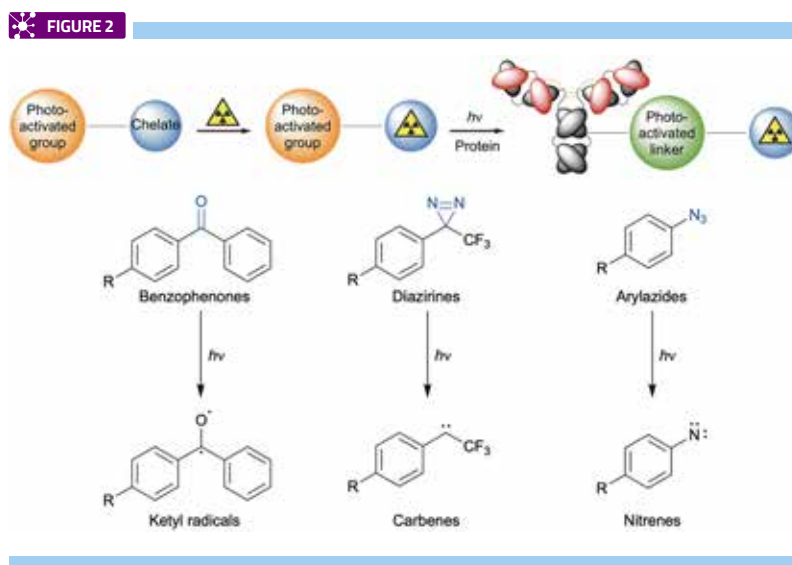
Switching from classical thermochemically-mediated reactions to photochemically-induced conjugation has the potential to solve some of the aforementioned issues associated with making radiolabelled antibodies.⁵⁻⁷ The hypothesis was simple: by using different reactive units that become activated under irradiation with mild ultraviolet (UVC) light, and are compatible with standard formulation buffers and labelling conditions, it may be possible to combine the

“By using different reactive units that become activated under irradiation with mild ultraviolet (UVC) light, and are compatible with standard formulation buffers and labelling conditions, it may be possible to combine the bioconjugation and radiolabelling steps into a simultaneous, one-pot procedure”

bioconjugation and radiolabelling steps into a simultaneous, one-pot procedure. The world of photochemistry provided several viable options for selecting reactive groups which may potentially be combined with good manufacturing process (GMP)-grade antibodies. These include many photoactive reagents based on benzophenones, diazires and aryl azides that were originally popularised as tools for photoaffinity labelling.^{8,9} To narrow the choice, parallel requirements were that the photoactive groups should also generate highly reactive intermediates that are stable to the background media (specifically, water, oxygen and various salts at high ionic strength), and undergo fast, chemoselective bimolecular reactions with proteins. Furthermore, the irradiation conditions should not induce damage to the underlying structure of the protein.

Photoradiosynthesis with aryl azides

While many photoactive groups exist, the constraints of working in biologically-compatible aerated media with high salt content, a narrow acidity window (ca. pH 5-9) and the need to perform biomolecular reactions without pre-association of the chelate to the antibody



limits the choice. Photoaffinity labelling has been successful with reagents that generate carbenes, nitrenes and diradicals upon irradiation (Figure 2).¹⁰ However, the extreme reactivity of carbenes and radicals makes it very difficult to control the conditions so that productive biomolecular

↑ ABOVE: Classic photoreactive groups used in photoaffinity labelling

EXPERTVIEW



Daniel Appledorn

Head of Product Development,
Cell Imaging, Essen BioScience,
a Sartorius company

"We expect that drug discovery will continue to evolve to a balanced approach that leverages the strengths of both phenotypic and target-based strategies"

Expediting therapeutic antibody discovery and development

A significant hurdle in monoclonal antibody discovery and development is the time to result. Here, we discuss how advancements in cell model systems and information-rich assay technologies provide more biologically relevant information early in the candidate screening process.

Since the introduction of the first antibody therapy, significant progress has been made in monoclonal antibody (mAb) development. Improved identification of therapeutic targets and the development of chimeric and fully human antibodies to decrease immunogenicity improved the efficacy and safety to make mAb therapy the gold standard in many disease applications. These early successes, the significant progress in mAb design and efficacy, and the more recent explosion of oncology targets makes the antibody drug industry one of the fastest-growing in the pharma sector.

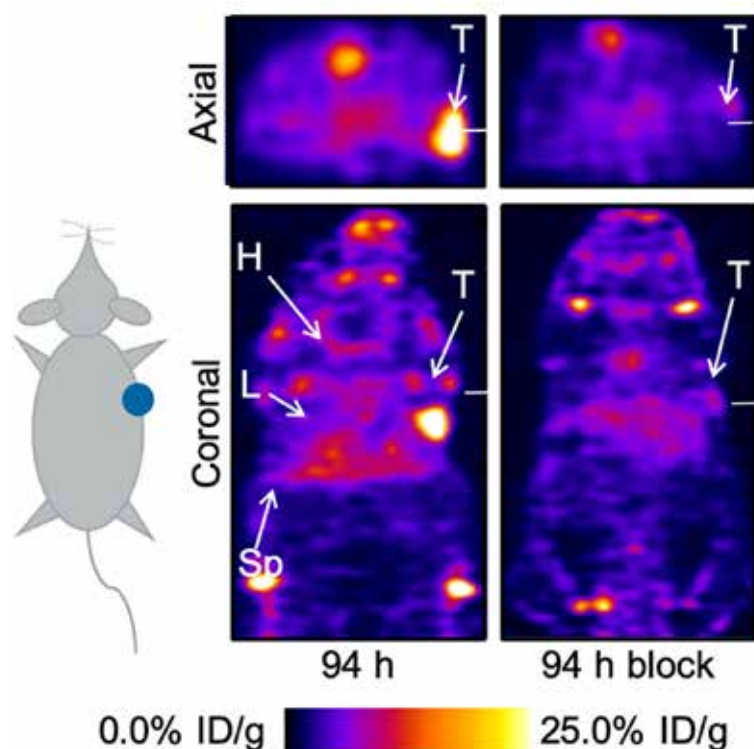
One look at the pipeline of any major pharma company reveals a common theme: drugs targeting immune cell markers, or immune cells themselves, will be at the forefront of research over the next few decades.

Fundamental to success will be identification of the right tumour-associated targets. There is a tendency to take a holistic approach to identify new targets. High content cell-based assays provide rich biological information earlier in the drug discovery process and since most are multi parameter and analyse single cells, they can be considered inherently "phenotypic." Recent advances in assay technology provide the ability to perform these multiplexed, multiparameter assays at high-throughput screening speeds. By collecting more information in primary screens, researchers can simultaneously identify hits based on specificity and cross-reactivity. Inclusion of more information early in the screening process builds confidence in potential hits. Using appropriate *in vitro* cell-based analytical technologies that translate

well to the clinic increases the likelihood that candidate molecules will successfully proceed to the discovery and development process.

We expect that drug discovery will continue to evolve to a balanced approach that leverages the strengths of both phenotypic and target-based strategies. The implementation of complex cell models using primary cells or human-induced pluripotent stem cells in multicellular, 3D models that more closely resemble the human microenvironment will provide a new depth of understanding in drug discovery. Development of these more sophisticated models and advancing the technologies required to evaluate them will significantly accelerate the antibody screening process, providing drug candidates with the promise of clinical success. ■

FIGURE 3



ABOVE: Small-animal immuno-PET images of $^{89}\text{ZrDFO}$ -azepin-trastuzumab in mice bearing subcutaneous SK-OV-3 human ovarian tumours. H=heart, L=liver, Sp=spleen, T=tumour



“In the future, the synthesis of complex drugs might be as simple as switching on the light”

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coupling to the antibody occurs at a rate that exceeds background quenching reactions with the solvent, dioxygen, or other species in the mixture. In contrast, aryl azides (and more generally aromatic azide species) also produce several short-lived, highly reactive intermediates; the parent of which is photolytically produced by loss of $\text{N}_2(\text{g})$ as open-shell singlet nitrene (PhN^1) that has a half-life in the order of a nanosecond.¹¹ Intramolecular rearrangement is the kinetically favoured pathway for the PhN^1 and rearrangement first produces a bicyclic benzazirene, which undergoes ring insertion of the nitrogen atom and expansion to form a seven-membered ketenimine ring. Density functional theory calculations have shown that this ketenimine reacts chemo-selectively with primary amines such as the $\epsilon\text{-NH}_2$ side-chain of a lysine residue, which are present in abundance on the solvent-exposed surface of antibodies. The reaction is photoinitiated at 365nm but the bimolecular coupling step remains under thermal control. Calculations (B3LYP/6-311++G(d,p)/PCM) of the reaction pathway confirmed that overall the coupling reaction is thermodynamically feasible ($\Delta_r G = -166 \text{ kJ mol}^{-1}$) with a low thermodynamic barrier to nucleophilic attack of a primary amine on the ketenimine ring ($\Delta_{\text{TS}} G = +54 \text{ kJ mol}^{-1}$). Much like ‘The story of the three bears’ by Robert Southey, it is perhaps fortuitous that photoinitiated activation of aryl azides generates reactive intermediates that

are not too reactive but operate in just the right kinetic window to facilitate rapid photochemical conjugation whilst mostly avoiding competitive non-productive quenching reactions.

Immuno-PET studies with various radiotracers including $^{89}\text{ZrDFO}$ -azepin-trastuzumab for molecular imaging of HER2/*neu* receptors in ovarian, breast, gastrointestinal and other cancers confirmed that photoradiosynthesis can produce viable imaging agents (Figure 3). Additional experiments also confirmed that standard IgG_1 molecules remain biochemically viable and are not photodegraded by irradiation with light at 365 or 395nm, which makes aryl azides versatile reagents for future developments of photochemically-mediated protein ligation reactions.

The future is bright

Radiopharmaceuticals represent just one potential area wherein photochemistry may have an impact in clinical drug development. Of the ‘Top 10 blockbusters’ in 2018, seven were classified as biologics with most based on monoclonal antibodies. Antibodies are certainly important, but experience has found that as a monotherapy, antibodies are limited by poor tissue penetration and other morphological, genetic and pharmacological factors that limit their therapeutic index. To circumvent some of these issues and to improve potency, pharmaceutical companies and research groups alike are turning their attention toward antibody-drug conjugates (ADCs). There are now four US FDA-approved ADCs and the development of extremely expensive drugs like brentuximab vedotin (Adcetris) or trastuzumab emtansine (Kadcycl) requires the development of efficient, reliable and sophisticated conjugation chemistries that provide not only chemoselective functionalisation but also regioselective, stereoselective and stoichiometrically precise products. Photochemistry may not be able to solve all of the challenges, but as early experiments using radioactivity have shown, in the future, the synthesis of complex drugs might be as simple as switching on the light. ☺

ACKNOWLEDGEMENTS

JPH thanks the Swiss National Science Foundation (SNSF Professorship PP00P2_163683), the Swiss Cancer League (Krebsliga Schweiz; KLS-4257-08-2017), and the University of Zurich (UZH) for financial support. This project has received funding from the European Union’s Horizon 2020 research and innovation programme from the European Research Council under the Grant Agreement No 676904, ERC-StG-2015, NanoSCAN. Thanks are due to all members of the Radiochemistry and Imaging Science group at UZH for helpful discussions and productive collaborations.

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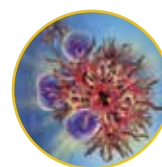
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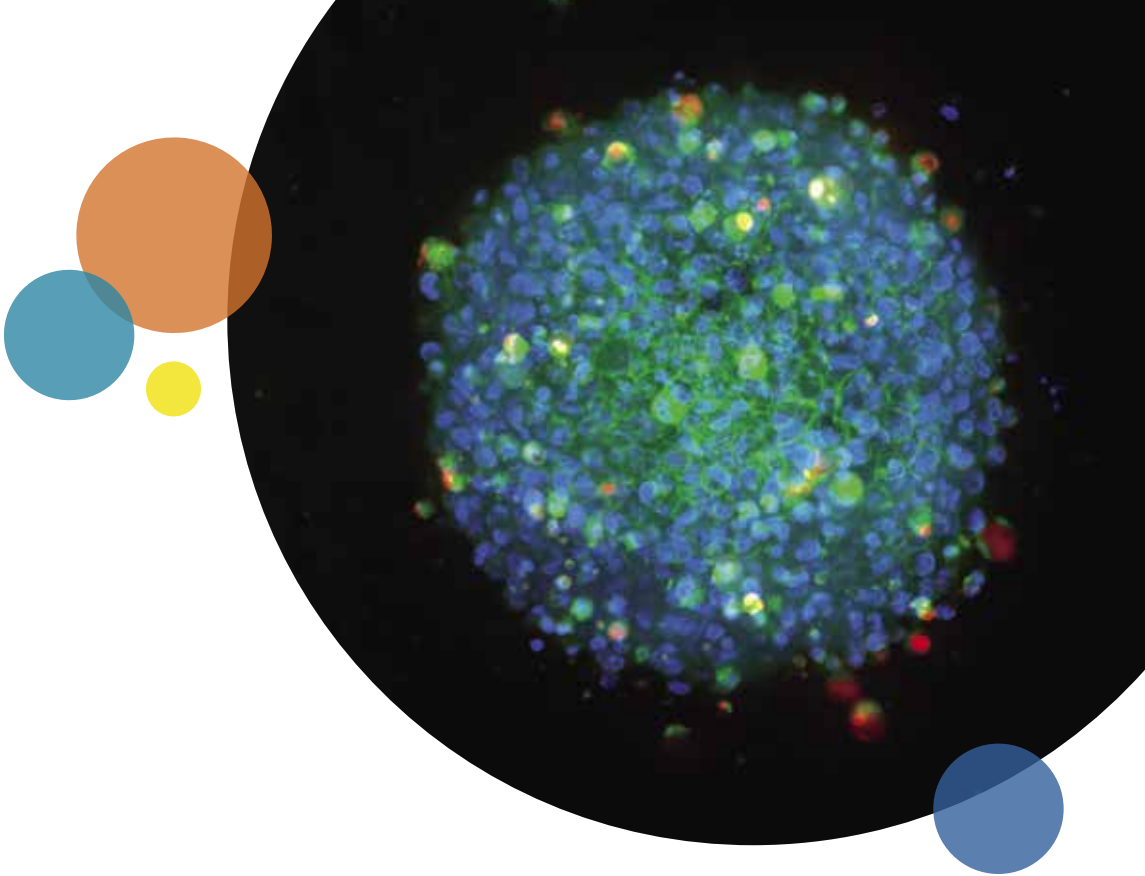
The potential of three-dimensional cell culture models is now well-recognised by scientists, however there are some challenges. Margaritha Mysior and Jeremy Simpson address some of the common challenges of working with spheroids and organoid models – including how they are developed, how to obtain high-resolution, high-content images and how to develop three-dimensional high-resolution imaging assays.

Siobhán Leonard, Victoria Juskaite and Laura Murch from LifeArc, an independent medical research charity in the UK, explain how they're using the latest imaging technologies to progress drug discovery campaigns and identify the most promising candidates for antibody-based therapies.

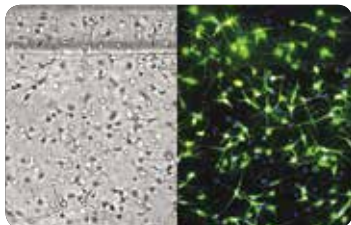


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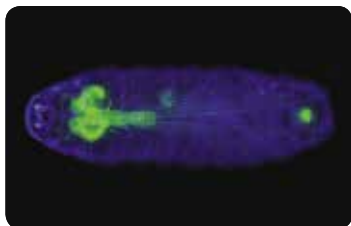
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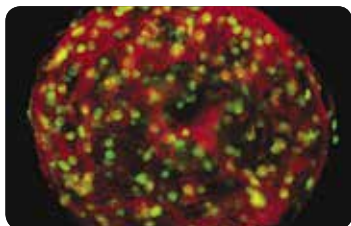
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High-content screening of complex physiologically-relevant cell models

High-content screening (HCS) approaches have become increasingly common in drug discovery with HCS technologies integrated across the drug discovery pipeline. Here we discuss recent developments in this arena.

RECENT YEARS have seen an increasing demand for drug discovery and development processes to use more predictive, higher complexity, physiologically-relevant three-dimensional (3D) cell models which better mimic *in vivo* environments than simpler two-dimensional (2D) models. By reproducing important parameters of the *in vivo* environment, 3D models can enable improved assessment of drug toxicity and target validation. Such 3D cellular models may include spheroids, organoids or induced pluripotent stem cell (iPSC)-derived cell models. Another important and expanding field for 3D cell culture-based models is that of 'organ-on-a-chip', whereby living human cells are assembled onto microchips using microfluidic technologies. This technology has the potential to revolutionise drug development, disease modeling and personalised medicine. These microchips offer an alternative to traditional animal testing and can potentially offer a quicker path to clinical trials.

While the development of quantitative assays using 3D cell models has emerged as an attractive investigative tool, challenging 3D high-content image acquisition and analysis workflows have hindered wider adoption by the screening and automated imaging communities. Next-generation high-content, high-throughput tools for microscopy offer innovative and automated techniques for evaluating this complex biology. One company that has led the way with this technology is Molecular Devices. With technology such as the ImageXpress Micro Confocal High-Content Imaging System and MetaXpress 3D Analysis Module with 3D Viewer, screening 3D cell models within a single, integrated interface can dramatically reduce the time to discovery. Implementation of these complex 3D assays also requires high resolution to capture publication-quality images and data.

Enhanced assay sensitivity can be achieved by taking advantage of the optical properties of confocal imaging, capturing images with a high signal-to-noise ratio while reducing out-of-focus light for crisper images and accurate cellular detail.

Molecular Devices is partnering with companies who specialise in physiologically-relevant cell models such as MIMETAS, who offer the OrganoPlate, a unique 3D organ-on-a-chip platform. This is a fully compatible microfluidic culture plate, enabling testing of compounds in any throughput on miniaturised organ models. Molecular Devices also work closely with HCS Pharma, who create innovative 3D cellular models which enable researchers to perform phenotypic screening on more relevant 3D cellular assays that also consider the extracellular matrix (ECM). To reach this goal, HCS Pharma have acquired BIOMIMESYS technology, which is a unique and natural hyaluronic acid-based hydrosc scaffold, biofunctionalised with other ECM components to better mimic the microenvironment of every organ. All these technologies are being imaged and analysed with the ImageXpress Micro Confocal High-Content Imaging System.

There is a trend towards simplicity in high-content screening approaches. Researchers create the greatest value for their organisation when they focus their efforts and time on their research and not on learning how to use the suite of complex instrumentation within their laboratory. With this in mind, the ImageXpress Pico Automated Cell Imager was recently launched as an easy-to-use imaging system with over 25 pre-configured analysis protocols available. In addition, it features a browser-based, icon-driven software which enables untrained scientists to easily access their data anytime and anywhere.



Andy Bashford, PhD, European Imaging Application Scientist, Molecular Devices

One of the challenges with the increasing complexity of HCS approaches, particularly with the movement towards complex 3D models and the resurgence of multi-parametric phenotypic screening, is the increased volumes of both image data and metadata, which needs to be stored and archived safely, resulting in greater reliance on a high-quality, redundant IT infrastructure. This challenge is further compounded by the necessity to conserve data long term. Finally, while cloud-based data management has been discussed as a possible solution, at this point such infrastructure is unlikely to adequately address the demands of these higher-complexity assays, or to overcome the reluctance of users to store their data on third-party servers. That said, Molecular Devices' HCS solutions offer unique capabilities to reduce the overall "data burden" that comes with adoption of these complex screening approaches. For example, they have developed targeted imaging workflows to pre-scan samples at low magnification in order to identify hits, and then capture more detailed data for only those hits at higher magnification – ultimately streamlining hit evaluation and saving disk space. ■



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High-content imaging: challenges of the 3D world

Margaritha M. Mysior and Jeremy C. Simpson

UCD Cell Screening Laboratory, School of Biology & Environmental Science, University College Dublin, Ireland

The potential of three-dimensional cell culture models is now well-recognised by scientists; however, there are still some challenges. Margaritha Mysior and Jeremy Simpson address some of the common challenges of working with spheroids and organoid models – including how they are developed and how to obtain high-resolution, high-content images and how to develop three-dimensional high-resolution imaging assays.



BIOGRAPHY



MARGARITHA MYSIOR graduated with a BSc and MSc degree in Molecular Biology from the University of Applied Sciences Gelsenkirchen, Bocholt and Recklinghausen, Germany. The final year of her MSc degree was in the lab of Jeremy Simpson at University College Dublin, Ireland, characterising several newly identified regulatory proteins of the Golgi-ER retrograde pathway in mammalian cells. She started her PhD in the same lab in 2017 and is currently developing a number of imaging-based assays to study the endomembrane system in a three-dimensional cell model.

ALTHOUGH the famous phrase “a picture paints a thousand words”, attributed to the marketing executive Frederick R Barnard, first entered common use in the English language almost 100 years ago, its origins are thought to date back several centuries. However, this old adage has never been more relevant than now in the twenty-first century. Fluorescence microscopy images and their exploitation to understand cell function is arguably the most fundamental resource employed today in research laboratories around the world. Their value lies in the fact that visualisation of molecules, organelles and their interactions and behaviour is now easily facilitated through an ever-expanding palette of fluorescence reporters and tools. These can be applied in real time and in living material, giving researchers unprecedented access to the inner workings of cells.

However, the paradigm shift in recent years has been the transition to quantitative imaging, such

that now the most complex of phenotypes observed in an image, or indeed in some cases not even visible to the human eye, can be described in mathematical ways. This evolution of quantitative microscopy is referred to as high-content imaging, emphasising that the image itself is a rich source of information. In turn, the quantitative interpretation of images is achieved through high-content analysis (HCA).¹ The power of this technology is that it can be applied in a highly reproducible and high-throughput manner allowing researchers to delineate cellular pathways on a systems-wide scale, thereby enabling drug discovery and development in the global pharmaceutical industry.²

To facilitate the HCA process, the vast majority of experiments reported to date have utilised cultured cells effectively growing in two dimensions in flat, optically-transparent multi-well plates. This format is convenient from a cell culture perspective and presents relatively few problems for advanced automated microscopes to gather millions of

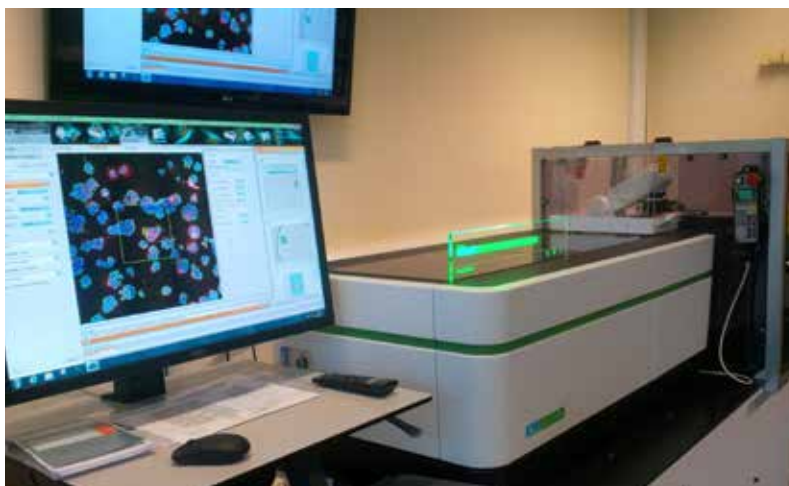
in-focus images in short periods of time (**Figure 1**). However, while such experiments are certainly informative, this format does not accurately represent the arrangement of cells in complex tissues and organs, and therefore has limitations.

One can envisage that events such as cell-cell signalling and communication – the interplay between the different cell types found in tissues and the mechanisms by which therapeutics transit across cellular layers – are not necessarily reliably recapitulated from simple cell monolayer models. It is therefore clear that the next phase in our deeper understanding of such events in cells will only be realised through models that more closely resemble the *in vivo* situation.

Methods of producing three-dimensional cell models

Although three-dimensional cell culture models have been described for several decades, it is only in the last few years that their potential has been more widely appreciated by biologists.³ This is largely a result of new technical developments that facilitate easier production in the laboratory, coupled with a new generation of automated confocal microscopes that can rapidly capture information from multiple planes within them. Most multicellular three-dimensional models take the form of spheroids, which can be of a solid cell mass or hollow core format, or organoids, which are morphologically similar to spheroids but derived from multiple cell types. Most commonly, spheroids and organoids can be assembled in one of four ways: 1) simple aggregation in U-bottomed wells, 2) aggregation in a liquid droplet, 3) self-directed assembly in, or on, a matrix or scaffold, and 4) in a microfluidic or micropatterned device. Each of these methods has its respective advantages and disadvantages, typically linked to the level of control of spheroid/organoid assembly and scale of their production. From a fluorescence imaging perspective, the aggregation methods in plates or droplets have the advantage that typically a single spheroid per well is formed, meaning that its identification in the imaging process, assuming that it is centrally located, is relatively straightforward. However, this approach is generally considered as low-throughput and is not easily scalable for high-throughput imaging-based screens. Matrix- and scaffold-based methods allow for much higher numbers of spheroids to be produced per well, typically upwards of 500 assemblies per well in a 96-well plate, for example; although this approach suffers from problems associated with autofluorescence from the matrix and the fact that spheroids assemble randomly across the well, including at multiple heights. This means that large numbers of confocal image slices may be required to capture the full depth of the spheroid, resulting

FIGURE 1



in lengthy image acquisition times. Microfluidic and micropatterned devices are attractive for spheroid generation as they can be custom-designed and provide a highly controlled environment, but they can be time-consuming and technically challenging to manufacture, and/or expensive to purchase on a large scale. Regardless of the method of spheroid generation selected, they present several opportunities for the next generation of cell-based assays that need to consider the local microenvironment. The challenge, therefore, is how to both transition well-established assays in two-dimensional form into these cell models, as well as develop novel assays with better tissue and/or clinical relevance.

The challenges of performing assays in three-dimensional models

There are several obstacles to establishing assays in three-dimensional models. Many of these relate to the heterogeneity of spheroids within any given population, with respect to the cell numbers within each spheroid, as well as their shape, size and distribution within the structure. In addition, many cell-based assays involve multiple manipulation steps, which can add complexity and unreliability to the assay. Also of note is that manipulations and perturbations applied to the cells (for example, transfections and drug treatments), which are highly routine in two-dimensional models, may not be equal across all cells within a spheroid. From an imaging perspective, consistent staining of structures of interest is essential to avoid quantification errors between cells within the same spheroid. Consideration should also be given to the fact that resolution is typically three-fold inferior in the z-dimension, compared to that in the xy plane. Furthermore, optical aberrations can become



ABOVE: Fully-automated high-content screening microscope

BIOGRAPHY

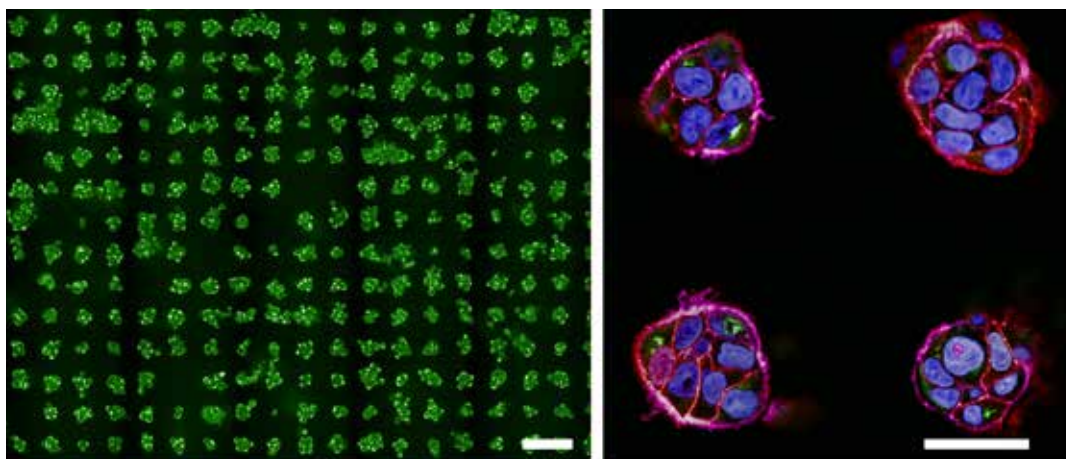


JEREMY SIMPSON carried out his PhD work at the University of Warwick, UK and post-doctoral work at the Scripps Research Institute in San Diego, USA, the ICRF in London, UK, and the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany. In 2008 he was appointed Professor of Cell Biology at University College Dublin, Ireland. His lab currently applies high-throughput imaging technologies to study membrane trafficking processes and how cells interact with nanomaterials. He has authored over 90 peer-reviewed articles, runs the UCD Cell Screening Laboratory (www.ucd.ie/hcs), and is a principal investigator in the CÚRAM Centre for Research in Medical Devices (www.curamdevices.ie).

RIGHT: Arrays of spheroids. Left panel shows a portion of a single well from a micropatterned 96-well plate containing over 250 mini-spheroids. Scale bar is 100µm. Right panel shows zoomed view of four spheroids, labelled for nuclei (blue), actin (purple), cell-cell junctions (red) and the Golgi complex (green). Scale bar is 40µm.



FIGURE 2



“Three-dimensional spheroid and organoid models present an exciting opportunity to bridge the knowledge gap between our understanding of cell function obtained from classical monolayer cultures and that coming from tissues”

significantly deep inside spheroids, particularly in structures greater than 150µm in diameter. Some of these issues can be resolved by greater standardisation of spheroid assembly, particularly using production methods that offer a higher degree of control. Standardisation of spheroid size and shape is undoubtedly of critical importance in drug development and delivery screens; with, for example, the efficacy of penetration of therapeutics across multicellular layers understood to be directly influenced by the arrangement of the cells. While not suitable for all applications, micropatterning of spheroids in highly regular arrays offers exciting possibilities for assay standardisation in drug delivery applications, as it allows highly uniform production of spheroids of defined size and potentially shape (Figure 2).

Data, data, and still more data

Once cell-based assays and appropriate imaging regimes have been established in a three-dimensional format, the next challenge to be addressed is how to extract meaningful data from the images. Use of HCA algorithms to precisely segment individual cells and subcellular structures from monolayer-grown cells is now routine, and quantification is highly reliable. The situation in spheroids, however, is more complex, as individual subcellular objects of interest must first be associated with the correct cell, and then each cell with the spheroid; and all of this with consideration to the three-dimensional space. To be successful, it is therefore essential that a sufficiently high image-sampling frequency is applied in the z-plane through the complete volume of the spheroid. If this is achieved, HCA potentially has the power to provide rich quantitative information describing subcellular morphologies and phenotypes as well as, for example, the efficacy of penetrance of therapeutics to particular

cells within the different layers of the spheroid or organoid. Such information is undoubtedly of huge potential benefit to the drug discovery community. While there is excitement about the knowledge that such experiments can deliver, this deep level of content is extremely challenging in terms of data handling. For example, imaging of approximately 750 small four-day-old spheroids, with a diameter of approximately 60µm, in a single well of a 96-well plate requires the acquisition of at least 50 optical slices in the z-direction in order to capture the complete volume of the objects. For a dual colour channel image this equates to 45Gb of data per well and 4.3Tb of data per plate. Larger spheroids have significantly greater depth, requiring many more z-planes to be acquired, and the data volume can easily approach 12Tb for an entire 96-well plate. Clearly, such massive datasets not only present problems from the perspective of image analysis, but there are implications for the feasibility of how such data volumes can be stored and mined.

Three-dimensional spheroid and organoid models present an exciting opportunity to bridge the knowledge gap between our understanding of cell function obtained from classical monolayer cultures and that coming from tissues. This gap is arguably of critical importance in the field of drug discovery and delivery, with still too many pharmaceuticals failing at late stages in the development pipeline process. Although there are clearly several challenges associated with transitioning fluorescence assays into three-dimensional models, they nevertheless offer a new avenue to be explored, and the pharmaceutical industry in particular would be foolish to ignore their potential. Perhaps in the not too distant future, even ten thousand words may be insufficient to describe the information that spheroid images (or pictures) hold. 📷

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Image-based characterisation of cancer biotherapeutics

Siobhán Leonard, Victoria Juskaite and Laura Murch

Scientists, Target Validation Group, LifeArc

Scientists from LifeArc, an independent medical research charity in the UK, explain how they're using the latest imaging technologies to progress drug discovery campaigns and identify the most promising candidates for antibody-based therapies.

THERAPEUTIC antibodies have progressively changed the paradigm of cancer care in recent years, with at least 28 monoclonal antibodies¹ and four antibody-drug conjugates (ADCs)² in clinical use to date. The exponential growth of this field has coincided with significant advancements in imaging platforms, facilitating in-depth pre-clinical assessment of novel therapies.

LifeArc has experience in the successful humanisation of therapeutic antibodies (Keytruda, Entyvio, Actemra and Tysabri) and has harnessed the capabilities of kinetic and high-content image analysis systems to identify lead candidates and characterise their impact on physiologically relevant two-dimensional (2D) and three-dimensional (3D) *in vitro* models.

Kinetic platforms offer a powerful method to dissect basic biology, which is crucial to the advancement of promising anti-cancer agents as it enables dynamic changes in cell viability, migration, invasion, immune cell proliferation and immune cell killing to be rapidly assessed in 96- and 384-well formats. High-content systems provide a means to further investigate complex scientific questions with sophisticated sensitivity and, when coupled with intuitive analysis workflows, this intricate, multiparametric data can have a significant impact on the progression of drug discovery programmes.

Antibody internalisation

The majority of successful therapeutic antibodies for oncology are selected for their capacity to induce antigen-dependent cytotoxicity or to provoke immune-mediated tumour cell death. Critical to the success of such mechanisms is antigen availability. Investigating antibody internalisation achieves deeper understanding of antibody and antigen turnover, which allows the best possible drug candidates to be progressed. In addition, ADCs, which enable highly specific delivery of a toxic payload to a target tumour cell, are emerging as effective therapeutics in oncology.

The development of robust methodologies that screen for candidate internalisation to the lysosome, where the linker will be cleaved to release the attached cytotoxin, are also essential to an ADC programme. While flow cytometry can be used to monitor antibody internalisation, this method proves difficult to miniaturise and optimise for screening.

In order to facilitate high-throughput analysis of hybridoma supernatant and humanised variant internalisation, we routinely utilise an antibody labelling reagent, which emits a fluorogenic signal when processed via acidic lysosomes and endosomes. The antibody/labelled complex can be directly

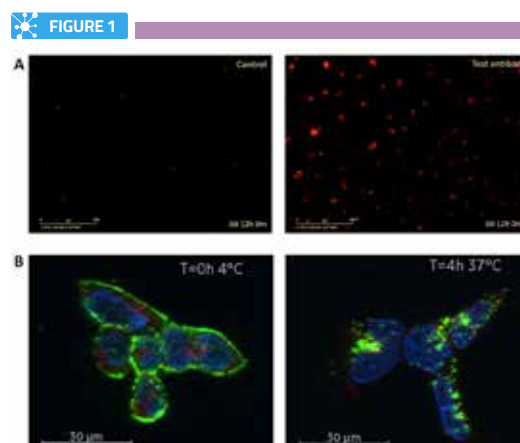


FIGURE 1 ABOVE: Antibody internalisation in HEK293 cells overexpressing a receptor of interest. **(A)** An isotype control or a test antibody against the target of interest was incubated with InCuCyte Human FabFluor-pH Red Antibody Labeling Reagent before addition to cells, then monitored over time for red object area on the InCuCyte S3 Live-Cell analysis system using a 10x objective. The labelling reagent only emits a red fluorescent signal at low pH and thus indicates antibody internalisation to the acidic lysosome. **(B)** Cells stained with an AlexaFluor488-conjugated antibody against the target shown in green, lysosomes stained with LysoTracker Deep Red shown in red and nuclei stained with Hoechst 33342 shown in blue. The antibody appears restricted to the cell membrane at the initial time point (0 hours at 4°C); after a 4-hour incubation at 37°C clear internalisation is observed with the appearance of punctate structures which co-localise with lysosomes.

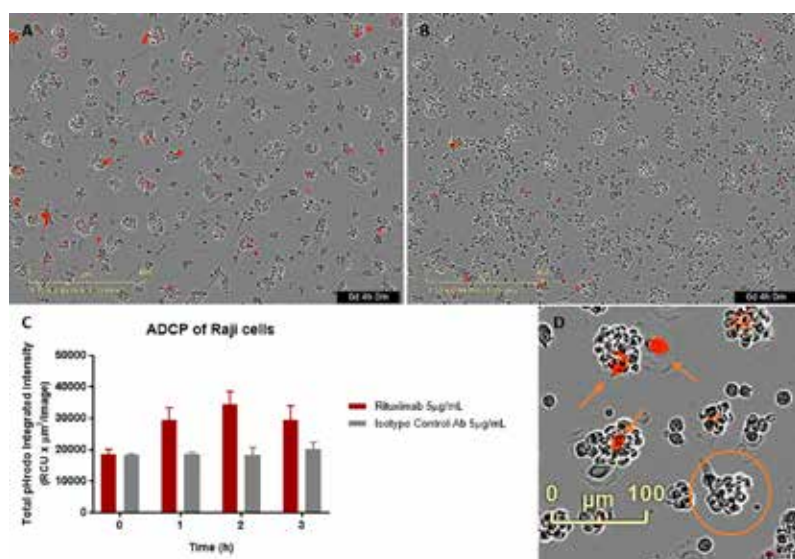
BIOGRAPHY



SIOBHÁN LEONARD joined LifeArc in 2016 as a Scientist within the Target Validation Group in the Centre for Therapeutics Discovery. Siobhán gained a BSc in Biomedical Science, a PhD in Cellular and Molecular Biology and carried out a post-doctoral fellowship at the National University of Ireland, Maynooth. With eight years experience in early-stage drug discovery, Siobhán is involved in designing cell-based assays to support the pre-clinical development of antibody, antibody drug-conjugate and small molecule programmes at LifeArc.

“Investigating antibody internalisation achieves deeper understanding of antibody and antigen turnover, which allows the best possible drug candidates to be progressed”

FIGURE 2



ABOVE: Raji target cells labelled with IncuCyte pHrodo Red Cell Labelling Kit were pre-treated with 5μg/mL Rituximab (A) or control antibody (B) and subsequently seeded onto a layer of primary human monocyte-derived macrophages. Plates were incubated on the IncuCyte 53 Live-Cell analysis system and images taken each hour using the 10x objective. Example images shown after 4-hour incubation for Rituximab treated (A) and isotype control treated (B) conditions. ADCC activity was quantitated by measuring the total pHrodo red fluorescence intensity in macrophage cells and plotted for comparison over time (C). It was noted that the target Raji cells form small clusters over time, as they would during routine culture (highlighted in orange circle (D)). The monocyte-derived macrophages interact with, migrate over and phagocytose the target cells (examples of this highlighted by orange arrows in (D)).

BIOGRAPHY



LAURA MURCH is a Scientist within the Target Validation Group at LifeArc and is responsible for designing cell-based assays to characterise and validate biotherapeutic and small molecule candidates for various drug discovery programmes. Laura gained a BSc in Human Biology, a PhD in Leukaemia Biology, and carried out a post-doctoral fellowship in Rheumatoid Arthritis at the University of Birmingham. Laura has worked in R&D for nine years and prior to joining LifeArc in 2018 held posts at MedImmune Ltd and Crescendo Biologics Ltd.

added to cells and monitored in real time to provide vital kinetic data in a rapid manner (Figure 1A). While providing robust results, this information also allows more complex analysis of the intracellular trafficking of candidate antibodies using high-content imaging platforms to be conducted with an informed approach (Figure 1B).

We collaborate closely with academics to develop project-specific assays for high-throughput analysis in order to progress drug discovery campaigns. The coupling of kinetic and high-content imaging systems in the screening of antibodies expedites the process of candidate selection by presenting an in-depth picture of intracellular biology and provides further confidence in potential therapeutics. Given the high costs of attrition in the clinic, it has become increasingly important to expand our knowledge of modulators for cancer therapy, and while an understanding of antibody engagement with the target antigen is critical to development, the impact of the Fc domain of a potential biotherapeutic on immune effector functions should also be evaluated.

Fc effector function

Interrogating the potential impact of immune cell effector function includes screening candidates for activation of monocyte or macrophage engulfment

of target cells (antibody-dependent cellular phagocytosis (ADCP)), NK cell-mediated target cell death (antibody-dependent cellular cytotoxicity (ADCC)) and activation of the complement cascade to induce target cell death (complement-dependent cytotoxicity (CDC)).

In order to set up physiologically relevant assays for candidate screening, Fc effector function assays typically require the isolation and use of primary cells or sera from human blood. The isolation and culture of such material, however, is time consuming and the relatively small cell numbers procured from such isolations can present limitations when setting traditional endpoint assays such as flow cytometry analysis. Assay readouts, such as luminescent cell viability dyes, have further drawbacks; for instance, being unable to distinguish between target cell and effector cell death.

In contrast, kinetic imaging systems enable real-time monitoring of co-culture assays and can be multiplexed with more traditional approaches to maximise the insight that can be garnered from a single experiment. Additionally, these assays are typically amenable to miniaturisation, which further increases the output from a single primary cell isolation.

In the course of our work, we have focused on developing screening assays for ADCP, ADCC and CDC Fc effector function. The use of apoptosis assay reagent allows for label-free analysis of cell death to be monitored in real time. We routinely combine this with tumour cell-line labelling to allow specific tumour cell death to be analysed. These dyes negate the use of cell perturbing or labour-intensive cell labelling steps for target cells ahead of assay set up. For analysis of ADCP by monocyte-derived macrophages, we have developed a 384-well plate assay to visualise target cells being engulfed by macrophages (Figure 2). This allows specific tumour cell death to be analysed and can be multiplexed with other endpoint viability analyses.

Given the feasibility of high-throughput screening versions of ADCC, ADCP and CDC assays using kinetic imaging platforms, it is now possible to gain insight into the Fc effector function at earlier stages of development for larger numbers of candidates using physiologically relevant cell types. As such, this increases our ability to identify lead candidates with profiles that suit the intended mode of action in the clinic.

Impact on the immune cell compartment is not limited to Fc effector function, but also includes interrogating the effect of potential therapies on T-cell proliferation as well as tumour cell-killing assays.

T-cell activity

In addition to assessing antibody internalisation and Fc effector functions, LifeArc routinely utilises

imaging for kinetic image-based analysis of effector T-cell function. Effector T-cells have the potential to control tumours, and therapies that boost T-cell killing of tumour cells have been/are currently in development, including immune checkpoint inhibitors, adoptive T-cell therapy and chimeric antigen receptors. Development of such therapies requires establishment of robust assays to measure effector T-cell activity, which can be analysed by assessment of cell proliferation, cytokine release, expression of cell-surface markers and tumour cell-killing ability. While these functions may be analysed by conventional flow cytometry methods, imaging offers unique advantages, such as visualisation of T-cell morphological changes including clustering as well as the possibility of multiplexing with endpoint assays.

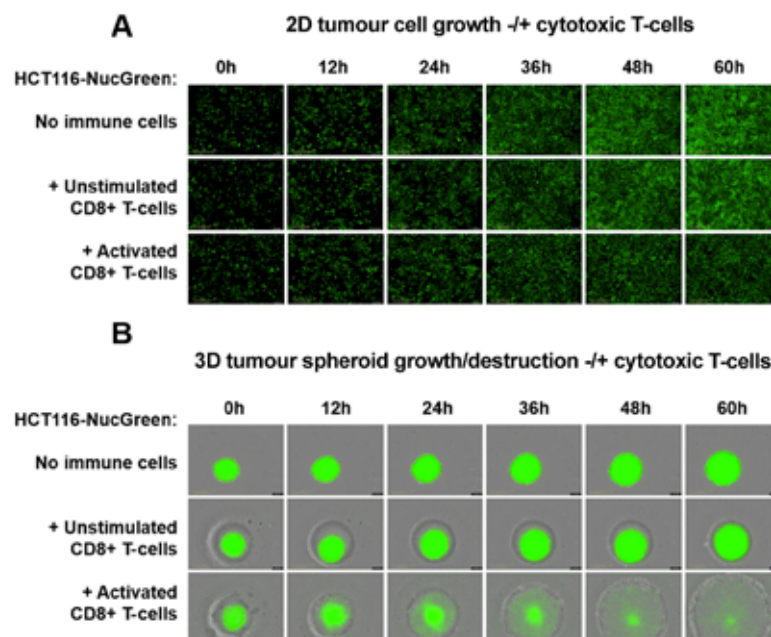
Imaging-based methods are particularly useful to assess therapeutic effects on the immune cell-killing of tumour cells, which is often analysed by co-culturing tumour cells with peripheral blood mononuclear cells (PBMCs) or cytotoxic T-cells. This requires tumour cells to be distinguishable from immune cells, which can be achieved by using fluorescently labelled tumour cells in imaging assays. We have established kinetic image-based immune cell-killing assays by using stable tumour-cell lines expressing nuclear-restricted green fluorescent protein (GFP). Co-culture of these cells with activated cytotoxic T-cells clearly demonstrates reduced tumour cell growth (**Figure 3A**). In addition to this, the assay may be combined with cytotoxicity or apoptotic reagents for multiplexed measurement and automatic quantitation of tumour-cell apoptosis or cytotoxicity in a single well.

While 2D cell cultures are valuable, this system does not recapitulate the physiological 3D environment and as a result sometimes provides ambiguous and non-predictive data for *in vivo* responses, which is a contributory factor to drug failure in clinical trials.³ We measured T-cell mediated destruction of tumour spheroids with our assay (**Figure 3B**), which, as with monolayer co-culture assays, may be combined with apoptosis or cytotoxicity reagents.

Summary

The use of kinetic and high-content imaging platforms to characterise biotherapeutics provides enhanced insight into therapy effects, particularly when paired with physiologically relevant models and 3D systems. Imaging assays deliver a wealth of content through multiparametric analysis of cell number, morphology and area, in addition to fluorescent readouts. Furthermore, these insightful assays can often be multiplexed with more 'standard' assay formats; for example, flow cytometry for effector/target-cell analysis or testing

FIGURE 3



ABOVE: Monitoring tumour cell or spheroid growth in immune/tumour cell co-culture assays. **(A)** Tumour cell growth is reduced by activated cytotoxic T-cells in a co-culture assay. HCT116 cells stably expressing the nuclear-restricted green fluorescent protein (GFP) (HCT116-NucGreen) were plated alone or in the presence of unstimulated or activated cytotoxic CD8⁺ T-cells. Tumour cell growth (shown in green) was monitored for 60 hours. **(B)** Tumour spheroids are destroyed by activated cytotoxic T-cells. HCT116-NucGreen spheroids were incubated alone or in the presence of unstimulated or activated cytotoxic CD8⁺ T-cells. Tumour spheroid growth or destruction (shown in green) was monitored for 60 hours. Images taken in brightfield and green channel. Note the presence and proliferation of activated immune cells in the periphery of the spheroid. Images were taken using a 10x objective.

supernatants for cytokine release profiles in ADCC and T-cell killing. The ability to miniaturise many of these delicate live cell assays without sacrificing data quality provides a powerful means to accelerate drug discovery campaigns.

Coupled with the advancements in automation, imaging-based approaches in combination with established endpoint assays enables creation of an intricate cellular picture of each candidate at a much earlier timepoint from similar numbers of experiments. Ultimately, the routine use of imaging technologies in primary screening campaigns will lead to better characterised biotherapeutics being selected as lead candidates for cancer therapy. 



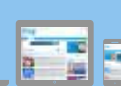
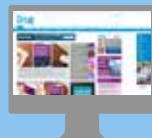
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BIOGRAPHY



VICTORIA JUSKAITE is a Scientist within the Target Validation Group at LifeArc, located at the Stevenage Bioscience Catalyst science park. Victoria obtained her BSc in Biomedical Science, followed by an MRes in Biomedical Research and PhD in Molecular Cell Biology at Imperial College London. After completion of her PhD in 2018, Victoria joined LifeArc where she is responsible for developing cellular assays for target validation and to support small molecule and antibody drug-discovery programmes.



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Bringing native analysis into the fast-moving world of biologics

Christopher Nortcliffe

Applications Specialist, LC/MS Biologics and Biopharma, SCIEX

For the past thirty years, native mass spectrometry has grown in both scope and reach in labs across the globe to encompass larger and more challenging molecular complexes. However, up until now, the throughput of these techniques has been slow and manual. Christopher Nortcliffe discusses ways that native analysis is advancing the field of mass spectrometry.



BIOGRAPHY



CHRISTOPHER NORTCLIFFE completed his undergraduate master's at Edinburgh University before joining a Doctoral training in Proteomics and Cell Technology. During this time, he focused on analytical techniques with an emphasis on mass spectrometry using QToF5 and FT-ICR. He conducted his PhD under Professor Perdita Barran at Manchester University, titled "Analysis of Non-Covalent Complexes using Mass Spectrometry Techniques". This focused on the application of ion mobility-MS. Upon finishing his studies, he started working at SCIEX in 2016 as an Application Specialist in Biologics and Biopharma.

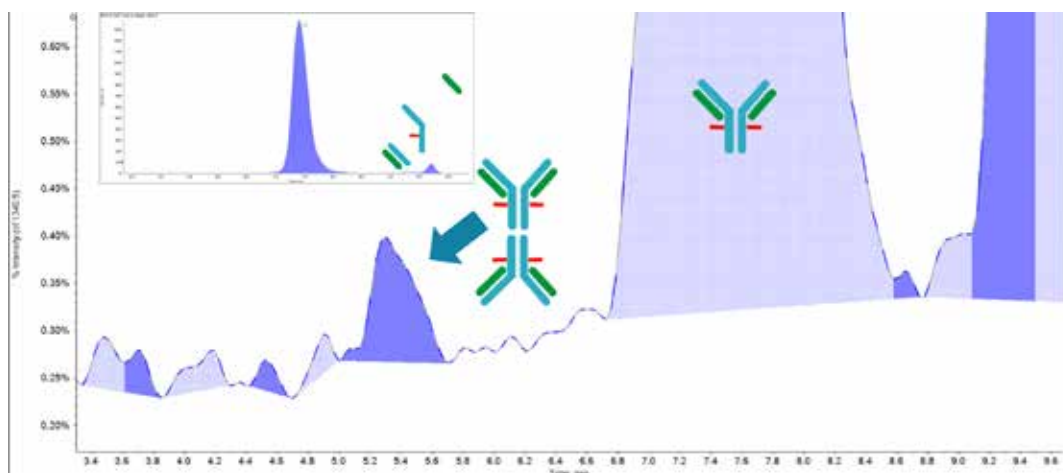
THE INTEGRATION between column and instrument manufacturers seeks to unlock new rapid native analysis, bringing the technology firmly into the hands of the pharmaceutical industry. The analysis of proteins in the gas phase underwent an expansion of Cambrian magnitude with

the development of soft ionisation techniques such as electrospray ionisation (ESI) and matrix-assisted laser desorption ionisation (MALDI).¹⁻⁵ These techniques allowed large fragile biomolecules to be transmitted into the gas phase along with the retention of some protein-protein and protein-ligand interactions. However, despite ►

RIGHT: Separation of the NIST mAb with distinct peaks for dimer, monomer and fragment species



FIGURE 1



“ Modified instrumentation specifically tuned to transmit large complexes into the gas phase has allowed immense macromolecular structures to be studied including whole intact viral capsids ”

the 'softness' of these techniques, most of the work carried out still relied upon the use of denaturing solvents such as acetonitrile and methanol. Therefore, although the biomolecules entered the gas phase intact, all their structural and stoichiometric data had been lost.

Development of new volatile buffer techniques and improvements in instrumentation have enabled the transmission of proteins and non-covalent complexes into the gas phase whilst retaining their solution phase structure. This technique was coined native or 'native-like' mass spectrometry.⁶ However, there was debate over the viability of the data from these techniques when compared with more established techniques such as NMR, X-ray crystallography and electron microscopy. Combining native mass

spectrometry with fragmentation techniques such as collision-induced dissociation and ion mobility allowed it to find its niche. This enabled these complexes to be analysed far more rapidly than similar structural techniques and could capture dynamic interactions taking place on time scales that were previously inaccessible.⁷

Numerous key developments in the field of native mass spectrometry have developed into new areas of structural biology and have answered fresh questions. Fragmentation techniques have developed such as electron capture dissociation (ECD) and electron transfer dissociation (ETD), which allow extensive coverage of biomolecules during fragmentation. These have been particularly useful when employed with protein cross-linking studies. A recent addition to this field of surface-induced dissociation pioneered by the Wyzocki group has paved the way for subunit stoichiometries and folding to be interrogated to exciting new frontiers.⁸ Other improvements have arisen since instrumentation with ion-mobility has become commercially available from several vendors in various orientations, including differential, drift time and travelling wave. These hybrid techniques give an orthogonal degree of separation to mass spectrometry for the separation of complex samples. Modified instrumentation specifically tuned to transmit large complexes into the gas phase has allowed immense macromolecular structures to be studied including whole intact viral capsids.⁹

Despite progress being made in the field, most of the work has taken place in academic institutions and the technique hasn't really migrated into the pharmaceutical industry, despite the abundance of mass spectrometry technology already in use. A key reason for this

BELOW: Reconstructed mass spectrum of carbonic anhydrase with the addition of three ligand species. Degree of binding was determined via peak area of bound versus unbound species.



FIGURE 2

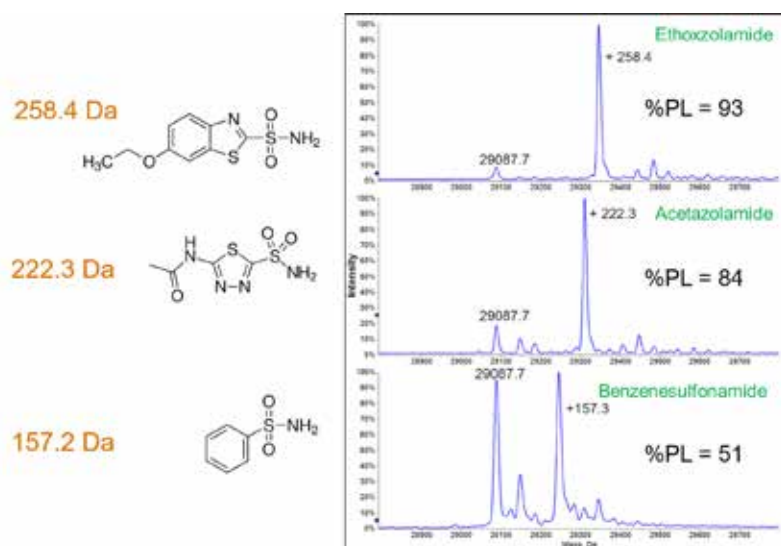
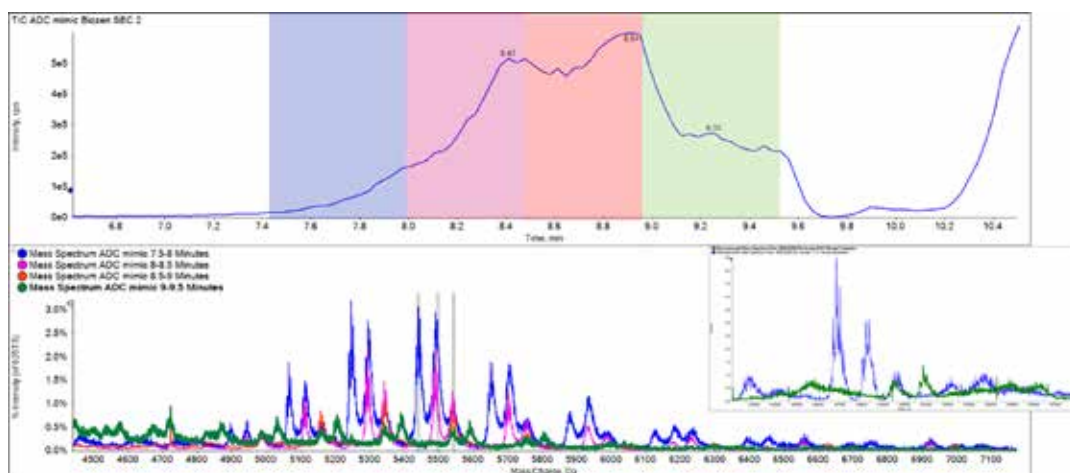


FIGURE 3



LEFT: Chromatogram of an ADC mimic showing four distinct elution zones. Mass spectrum extracted from each elution zone shows the separation of different DAR isoforms based upon size.

“There is now pressure for samples to be analysed in a native way with the development of new biotherapeutics”

is the individual way most native experiments have been performed on very low flow techniques. A common way to undertake a native experiment is by filling a pulled glass capillary with the sample before applying a voltage differential. These tips must be filled individually by hand, are fragile and prone to blockages caused by aggregates or insufficient sample preparation. Steps have been taken to give this process higher throughput, for example the development of the Advion

Nanomate.¹⁰ However, this requires a specific piece of instrumentation to be set up for native analysis and can be expensive to run in a high-throughput manner.

The pharmaceutical industry's reliance on high-throughput batches and LC technology means the benefits of native analysis have so far been outweighed by operational logistics. However, there is now pressure for samples to be analysed in a native way with the development of new biotherapeutics. Three major areas are: new ▶

EXPERTVIEW



Ferran Sánchez

Pharma/CRO EMEA Market Development Manager, SCIEX

“Using the appropriate combination of analytical methods and techniques can result in significant time and cost savings during product development”

When all you need is range

Ferran Sánchez discusses the complexities surrounding characterisation of biotherapeutics and how mass spectrometry can aid the process.

Biologics are the fastest growing segment of pharmaceutical development with new molecules coming to market at an extraordinarily rapid pace. Never before have we seen so many products coming through discovery, development and manufacturing. The timelines are tight, the pressure is on and mistakes are costly.

The ability to routinely and accurately characterise and measure the protein mass is a critical step in demonstrating the quality and stability of biologics. Using the appropriate combination of analytical methods and techniques can result in significant time and cost savings during product development, obtaining regulatory approval and scaling-up manufacturing. However, biotherapeutics are

inherently complex and these large class protein biologics, such as monoclonal antibodies (mAb), are significantly more difficult to characterise than small molecules.

Like all other therapeutic proteins, mAbs can undergo degradation processes during production, transportation and storage. Antibody aggregation is a common degradation process induced by the change of pH, temperature or agitation. Aggregation analysis of therapeutic mAbs is crucial due to potential consequences of decreased drug efficacy and increased immunogenicity concerns. Therefore, there is a great need for characterising protein aggregation during antibody development.

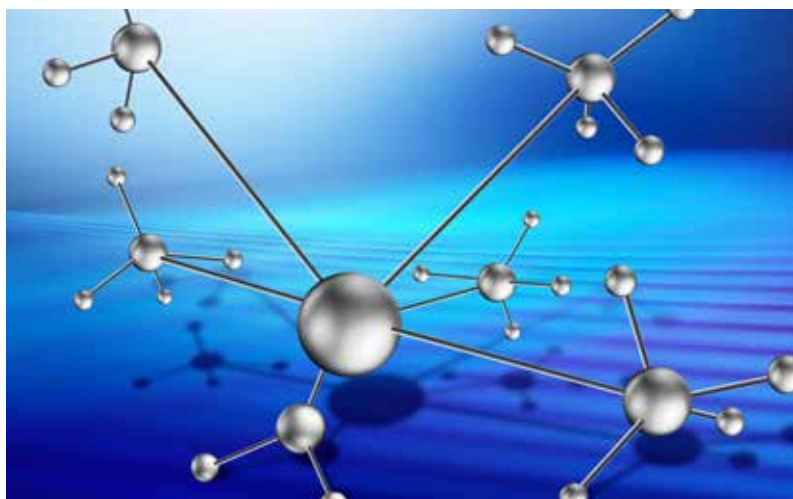
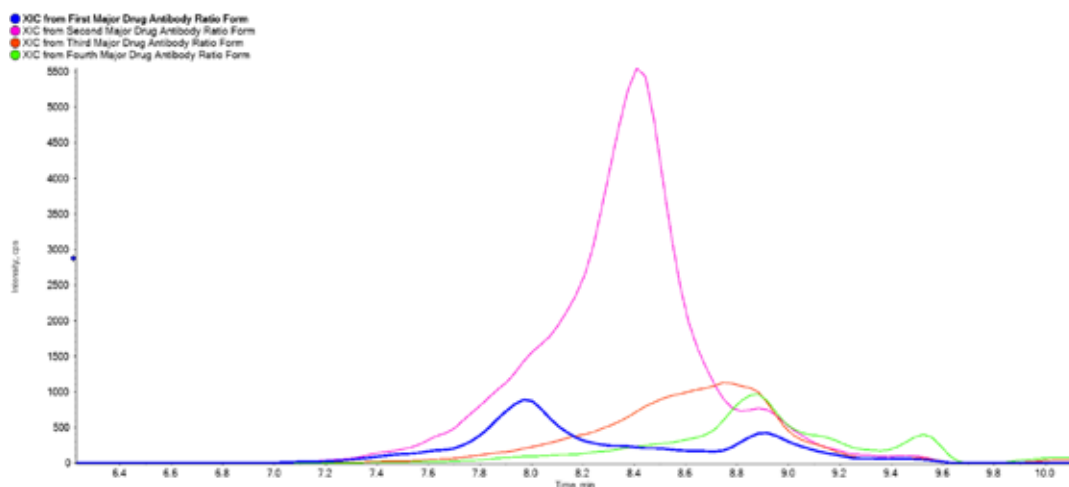
Serving as an orthogonal method for size exclusion chromatography

combined with multi-angle laser light scattering (SEC-MALLS), native mass spectrometry (MS) analysis can be useful for structural characterisation of intact protein aggregates, due to its ability to preserve protein structures and non-covalent protein interactions. By applying an MS-compatible volatile aqueous buffer at the mobile phase, protein aggregate heterogeneity can be maintained. Compared to reversed-phase conditions, native MS typically generates mass envelopes at higher m/z with fewer populated charge states due to the higher pH and nondenaturing properties of the mobile phase. This can be beneficial since the overall sensitivity in native mode can be lower, and so fewer populated charge states enable greater detection for complex protein analysis. ■

RIGHT: Extracted ion chromatogram of four DAR isoforms across a single charge state showing differences in elution profile for BioZen SEC 2 column



FIGURE 4



SEC separates molecules based upon their size with large molecules unable to enter the pores of the column and therefore experiencing a shorter path length than smaller molecules. Due to this, any non-volatile salts present in the sample buffer should be held on the column to be eluted to waste later, whilst the biological sample elutes off the column earlier. Larger protein aggregates can also be separated from monomers.

Figure 1 shows the example of aggregate separation utilising the BioZen Sec 2 column. There is a clear peak eluting around 5.3 minutes, which shows only signal from the protein dimer with no monomer eluting. There is then a major peak around 7.5 minutes with evidence of monomer followed by two distinct fragment species. The m/z of each of these species can then be confirmed using the mass spectrometer.

Figure 2 outlines a series of experiments utilising carbonic anhydrase along with a series of known carbonic anhydrase inhibitors. Not only are the protein-ligand interactions maintained into the gas phase, but the stoichiometry of binding can be used to determine a binding strength.

Figure 3 and **Figure 4** show work on an ADC mimic obtained from Sigma Aldrich, which has a fluorescent molecule bound to cysteine residues. Under standard reverse phase conditions, the heavy and light chains of the antibody separate, and the overall DAR ratio can't be observed. Under native conditions the complex is kept intact and the separation of various DAR forms through the SEC shows the different elution profiles of each form, contributing to the understanding of ADC formation.

In conclusion, this new workflow seeks to address issues with certain biomolecules and lowers the hurdles associated with the adaption of native mass spectrometry.

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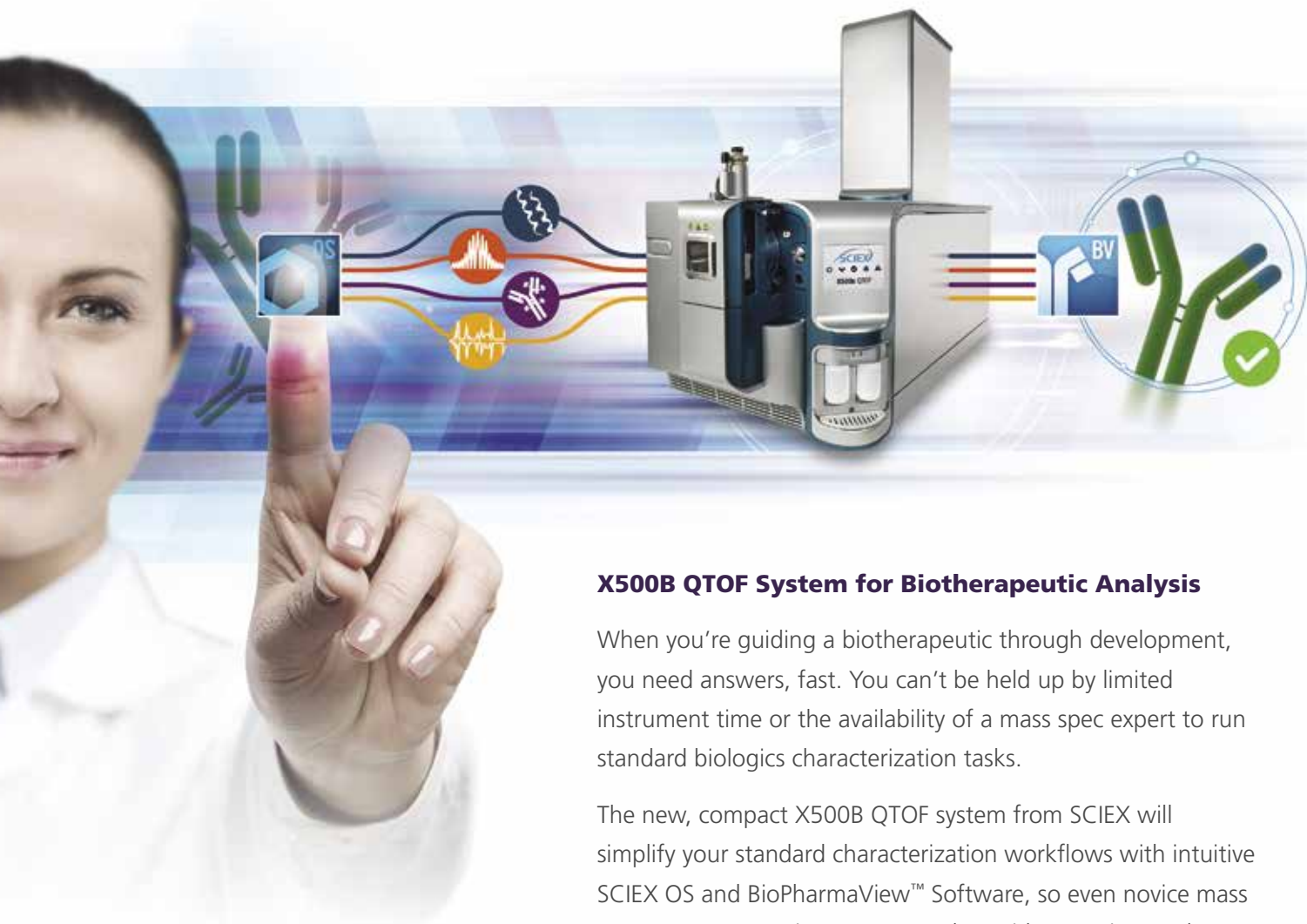
antibody drug conjugates with cysteine linked payloads; increased desire to understand and control protein aggregation; and the analysis of the specific interactions between small molecule drugs and protein targets. These burgeoning areas rely upon non-covalent interactions, which cannot be maintained through traditional LC-MS approaches.

Potential solutions are being developed by the union of mass spectrometry and chromatography. The key factors driving this are:

- High-throughput methods that fit within the already established pharmaceutical pipeline
- Methods on routine instrumentation without need for modification or specific add-ons
- Robustness and reliability leading to confidence in results.

Size exclusion chromatography (SEC) is not a new technique and has been utilised for many years in the biopharma industry with UV detection.

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Detection of anti-drug antibody (ADA) using single molecule counting (SMCTM) technology

Introduction to immunogenicity

All biological therapeutics have the potential to induce an immune-mediated response ranging from benign to severe adverse effects. These effects can encompass diminished clinical efficacy of the biotherapeutic being administered to hypersensitivity, an allergic reaction or even cytokine storms.

Consequently, regulatory agencies are looking to understand the implications of immunogenicity and are directing the industry to integrate programmes for immunogenicity risk management starting in early-phase drug development in clinical and pre-clinical. The US Food and Drug Administration (FDA) and pharmaceutical experts in the area of immunogenicity testing have recently published guidelines for the design and optimisation of immunoassays used in the detection of antibodies against biopharmaceutical drug products in patient samples in the absence of a drug and, more importantly, when a drug is present. The FDA recommends that screening and confirmatory IgG and IgM assays achieve a sensitivity of 100–500ng/mL (FDA Guidance 2016).

The increased sensitivity recommended is based on the current state of the science observed in the FDA's filings as well as publicly available studies.

A more sensitive detection method may lead to earlier detection of a primary immune response or detection of IgG4, which the FDA can request on a case-by-case basis.

It has been seen that patients develop persistent anti-drug antibody (ADA) responses having levels lower than 100ng/mL (AAPS Journal, Vol. 15, No. 1, January 2013).

Traditionally, ELISAs or electrochemi-luminescence (ECL) have been used to identify the presence of ADA. Though effective for detection, ELISA methods often fail to adequately measure a specific antibody response in the presence of circulating protein therapeutics due to the limitation on sensitivity and problems presented on a plate-based format.



Multivalent IgM ADA binding to the antigen on a plate surface or in a microwell (spatial restriction) can prevent binding of the detecting reagent. This could lead to loss of early detection of the primary ADA response as IgM is the first isotype generated.

According to the FDA: 'ADA assays need to be sufficiently sensitive to detect low levels of ADA before the amount of ADA impacts the PK, PD, safety, or efficacy.' The SMCTM technology offers a magnitude fold increase in sensitivity over current existing technologies.

Immunogenicity and SMC technology

SMC™ technology enables the development of ADA assays by the labelling of the drug with capture and detection reagents, and utilisation of buffer reagents to develop and optimise the assays. The technology allows the ability to develop a homogenous species-independent assay format that is simple, easy to design and validate. The reduced number of wash steps aids in the detection of low affinity antibodies and decreases assay time. This assay format is often referred to as a “bridging assay” since the ADA acts as a bridge between the drug labelled capture and detection.

SMC™ technology, employing the SMCxPRO™ high-sensitivity instrument, uses digital counting for low-level protein detection and offers several advantages with a unique platform design, in addition to specialised chemistry for enhanced specificity.

Controlling the SMCxPRO™ system is an integrated software package that is 21 CFR part II compliant. The software has easy to use, flexible command and data interpretation functions and is also LIMS compatible.

By using a 642nm laser focused 250µm above the base of an Aurora plate, a rotating objective scans through the free-floating suspension exciting fluorochromes as they pass through the interrogation space.

A low noise Avalanche Photodiode (APD) counts individual photons as they are emitted. The focused interrogation space of acquisition reduces cross talk from well to well, flare from meniscus diffusion of light, as well as inherent interference from turbid solutions.

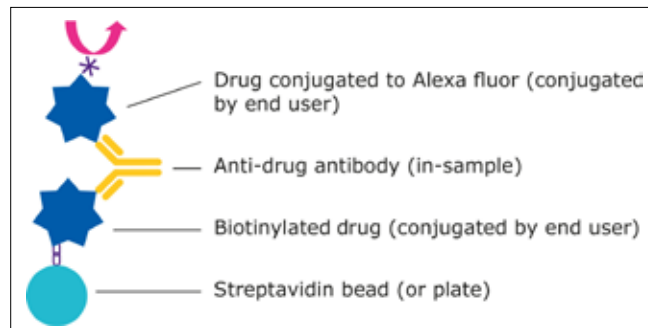


Figure 1: Bridging assay format for ADA detection in sample. Immunocomplex drug is Alexa and Biotin conjugated, and is captured on a magnetic streptavidin bead.

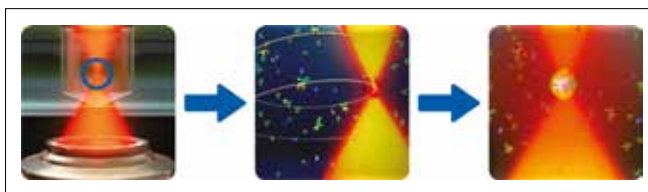
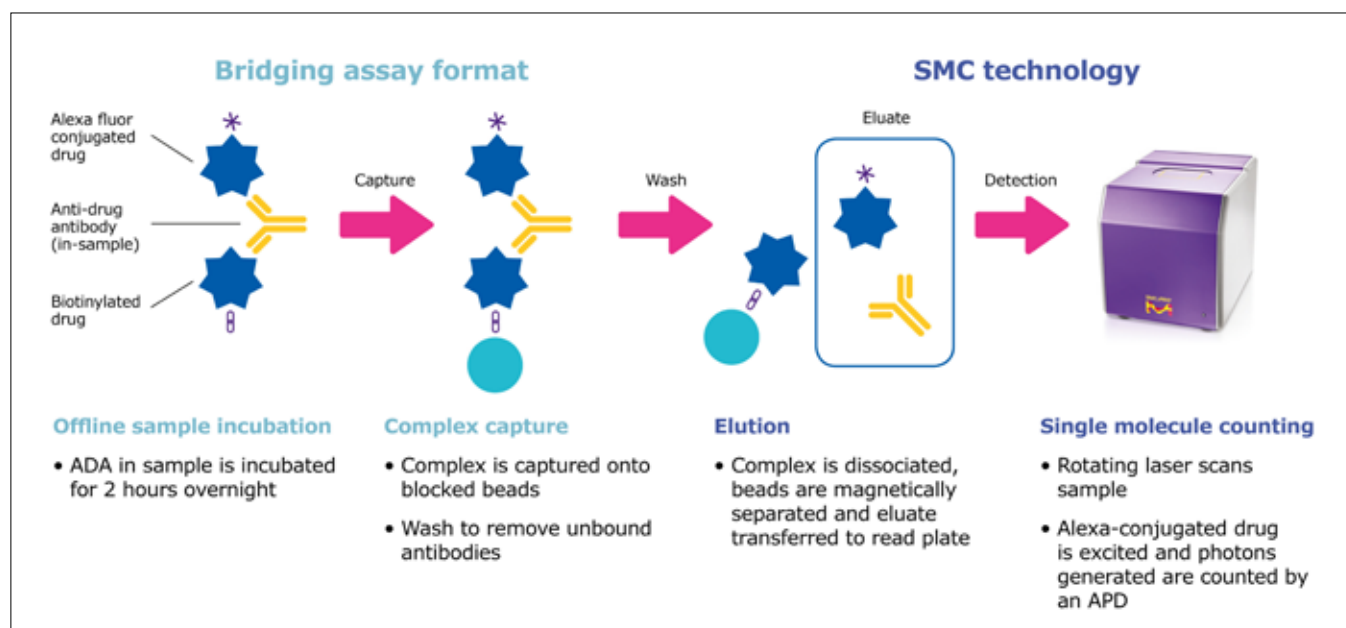


Figure 2: Counting of Alexa conjugated drug as it traverses through interrogation window.

Simple workflow

Upon completion of the derivatization of the drug for use as capture and detection, the workflow for assay development is as follows:



In developing an immunogenicity assay, optimisation is required to fully validate for the immunological system being studied. Considerations such as those listed below can be easily studied with the SMC technology platform:

- Drug tolerance
- Cut point/matrix tolerance
- Sensitivity/dynamic range of the assay
- Reproducibility.

Further optimisation

Further optimisation of different variables can take place to produce the most sensitive assay. These include:

- Drug concentration (capture and detection reagent)
- Assay diluents (to mitigate HAMA or other interfering factors)
- Sample volume
- Number of wash steps
- Incubation time
- Standard/sample diluent
- Determination of minimum required dilution (MRD)
- Evaluation of drug interference/tolerance

Drug tolerance

In bridging assays of this type, it is important to minimise the amount of free (unlabelled capture or detection reagent) drug to quantify and drive the equilibrium in favour of quantifying ADAs in samples.

Drug tolerance is an important consideration in immunogenicity and is a challenge faced where the ability to quantify ADA in matrix is reduced in the presence of high drug concentration as a result of competition. Several methods have been used to overcome this challenge, which includes acid dissociation.

By using a platform such as SMC™, with better sensitivity, may help overcome this by simple dilution, thereby eliminating the need for acid dissociation.

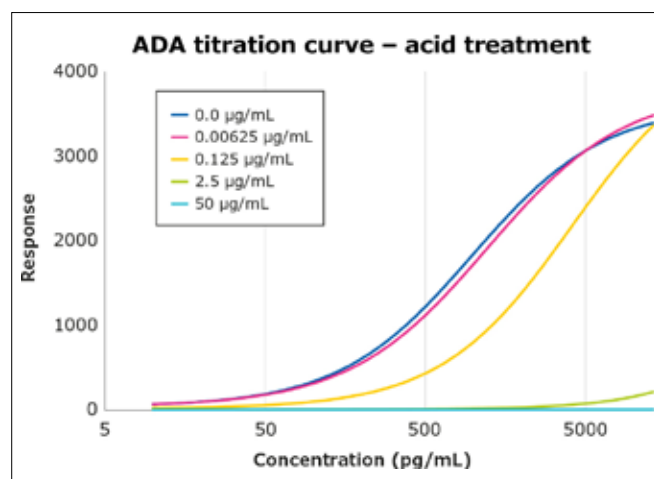


Figure 3: Example of drug tolerance on SMC Technology

Further implications of immunogenicity – adaptive immune response assessment

Screening assays do not necessarily need to identify isotypes but they need to be capable of binding multiple relevant classes or sub-classes. A number of isotypes play a major role in the immunogenic response. For instance:

- IgE-specific assays may be informative for products with a history of high risk of anaphylaxis
- IgG4-specific assays may be informative for products that are chronically administered, or on erythropoietin-treated patients with pure-red cell aplasia
- IgE and IgG4-specific assays may be requested on a case-by-case basis by the FDA due to hypersensitivity. The complement cascade can also be mediated by IgM and IgG.

These responses ultimately lead to generation of an inflammatory response through the formation of anaphylatoxins, such as C1q, C4a, C3a, and C5a.

Engagement of FcR or CR (complement receptor) on cells, through immune complex cross-linking, results in the production of chemokines and growth factors that have a cascade effect on trafficking and growth of T and B cells.

This leads to release of cytokines and chemokines (IL-2, IL-4, IL-5, IL-6, IL-10, IL-17, IL-21, IFN-γ) which ultimately leads to tissue damage.

We have several products that can assist in the assessment of these responses and are available for the Luminex platform using MILLIPLEX kits.

Human high sensitivity T-cell

 (Cat. No. HSTCMAG-28SK)
 (Cat. No. HSTCMAG28SPMX13) ♦
 (Bulk Cat. No. HSTCMAG28SPMX13BK) ♦
 (Cat. No. HSTCMAG28SPMX21)
 (Bulk Cat. No. HSTCMAG28SPMX21BK)

Fractalkine / CX3CL1	IL-12 (p70) ♦
GM-CSF ♦	IL-13 ♦
IFN γ ♦	L-17A/CTLA8
IL-1 β ♦	IL-21
IL-2 ♦	IL-23
IL-4 ♦	I-TAC/CXCL11
IL-5 ♦	MIP-1 α /CCL3
IL-6 ♦	MIP-1 β /CCL4
IL-7 ♦	MIP-3 α /CCL20
IL-8/CXCL8 ♦	TNF α ♦
IL-10 ♦	

Human immunoglobulin isotyping

 (Cat. No. HGAMMAG-301K)

IgA	IgG3
IgG1	IgG4
IgG2	IgM

Human IgE – singleplex

(Cat. No. HGAMMAG-303E)

IgE

Human complement panel 1

(Cat. No. HCMP1MAG-19K)

Adipsin/Factor D	C5a
C2	C9
C4b	Factor I
C5	Mannose-binding lectin (MBL)

Human complement panel 2

(Cat. No. HCMP2MAG-19K)

C1q	Factor B
C3	Factor H
C3b/iC3b	Factor P/properdin
C4	

Legend key for MILLIPLEX map kits

- ♦ Available in Cat. No. listed
-  Available for custom premix

Mouse high sensitivity T-cell

(Cat. No. MHSTCMAG-70K)
 (Cat. No. MHSTCMAG-70KPMX)
 (Bulk Cat. No. MHSTCMAG-70KPBK)

GM-CSF	IL-10
IFN γ	IL-12 (p70)
IL-1 α	IL-13
IL-1 β	IL17A/CTLA8
IL-2	KC/GRO α /CXCL1
IL-4	LIX
IL-5	MCP-1/CCL2
IL-6	MIP-2/CXCL2
IL-7	TNF α

Mouse immunoglobulin isotyping

 (Cat. No. MGAMMAG-300K)

IgA	IgG2b
IgG1	IgG3
IgG2a	IgM

Mouse IgE – singleplex

(Cat. No. MGAMMAG-300E)

IgE

SMC immunogenicity assay development kit

(Cat. No. 03-0175-00)

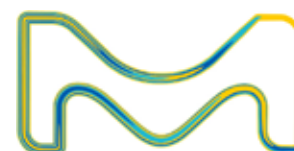
Combining our immunoassay portfolio to study the impact on the immunogenicity of a therapeutic can provide insight into the mechanism of the response. The SMC technology can offer increased sensitivity which may assist in the detection of low affinity antibodies and lead to earlier detection of the

primary ADA response, overcome matrix effects and may reduce drug tolerance.

The MILLIPLEX kits can offer insight into the mechanism of the response and also help understand the immune complex mediated responses to the ADA.

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STEM CELLS

In recent years scientists have developed many therapeutic molecules – such as bifunctional multi-targeting antibodies, fusion proteins and oncolytic viruses – and different types of stem cells are being modified to facilitate their efficient delivery to tumours, illustrating the great potential of engineered therapeutic stem cells. In this article, Aparajita Dubey, Associate Scientific Manager at Biocon Biologics Limited, outlines the exciting developments that are taking place with immunotherapies and demonstrates how stem-cell technologies are proliferating the paradigm shift in how we tackle cancer treatment.

Thanks to new developments with induced pluripotent stem cells enabling human neuron generation, scientists can more precisely analyse the effects that chemicals have on human cells. Trisha Rao, Montreal Neurological Institute, and Thomas Durcan, McGill University, expand on their research into the effects of cannabis on neuronal development and implications of its legalisation in Canada.

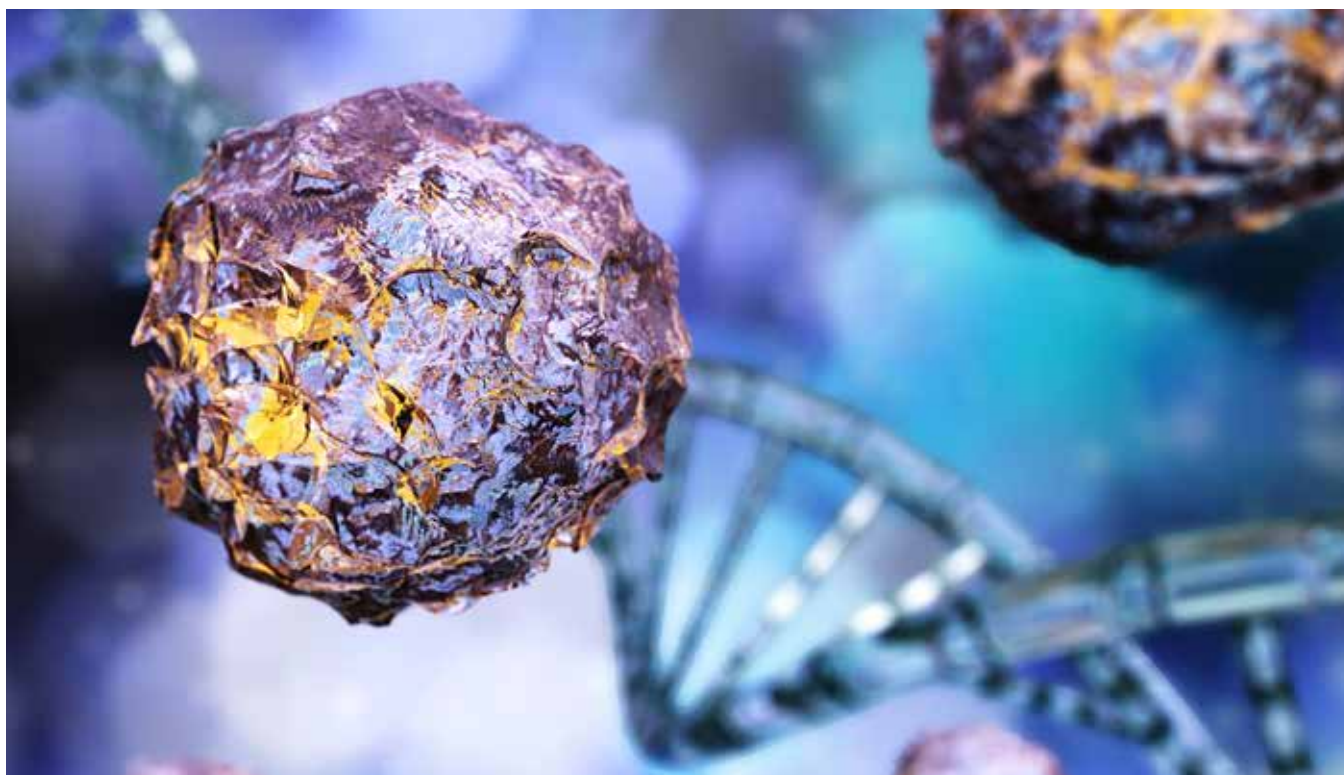


Stem-cell technologies in immunotherapy

Aparajita Dubey

Associate Scientific Manager, Biocon Biologics Limited, Bangalore, India

This article outlines the exciting developments that are taking place with immunotherapies and demonstrates how stem-cell technologies are proliferating the paradigm shift in how we tackle cancer treatment.



THE AMALGAMATION of stem-cell technology with an immunotherapy approach has produced a new method that rejuvenates cancer-fighting immune cells and strengthens the ability of ageing cells to replicate in the body and mediate tumour regression. This represents a milestone for immunotherapy; developing more vigorous anti-cancer cells and using a similar approach to produce large quantities of cancer-fighting cells from patients' own blood cells. Ageing cells have proven to be a major challenge in generating these cancer-fighting cells but induced pluripotent stem cell (iPSC) technology, which allows adult cells to be reprogrammed in the laboratory and returned to a stem cell state, is helping to overcome this.

In recent years, scientists have developed many therapeutic molecules – such as bifunctional multi-targeting antibodies, fusion proteins and oncolytic viruses – and different types of stem cells are being modified to facilitate their efficient delivery to tumours, illustrating the great potential of engineered therapeutic stem cells. This combination of immunotherapy and stem-cell technology has the potential to revolutionise cancer treatment regimes. Immunotherapy has changed our approach to cancer treatment by targeting the immune system rather than the tumour itself; this has been the consequence of better understanding the precise cellular interactions between cancer and the immune system. These undifferentiated stem cells are a powerful tool in clinical research

to help unravel the mystery of cancer progression and development of stem-cell technologies is the ultimate goal.

Cancer immunotherapy

Immunotherapy represents an entirely new approach to cancer treatment; targeting the immune system rather than the tumour itself. This therapy makes use of the body's own internal defenses to combat or check cancer growth and metastasis. Four common approaches to immunotherapy include the following:

- **Immune modulators:** these boost the patient's entire immune system; not only those immune cells active within the tumour microenvironment.
- **Tumour targeted monoclonal antibodies (mAbs):** these block the growth signal of tumour cells or induce apoptosis. They are designed to target specific antigens found on cancer cells.
- **Checkpoint inhibitors (CTLA4, PD1 and PDL1):** these release the breaks on the body's immune system and induce T-cells to actively combat tumour growth. This is directed against the cancer cells, which silence the body's T-cell functioning by taking advantage of the immune system's natural checkpoints designed to maintain equilibrium and prevent autoimmunity.
- **Adoptive T-cell therapy or adoptive cell transfer therapy:** this is designed to enrich tumour infiltration lymphocytes by multiplying T-cells removed directly from the patient's tumour microenvironment. These are analysed based on the receptors' specificity or to determine which receptors are more specific to the tumour. These cells are then administered to the patient after being generated in greater numbers to initiate a massive antitumour immune response.

Upcoming advancements in immunotherapy include improvements in translational research, patient stratification, target validation and the development of predictive biomarkers.

Stem cells as a potential target

Characteristics such as a high proliferative capacity, low immunogenic reactivity and differentiating capabilities mark stem cells out as potential therapeutics – along with challenges such as unclear expression profiles *in vivo* and their potential immune-reactivity.

Major engineered stem cells for delivery of anti-cancer agents

Many adult stem cells, namely mesenchymal stem cells (MSCs) and neural stem cells (NSCs), have intrinsic tumour-tropic properties, which makes them a novel targeted cellular delivery vehicle for anti-cancer agents. Stem cells, along with their ability to home to the primary tumour mass, can efficiently track micro-metastatic lesions and can be engineered to express or secrete a range of anti-cancer agents. MSCs have been isolated from several organs including bone marrow, adipose tissue, foetal tissues, dental pulp, umbilical cord, Wharton's jelly and other tissue types, among which bone marrow-derived stem cells play a major part in preclinical studies.

Recent advancements in CRISPR-mediated genome editing of primary cells such as T-cells and monocytes are taking us a step closer to engineering immune cells with the specificity necessary to develop the next generation of immunotherapies.

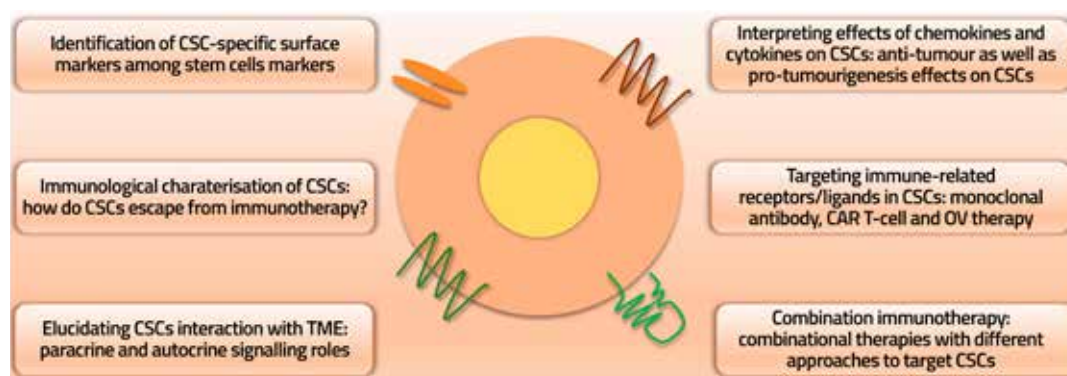
Stem-cell mediated delivery

Different approaches used for stem-cell mediated delivery of biological anti-cancer agents include:

- **Cancer stem cells (CSCs) or tumour-initiating cells (TICs):** These are new targets for ▶

“ This combination of immunotherapy and stem-cell technology has the potential to revolutionise cancer treatment regimes ”

FIGURE 1



← LEFT: Prospective immunotherapeutic approaches to target CSCs

immune intervention for both solid and haematologic malignancies.

- **Stem-cell based therapeutic platforms:** these include haematopoietic stem cells, reprogrammed pluripotent stem cells, stem cell-like memory T-cells and tumour-derived stem cells.

The identification of targets potentially associated with CSCs that are amenable to immunotherapy could play a major role in future

discovery efforts and the development of therapies to cure and control different forms of cancer.

CSCs have drug resistance to most chemotherapeutic agents and are also responsible for tumour cell heterogeneity. In order to achieve complete regression of tumours, CSCs must be targeted. Recent advances in immunotherapies are also applicable for targeting CSCs. Immune markers expressed by CSCs exhibit specific immune characteristics in various cancers, which can be used in

TABLE 1 Recent advances in targeting CSCs by immunotherapy

Immuno-therapy	Targeting approach	Cancer model
Adoptive T-cell therapy	CAR T-cells against EpCAM antigen. Peripheral blood lymphocytes expressing EpCAM-specific chimeric antigen receptors targeted EpCAM ⁺ CSCs	Prostate cancer
	CAR T-cells, targeting membrane bound IL-15	Leukaemia
	CD8 ⁺ cytotoxic T-cells, specific for the CSCs antigen ASB4	Colon cancer
	CIK cells transduced with CAR T-cells against CD123	Acute myeloid leukaemia
	Autologous CIK cells	Melanoma
	CIK cells-NKG2D ligands	HCC
	CIK cells-NKG2D ligands	Nasopharyngeal carcinoma
	NK cells from healthy donors	High-grade non-muscle invasive bladder cancer
	NK cells	Pancreatic cancer
	NK cells	Oral squamous carcinoma
	γδ and CD8 ⁺ T-cells	Breast cancer
DC-based vaccine	CSCs lysate-pulsed DCs	Malignant melanoma
	CSCs lysate-pulsed DCs	Squamous cell carcinoma
	DCs charged with total lysates of Panc-1 CSCs	Pancreatic cancer
	DCs loaded with NANOG peptide	Ovarian cancer
	ALDH ^{high} SCC7 specific CSCs-DCs	Squamous cell cancer
	ALDH ^{high} D5 CSCs-DCs	Metastatic melanoma
	ALDH ^{high} CSC-pulsed DCs	Metastatic melanoma
	ALDH ^{high} CSC-pulsed DCs	Squamous cell cancer
Oncolytic virotherapy	Oncolytic herpes simplex virus armed with IL-12	Glioblastoma
	Oncolytic adenovirus targeting CD133 ⁺ CSCs	Glioblastoma
	Oncolytic vaccinia virus (GLV-1h68) targeting ALDH ^{high} stem cell-like cancer cells	Breast cancer
	Oncolytic vaccinia virus targeting ID8-T tumour model that harbours CD44 ⁺ CD117 ⁺ cancer-initiating cells	Ovarian cancer
	Cancer-favouring oncolytic vaccinia virus stem-cell-like colon (CD133 ⁺ and CD44 ⁺) cancer cells	Colon cancer
	Oncolytic measles viruses: targeting CD133 ⁺ tumour-initiating cells	HCC
	Cancer-favouring oncolytic vaccinia virus: metastatic hepatocellular carcinoma (CD44 ⁺)	HCC
Others	Monoclonal antibody antigen Lgr5	Colon cancer
	IFN-β therapy: targeting type I IFN signaling	Triple negative breast cancer
	Blockade of the IL-8 receptor	Breast cancer
Combination therapy	DC-based vaccine in combination with anti-PD-1 and anti-CTLA-4	Melanoma
	Oncolytic herpes simplex virus in combination with anti-PD-1 and anti-CTLA-4	Glioblastoma
	STDENVANT (a vaccine comprising of GSC lysate, DCs, and TLR9 agonist CpG motif-containing oligo-deoxynucleotides) in combination with anti-PD-L1	Glioblastoma
	CSCs vaccine (streptavidin-granulocyte-macrophage-colony stimulating factor surface-modified bladder CSCs) in combination with anti-PD-1	Bladder cancer

Abbreviations: ALDH, aldehyde dehydrogenase; CAR, chimeric antigen receptor; CCR7, CC-chemokine receptor 7; CIK cells, cytokine-induced killer cells; CRC, colorectal cancer; CSCs, cancer stem cells; CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; CXCR1, C-X-C chemokine receptor 1; DC, dendritic cells; HCC, hepatocellular carcinoma; IFN-β, interferon-beta; IFN-γ, interferon-gamma; IL-8, interleukin 8; IL-12, interleukin 12; NK, natural killer; NKG2D, natural killer group 2D; PD-1, programmed cell death-1; PD-L1, programmed death-ligand 1; TLR9, toll-like receptor 9.

immunotherapies to target CSCs in the tumour microenvironment. The following strategies have been recently applied to target CSCs: adaptive T-cells, dendritic cell (DC)-based vaccines, oncolytic viruses, immune checkpoint inhibitors and combination therapies.

Immunotherapy targeting CSCs

Immunotherapy targets for CSCs include immune cells such as cytokine-induced killer (CIK) cells, natural killer (NK) cells, T-cells, and CD8+ T-cells. DC-based vaccines also target CSCs; oncolytic virotherapy (OVT) induces antitumour immunity through immunogenic cell death and activation of the T-cell. Combinations of immunotherapies are also used to target CSCs. Recently, strategies in combination therapy include DC-based vaccines, oncolytic viruses and immune checkpoint blockades.

Adoptive T-cell therapy

This is a type of personalised cancer therapy that uses cancer-bearing host immune cells with direct anticancer activity. Engineered T-cells with chimeric antigen T-cell receptors (CAR T-cells) against the antigens of CSCs have been recently developed and evaluated in various cancer models.

Combination immunotherapy

Combined immunotherapy approaches have recently been developed that aim to completely eradicate CSCs; an example of which is a DC-based vaccine in combination with anti-PD-L1 and anti-CTLA-4.

Significant features of the CSC model

Features of the model include: low abundance, self-renewal, high tumorigenicity, ability to reconstitute a heterogeneous population of cells, expression of multi-drug resistance proteins and upregulation of DNA repair proteins. Extensive clinical evidence establishes the importance of CSCs in cancer progression, relapse and metastasis, which suggests that targeted therapies could be a potential approach to achieve a comprehensive treatment regimen.

Induced pluripotent stem cells and immunotherapy

iPSCs specific to patient iPSCs could be potentially beneficial for immunotherapy approaches. Functionally-active T-cells can be induced to differentiate from pre-rearranged TCR genes retained in T lymphocyte-derived human iPSCs. Reprogramming of selected T-cells into iPSCs *in vitro* can lead to the production of tumour antigen-specific T lymphocytes. These iPSCs then differentiate back into T lymphocytes for infusion into patients after checking the safety-related aspects.

Targeting CSCs

Normal stem cells can also be used to target CSCs in cancer therapy. Normal stem cell and CSC interaction suppresses tumour proliferation, angiogenesis and metastasis, and reduces inflammation and apoptosis. The potential of NSCs and haematopoietic stem cells (HSCs) in anti-glioblastoma therapy was assessed and it was concluded that HSCs can be ideal for developing technologies aimed at controlling glioblastoma CSC activity. HSCs are unique since they are less prone to neoplastic transformation in neural tumours than neural stem/progenitor cells and can be engineered to facilitate the generation of cell systems that can trigger targeted CSC apoptosis.

Cancer stem cell limitations and advantages

Since CSCs are resistant to conventional approaches, this can lead to failure of colorectal cancer (CRC) therapy for patients. However, dendritic cells (DCs) could effectively supplement CRC therapy. Some major features of CSCs include metastasis, recurrence, relapse and resistance to conventional chemotherapy, which only destroys proliferating and mature cancer cells while quiescent CSCs survive. Clarification related to the maintenance of CSCs is important to understand cancer cell persistence and relapses. CSCs can be used as a potential targeting mechanism and also as a potential therapeutic strategy to eradicate cancer. Applying knowledge regarding CSCs could play a key role in future drug development; to address cancer resistance, relapse and progression. It's comparatively refractory to current therapies such as chemotherapies, radiotherapy and small molecule targeted therapies because CSCs can persist for longer durations in the body without being subjected to immune surveillance or other homeostatic mechanisms.

Conclusion

Stem-cell technologies along with immunotherapy have the potential to provide breakthroughs in cancer therapy. The ability of stem cells to migrate to solid tumours and micrometastatic lesions, facilitating site-specific anti-tumour agent delivery and overcoming the short half-lives of conventional chemotherapeutic agents, is dynamic. A more widespread clinical utilisation of stem cell-based therapies are expected in future. Adding to this approach, researchers are testing a stem cell-derived NK cell immunotherapy in people with incurable cancer. Cells derived from iPSCs are being used for the first time in this "off-the-shelf" NK immunotherapy clinical trial. This regime to rejuvenate cancer-fighting immune cells by strengthening ageing cells and developing large amount of cancer-fighting cells via immunotherapy is a great advancement in current and future cancer research. 

“Upcoming advancements in immunotherapy include improvements in translational research, patient stratification, target validation and the development of predictive biomarkers”

BIOGRAPHY



APARAJITA DUBEY is an Associate Scientific Manager at Biocon Biologics Limited, Bangalore, India. Aparajita has more than 11 years' experience in pharmaceutical research, mainly in cancer biology and immunotherapy within Biocon, Panacea and Jawaharlal Nehru University. As a biologist she has broad experience in the field of cell-line development, cancer immunotherapy, molecular biology along with medical writing and consulting experience at Prescient Healthcare. She has an MSc in Life Sciences and a PG Diploma in IP from National Law School University, Bangalore, India.

Using stem cells to study the effects of cannabis on neuronal development

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Thomas M. Durcan

McGill University, Montreal, Canada

Thanks to new developments with induced pluripotent stem cells enabling generation of human neurons, scientists can more precisely analyse the effects that chemicals have on human cells. This article describes research into the effects of cannabis on neuronal development and implications of its legalisation in Canada.



CANNABIS has been on the brains of Canadians more than usual in recent months. On 18 October 2018, Canada became the second country in the world after Uruguay to legalise cannabis. Prior to this date, cannabis was a controlled substance that could only be used for medicinal purposes with approval from a healthcare practitioner;¹ but now that consumers can purchase cannabis from authorised retailers, there is potential for increased

use among Canadians. The fact that cannabis use among Canadian youth aged 15 to 24 years was already three times higher than that of adults prior to its legalisation, makes this a particular concern.¹ By expanding access to cannabis, Canada is faced with higher rates of use among Canadian teens and young adults and higher rates of prenatal exposure to a product whose effects on neuronal development and the normal function of the brain are not yet fully understood. Cannabinoids, the major pharmacologically-active components of cannabis, act on the central nervous system (CNS) through the endocannabinoid system (ECS).² The ECS has important roles in brain development, which raises questions about the impact of cannabis exposure on this process.

The main receptors of the ECS are the G-protein coupled receptors cannabinoid receptor

“Neuronal organoids therefore present an attractive model for studying the effects of prenatal cannabinoid exposure on brain development”

1 and 2 (CBR1 and CBR2), which are targeted by both cannabinoids and the endogenous homologues of cannabinoids (endocannabinoids).² CBR1 is expressed throughout the CNS, while CBR2 is expressed primarily in immune cells. CBR1 is presynaptic and regulates neurotransmitter release in response to endocannabinoids. In adults, endocannabinoids modulate anxiety, depression, neurogenesis, reward, cognition, learning and memory,³ and are required for the stress response.⁴ In the developing brain, the ECS has roles at both the embryonic and adolescent stages. Genetic studies in mice have implicated the ECS in forebrain development, including progenitor cell expansion and neurogenesis, neuronal and glial specification, and axonal pathfinding.⁵ CBR1, in particular, has been shown to regulate neural progenitor survival, proliferation, differentiation and migration.⁶ In mice, CBR1 has roles in corticospinal neuron specification⁷ and development of long-range axonal connectivity by regulating corticofugal axon navigation and fasciculation.^{8,9} During adolescence, the ECS contributes to the development of several brain regions, including the cortex, amygdala, hippocampus and hypothalamus.⁴

Stem cell models

Understanding the effects of exposure to substances like cannabis on the developing brain has been limited by the use of traditional cell- and animal-based models, which are not fully representative models of the human nervous system. This is particularly problematic for studying neurological tissues, for which the majority of primary neurons are derived from either rodent brains or from human cells derived from brain tumours. However, the ability to generate different types of neurons from human induced pluripotent stem cells (iPSCs) has provided researchers with a multifaceted tool for investigating the development and function of human neurons.¹⁰ Stem cell models are preferable for studying neuronal development owing to their ability to be directed into any type of neuronal cell found within the human brain, through defined developmental cues. Furthermore, advances in culturing technology have enabled three-dimensional organoid models of neuronal stem cells – neuronal organoids or “minibrains” – that recapitulate the cellular, morphological and physiological properties of neurons.¹¹ Depending on culturing conditions, minibrain formation may be undirected, resulting in spontaneous self-organisation of structures that exhibit regional patterning, or formation being directed toward a specific region using defined molecular factors (ie, forebrain, midbrain and cortical). Since neuronal organoids can survive in culture for many months, they can be used to analyse the long-term effects of one-time

or repeated interventions. Novel approaches to culturing neuronal organoids have recently emerged that address some of the limitations of current culturing methods. Neuronal organoids can be engineered and cultured in a controlled manner using microfluidic chips that recreate the neural microenvironment (organoid-on-a-chip).¹² A common challenge encountered with *in vitro* organoid cultures is a lack of oxygen and nutrient supply due to the absence of vasculature. A recent study demonstrated that human cerebral organoids generated *ex vivo* can be transplanted into the brain of mice where they become vascularised and imaged in real time.¹³ Three-dimensional neuronal organoids present unique opportunities in contrast to their two-dimensional neuronal counterparts and may be a more physiological model for studying the effects of environmental factors and small molecules on the neuronal developmental process.

Exogenous substance exposure

Several studies have used neuronal organoids to model prenatal exposure to exogenous substances, including alcohol, nicotine and cocaine. In neuronal organoids engineered to model early stages of brain development, treatment with ethanol resulted in impaired neurite outgrowth and neural maturation, which correlated with transcriptional differences.¹⁴ In a similar neuronal organoid model, treatment with nicotine resulted in premature neuron differentiation, disruption of brain regionalisation and cortical development, and abnormal differentiation and migration of neurite outgrowths.¹² Premature neuronal differentiation was also observed in a model of prenatal cocaine exposure using self-organised neocortical organoids, along with inhibition of neocortical progenitor cell proliferation and interruption of neural tissue development.¹⁵ Interestingly, this study also had mechanistic findings, linking CYP3A5 to an induction of reactive oxygen species (ROS) generation.

Another psychoactive agent that has been investigated in cerebral organoids is 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT), a plant-derived compound belonging to a group of molecules found in medicines used by indigenous cultures in Central and South America.¹⁶ A proteomic analysis of cerebral organoids exposed to 5-MeO-DMT identified more than 900 differentially-expressed proteins by shotgun mass spectrometry. Another study found that exposure of forebrain organoids to bisphenol A (BPA), a component of commonly used plastics, decreased ventricular zone thickness and reduced proliferation of neural progenitor cells.¹⁷ Forebrain organoids were also used to model Zika virus infection and the results supported a suspected link between prenatal exposure ➤

BIOGRAPHY



DR TRISHA RAO is a Medical Writer in the Clinical Research Unit at the Montreal Neurological Institute in Montreal, Canada. She obtained her doctorate in cancer research from McGill University. Dr Rao develops scientific and medical content for a variety of projects, specialising in training materials.

“**Neuronal stem cell cultures present an ideal model for investigating the ECS and cannabinoids, offering systems that are relevant in terms of species and cell type**”



BIOGRAPHY



DR THOMAS DURCAN is an Assistant Professor in the Department of Neurology and Neurosurgery at McGill University in Montreal, Canada. He is also Associate Director of the iPSC platform at the Montreal Neurological Institute. Dr Durcan's research focuses on 2D and 3D neuronal stem cell models of neurological disorders.

to Zika virus and microcephaly.¹⁷ Collectively, these studies demonstrate that minibrains can serve as culture systems for modelling prenatal exposure to specific agents. Neuronal organoids therefore present an attractive model for studying the effects of prenatal cannabinoid exposure on brain development.

Mimicking exposure

Of the more than 100 cannabinoids found in cannabis, the two with highest concentrations – and therefore of primary interest – are delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD).¹⁸ THC is the predominantly psychoactive cannabinoid in cannabis. CBD does not have psychoactive properties, although it contributes to the pharmacologic actions of cannabis. In order to mimic cannabis exposure at different stages of development, the effects of THC and CBD treatment on neuronal organoid formation can be examined at corresponding timepoints. It will be of interest to compare and contrast these results with findings from developmental studies in mice; for instance, that prenatal exposure to THC caused deficits in corticospinal motor neuron development.⁵

More importantly, it will be necessary to address whether the morphological and biological phenotypes observed in these experimental models correlate with the clinical phenotypes of children who experienced prenatal cannabis exposure, such as impaired executive function, increased depressive symptoms and impaired response inhibition. It may even be possible to identify therapeutic targets that can be pharmacologically exploited to treat neurodevelopmental defects caused by exposure to cannabis. This possibility was addressed in a neocortical organoid model of prenatal cocaine exposure in which CYP3A5 was identified as a mediator of the observed neurodevelopmental deficits.¹⁵

Medicinal usage

Neuronal organoids also have applications in disease modelling and could therefore be used to investigate the medicinal use of cannabinoid products. There are several cannabinoid products that are currently used as medicines or are under clinical investigation to treat various conditions, including neurological disorders. In June 2018, the United States Food and Drug Administration (FDA) approved Epidiolex (cannabidiol) for the treatment of seizures associated with two rare and severe forms of epilepsy. Epidiolex is the first drug approved by the FDA that contains a purified drug substance derived from cannabis. According to the FDA labelling, the precise mechanisms by which Epidiolex exerts its anticonvulsant effect in humans are unknown.¹⁹

Neuronal stem cell models offer a novel model system to further characterise approved cannabinoid products like Epidiolex, in addition to testing investigational products at the preclinical stage. Cannabis also has proposed uses in treating certain neurodegenerative diseases in adult patients. For neurological disorders in general, whether developmental or degenerative, the ability to derive patient-specific neuronal stem cell models allows for individualisation of treatment with cannabinoids as well as identification of potential therapeutic targets. Neuronal organoids are also amenable to the culturing and plating conditions required for high-content screening.²⁰

As Canada transitions into an era of legalised cannabis, there is a need to better understand the molecular and cellular mechanisms of cannabinoids that underlie their physiological effects, particularly on neuronal development. Given the importance of the ECS in foetal and adolescent neuronal development, it will be imperative to elucidate how cannabinoids impact these processes.

Conclusion

Cannabis products have already entered drug markets, including those with neurological indications for which the mechanisms of action remain unclear. Neuronal stem cell cultures present an ideal model for investigating the ECS and cannabinoids, offering systems that are relevant in terms of species and cell type. Specifically, neuronal organoids have been used to model exposure to a variety of substances and the techniques employed in these studies can be applied to investigate exposure to cannabinoids. With promises of increased funding opportunities for cannabis research from the Canadian government, it is hoped that we can learn more about the effects of cannabis on neuronal development to inform regulatory and policy decisions related to its use for other countries looking to follow Canada's lead in the future, regarding legalising Cannabis. 🍀

REFERENCES

To view references, please visit:
drugtargetreview.com/2-19-Durcan

EVENTS DIARY

Keeping you up to date with forthcoming events in the drug discovery industry



june

11

Antibody Engineering & Therapeutics Europe

Date: 11-13 June
Location: Amsterdam, The Netherlands



16

HPLC

Date: 16-20 June
Location: Milan, Italy

HPLO 2019

23

EACR Conference on Cancer Genomics

Date: 23-26 June
Location: Cambridge, UK



26

SLAS Europe

Date: 26-28 June
Location: Barcelona, Spain



26

ISSCR

Date: 26-29 June
Location: Los Angeles, USA

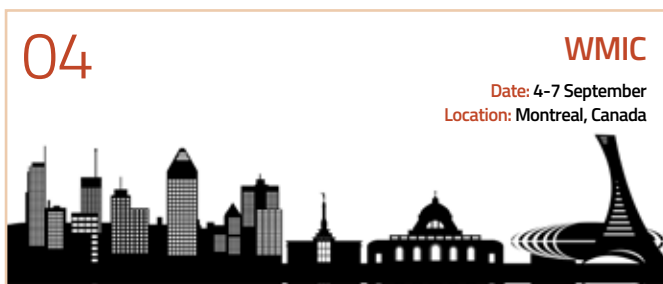


september

04

WMIC

Date: 4-7 September
Location: Montreal, Canada



09

Basel Life

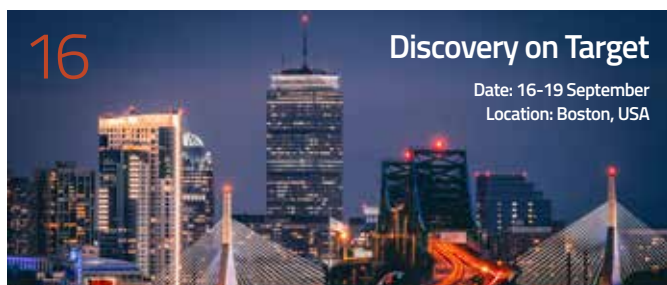
Date: 9-12 September
Location: Basel, Switzerland



16

Discovery on Target

Date: 16-19 September
Location: Boston, USA



26

ELRIG Discovery Technologies

Date: 26 September
Location: Cheshire, UK



october

19

Neuroscience

Date: 19-23 October
Location: Chicago, USA



november

05

ELRIG Drug Discovery

Date: 5-6 November
Location: Liverpool, UK



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