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INTRODUCING OXFORD GLOBAL'S R&D KEY 40 STORIES OF 2022

We are pleased to share with you selected highlights from Oxford Global's thought leaders and scientific experts from our flagship events and exclusive interviews in 2022. Last year marked an important milestone for us – the launch of 8 content portals with the aim to showcase cutting-edge research results in life sciences, key learnings and market updates via headline articles, commentary pieces, expert Q&As, whitepapers and annual events. Through the membership-only access portal features, we hold monthly discussion groups bringing together visionary leaders for an hour of discussion on topic areas that really matter for them as well as provide exclusive access to content from the in-person and online events we hold. There's more to come in the future as we stand by our promise to innovate, impact and elevate research in life sciences.

Since the launch of the 8 portals – **Biologics, Biomarkers, Immuno, Cell, PharmaTec, Formulation & Delivery, Omics & Discovery** – we have worked tirelessly to fulfil the above commitment to the community we serve and have released over 500 content pieces across the portals. In this ebook, a culmination of our efforts, we are delighted to present to you the 40 top stories and insights of 2022 from thought leaders and industry experts. We do hope you will enjoy taking a deep dive into these.

Eszter Sutowski Nagy,
Director of Editorial & Event Content, Oxford Global



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— Part I —

OMICS

Providing insights into multi omics
approaches to disease

GENOME EDITING

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DIGITAL PCR

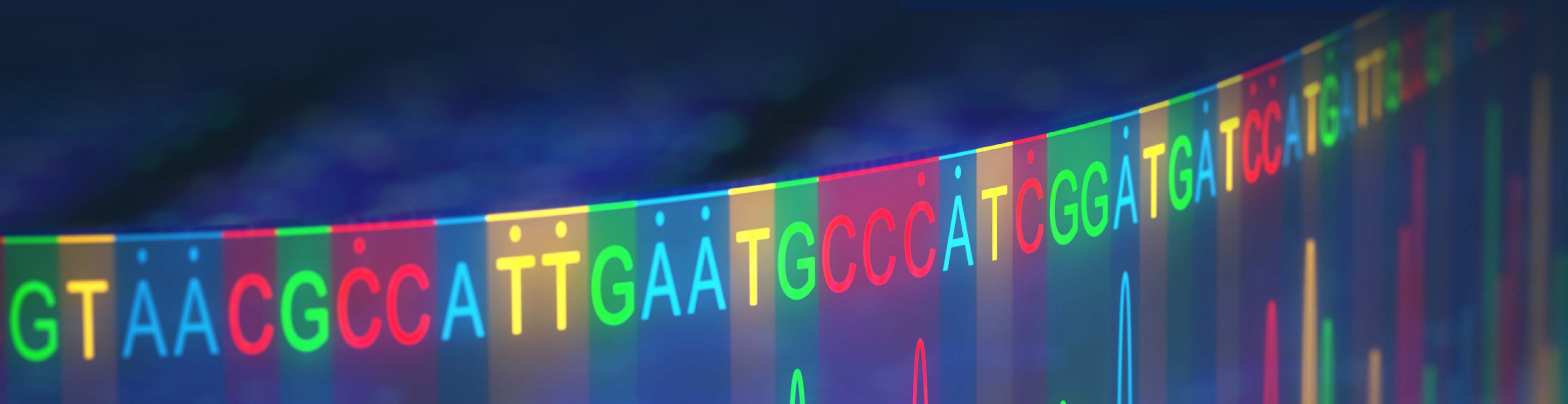
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NGS & CLINICAL DIAGNOSTICS

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NOVEL SYSTEM FOR MICE SPERM CRYOPRESERVATION USING MULTILAYER INSULATION AND LIQUID NITROGEN

We sat down with the Co-director of Yale Genome Editing Centre of Yale University, Xiaojun Xing, to discuss his breakthrough cryopreservation method and his thoughts on the genome editing landscape.

Transcribed by Oliver Picken

Could you give us a brief overview of your current research?

Cryoprotectant and cooling rate are two critical factors for successful cryopreservation of animal sperm. As an amateur researcher and inventor, I have been focusing on developing a low cost, simple cooling rate control method, thus improving the efficiency and quality of the cryopreservation process. I found that using multiple thin layer materials to insulate sperm samples and directly immerse them into liquid nitrogen can achieve an ideal cooling rate and enable the majority of sperm to survive after freezing and thawing.

What is the greatest benefit of this new cryopreservation method?

This new method will significantly reduce the cost of sperm cryopreservation. I believe it could replace computer-controlled devices in the future. For mice, the sample storage

capacity will increase to 5 to 10-fold due to the new method enabling the use of much smaller MicroStraw containers, 15 or 20 mm in length, 10-12 µl. Resources such as liquid nitrogen, storage tanks, storage room space can be reduced to as little as 1/10 compared to previous methods.

What is the greatest challenge you face in developing this technology, and what do you need to overcome it?

I am shy to say; the most significant challenge is my English. I am not good at writing funding application documents, so I have to invest my salary to support research and development. From a technology aspect, I was trying to solve an engineering problem with my reproductive physiology background. I had to teach myself about cryogenic heat transfer, plastics and manufacturing processes. I hope I can get funding and a research team to accelerate the application and commercialization of this new method for mice and many other species.

How do you see the genome editing landscape changing in the next five years?

I would say that protocols for genome editing based on CRISPR/CAS-9 will be further refined and improved; thus, the efficiency for genome editing will be higher and higher.

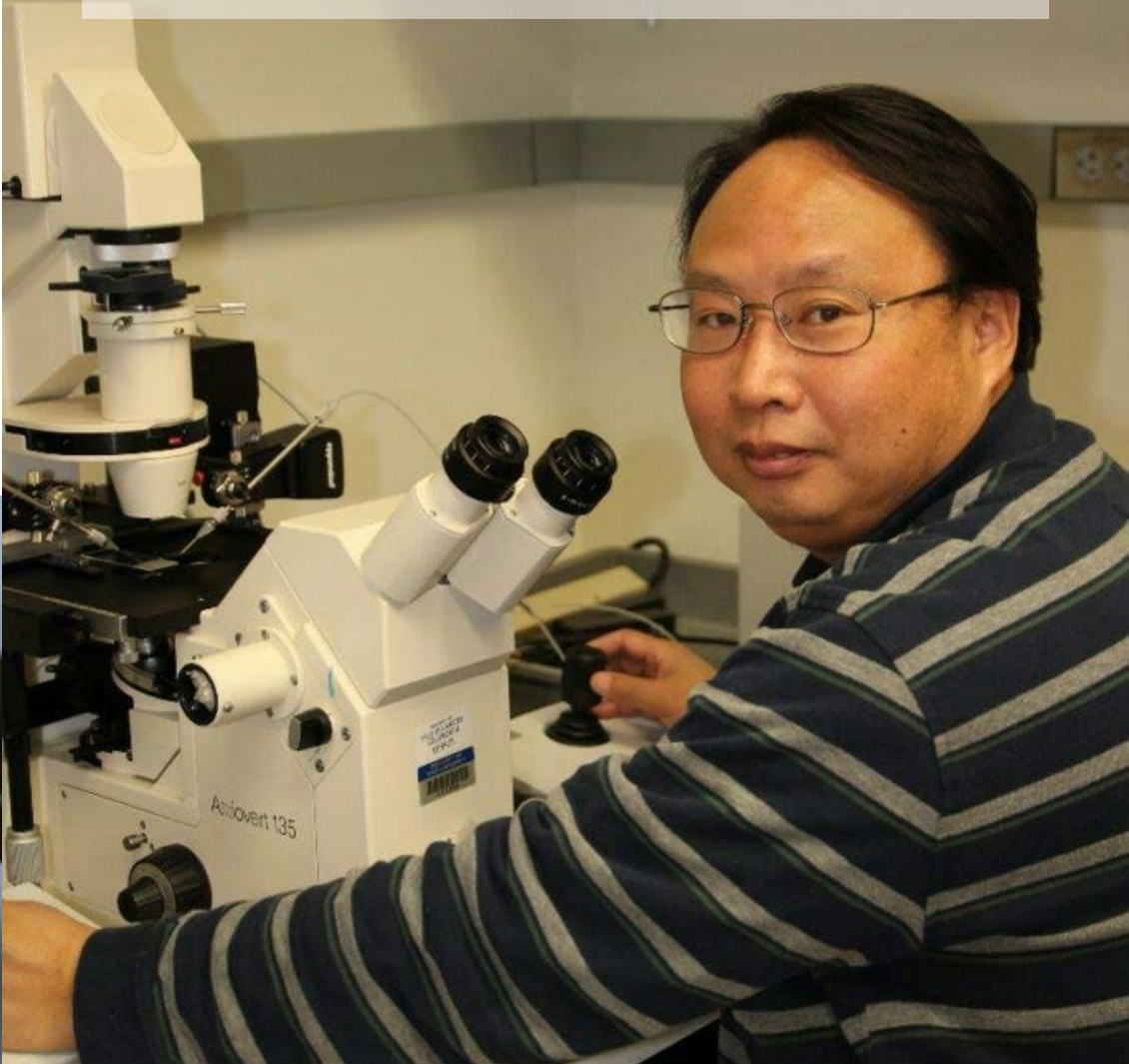
Any closing remarks on the genome-editing field at large, especially with advancements in technologies such as yours?

For genetically modified animal models, especially mice, one thing for sure is that the number needed to be cryopreserved each year will increase rapidly. Unlike livestock, where frozen sperm will be used for artificial insemination soon after freezing, animal model sperm will store for decades, even forever. With an up to a 10-fold increase in storage capacity, the new method will significantly reduce the storage pressure and burden. Also, it is imperative to alter this method to suit rats and other species. It needs many people's effort, not me alone.

Xiaojun Xing
Yale Genome Editing Center
Co-Director at Yale University

Mr Xiaojun Xing is the Co-director of Yale Genome Editing Centre of Yale University. He oversees the mouse embryo manipulating group in the centre to create, preserve and reanimate genetically modified mice. He has optimised and improved methods and procedures for microinjection, sperm cryopreservation and IVF. His improved microinjection method, referred to as Zero Angle Microinjection (ZAM), eliminates the injection angle and increases embryo survival rate.

His improvement advanced the progress of research, and he is the co-authors of two publications on Neuron and Nature. As an amateur researcher and inventor, he has focused for decades on cooling rate control during sperm cryopreservation. He invented a novel method and holds a USA Patent titled Multilayer Insulation of Biological Samples and Cooling by Immersing in A Cryogenic Liquid for Cryopreservation. He is also the inventor and producer of the first computer program-controlled sperm freezer in China, which adopted another patented method for cooling up to 10 thousand bundled together straws by immersing gradually into liquid nitrogen. He was an associate professor in China before coming to the USA in 2003. He earned his second master's degree from Purdue University in the field of animal reproduction physiology.





DISCUSSION GROUP

SPATIAL BIOLOGY: KEY QUESTIONS FOR THE FUTURE

Our February Discussion Group brought together more than 40 key opinion leaders to debate the future of spatial biology. Spatial transcriptomics, single cell resolution and tissue preparation were among the key topics raised.

Edited by Oliver Picken

Our February discussion group brought together over 40 people working on spatial transcriptomics, technology advancements and data analysis. To facilitate such a large group a pre-session survey was sent out, asking attendees what topics they wished to focus on. The results proved to be very interesting and highlighted areas including single cell resolution, spatial analysis in oncology and understanding the tumour microenvironment which were of particular interest to attendees.

This month's group was led by Raffaele Calogero, Professor of Bioinformatics and Genomics Unit at University of Turin. Between 2002-2010 he organized, in collaboration with Affymetrix, training courses for biologists on microarray data analysis. Since 2010, he is involved in bioinformatics training at EMBL (Heidelberg). He runs courses on NGS data analysis in collaboration with Illumina in Italy, and in collaboration with DUKE-NUS in Singapore.

Spatial Transcriptomics to Single Cell Resolution

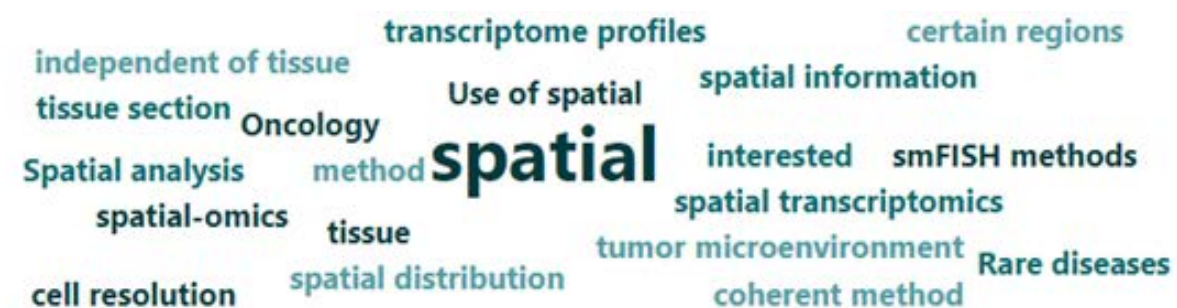
Currently, most spatial techniques do not provide insights

at single cell resolution. This is a concern, particularly in oncology, as many biological structures including tumours are made of heterogeneous cell clusters. One of the foremost goals of spatial transcriptomics is enabling single cell resolution on spatial platforms, which would eliminate the needs of running two independent experiments.

There are many technologies currently in development which are being designed with this goal in mind. Examples from the group included PhenoCycler-Fusion from Akoya Biosciences, which claims to be "the fastest spatial biology solution that enables ultrahigh-plex spatial phenotyping of whole slides at single-cell resolution", and upcoming platform updates from Visium and 10x Genomics.

Key Question: "Do we need single cell resolution?"

"To study the tumour microenvironment, at least at the oncological level, that is something that you need. You need to be sure that those cells do not belong to the tumour itself. What we do in our lab, if budget is sufficient, is run a single cell experiment plus the spatial. The problem is always the price of the overall experiment."



Tissue Preparation and Reproducibility

Preparing tissue for spatial transcriptomic analysis is key to reproducibility and accurate results but remains challenging and expensive. Xiaoxia Liu et al (2022), conducted a study on tissue preparation for spatial analysis. They found that out of 125 samples from 57 pairs of lung cancer, paired cancer and normal tissues from 54 samples were suitable for spatial transcriptomes. Out of these, 43% were successfully prepared for analysis.

Key Question: "I'm wondering, since you're from the cancer field, how reproducible are your results? I'm from a neuroscience field and in my case, it's very reproducible, but it is heavily dependent on where the tissue is from and how it was sliced because if you're only like 10 microns off, it results in an entirely different result."

"I would say that quality and reproducibility depend on the quality of the RNA you're working with. I like the idea of working on snap frozen samples, because the quality of the RNA is higher with respect to the one observed on FFPE samples. Visium for FFPE technology is now on the market, and it seems to be quite robust, although the results are dependent on the shelf storage time of the samples. It is important to remember that the RNA quality of FFPE slides degrades progressively over the years. Definitively, experimental reproducibility is firmly linked to the quality of the input RNA."

Spatial Transcriptomics: Immaturity and Data Analysis

Spatial transcriptomics is a relatively new discipline, and it is generally agreed that current methods and tools are immature. Raffaele explains that "spatial transcriptomics is not yet mature, in the sense that we have a lot of platforms. We're more or less in the same situation that we had few years ago within the single cell RNAseq framework, when a plethora of different platforms were placed on the market. Today, only a few of the initial platforms survived and now we have few clear market leaders. On these 'survived' platforms are run most of

the single cell experiments. The present problem for spatial transcriptomics is that if you decide to buy a specific platform today, and there are a lot of them now on the market, in a few years that investment may not pay out, because what you bought is no longer upgradable or supported."

Another challenge is analysing the large volumes of data gathered from spatial experiments, particularly when combined with other data such as single cell omics. Current spatial transcriptomic techniques also face the issue of unwanted technical artifacts, which are not well characterised, yet.

Key Question: "I think there is also immaturity of the spatial side when it comes to data and analysis. I've seen papers coming up with that are using the Google browser, I've seen people are using R and so on. So, what is everybody basically doing and kind of the immaturity of the domain as well?"

"What we do, in our lab, is picking the best of what is available within the open-source community. If we use clustering as an example, I can say that the most used tool by us is the Seurat tool. We tested many clustering tools, and Seurat clustering implementation is the one performing better than the others. What we do normally is we run a conventional single cell clustering analysis on spatial RNAseq data and subsequently, overlay the clustering results on the tissue slide. Although, it is a quite simple approach we grab quite interesting info in that way, especially if we integrate spatial data with conventional single cell RNAseq."

Final Thoughts & Conclusion

At Oxford Global, we couldn't be more pleased with the turnout and feedback from this discussion. This group was one of our most popular sessions so far and provided the perfect setting for exchanging ideas, sharing innovations and discussing the ever-evolving spatial transcriptomics landscape. If you would like to join one of our future groups, consider becoming an Omics community member. Future topics include single-cell analysis of the tumour microenvironment, target identification and single-cell proteomics.

SINGLE CELL PROTEOMICS: ADVANCEMENTS AND POTENTIAL

Our April 2022 Omics Series discussion group focused on the potential and future of single-cell proteomics.



DISCUSSION GROUP

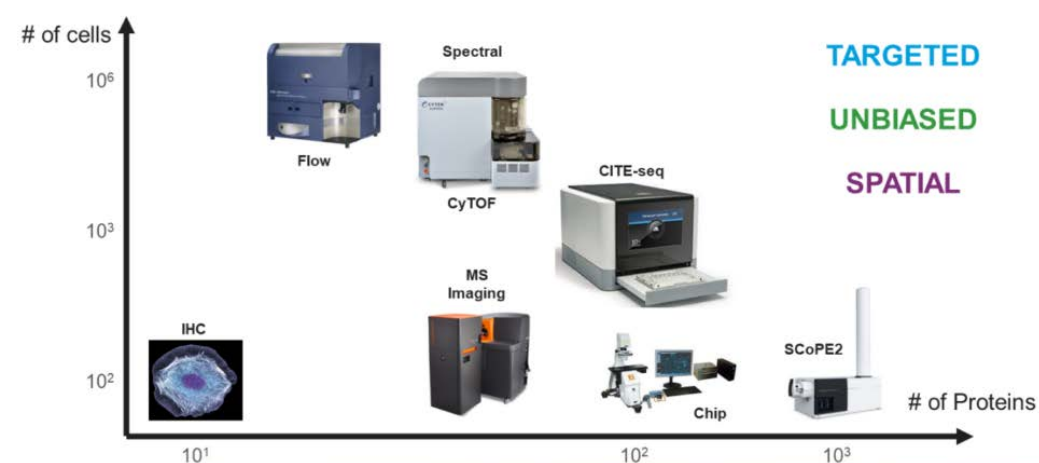
Edited by Oliver Picken

Oxford Global's discussion groups bring together a select group of 15-20 key industry leaders for approximately one hour for in-depth knowledge sharing and conversation.

This month the group was led by Yann Abraham, Senior Principal Scientist at Janssen. In this position, Yann plays a key role in analysing and integrating datasets generated through partnerships with institutions in the French biomedical research ecosystem, leveraging synergies with internal projects from the Janssen Therapeutic Areas and initiatives from the Data Science team within DS Computational Sciences.

Abraham opens the group by asking, "why do we want to do proteomics?" He explains that "proteins are the building blocks of life, and most biology is driven by the amount, localisation and activation statuses of proteins. Conversely, diseases most likely occur when the proteins are incorrectly expressed, localised, or activated. Therefore, understanding proteins is key to advancing biology and advancing the way we cure diseases."

Measuring proteins at the single cell level



Single-cell proteomics allows you to do two equally important things to answer the questions posed by recent literature. First, they will enable you to document or study changes in composition. Second, Proteomics also allows you to examine changes in functionality, which is challenging to do with other methods.

Single Cell Proteomics Technology

One of the limiting factors of single-cell proteomics over the past few years has been the lack of efficient technology. Fortunately, this is changing fast. Abraham notes that you can "classify technologies by two axes: number of cells and number of proteins. So basically, when you're doing immunohisto chemistry, you are doing single-cell proteomics."

When discussing single-cell proteomics, many people will think about flow cytometry. This technology allows you to measure more markers on significantly more cells. CITE-seq is also a recent development which allows sequencing to detect proteins through DNA barcodes. Spectral cytometry combines the best of both worlds between flow cytometry and CITE-seq. It can achieve similar flow heights as flow cytometry and an equal number of molecules as CITE-seq.

Now, there is a way to measure patterns in an unbiased way: mass spectrometry. Abraham explains that "recently, some people have pushed the boundary of what mass spectrometry can do by developing a technology to measure the protein content of a single cell at a reasonable scale. We're still talking a few 100 cells at a time, we are not quite there yet, but the main advantage of this technology is that it's unbiased."

OMICS

Abrahams elaborates that "all you need to do is a sequence of a protein and you can, under certain circumstances, detect the presence or the absence of particular proteins without the need of an antibody." The most recent development for the field of single cell proteomics has been the so-called spatial technologies, either antibody-based (Chip cytometry, Imaging Mass Cytometry) or unbiased (Image Mass Spectrometry), enabling deep profiling of tissues and tumors.

The Future of Single Cell Proteomics

Abraham highlights two significant developments he expects to see in the coming years. "There's a technology called SELEX. Using the property of some RNA sequences to adopt 3D conformations that have protein-like properties, one can use *in vitro* evolution (repeated cycles of mutation and selection against a target) to raise a sequence that will recognize a particular target or even a particular conformation much in the way an antibody would. Such aptamers can be fine tuned to recognize specific isoforms, and detected either directly through sequencing, or by tagging them. So, one of the major advancements I see in the field is the extension of what you can target."

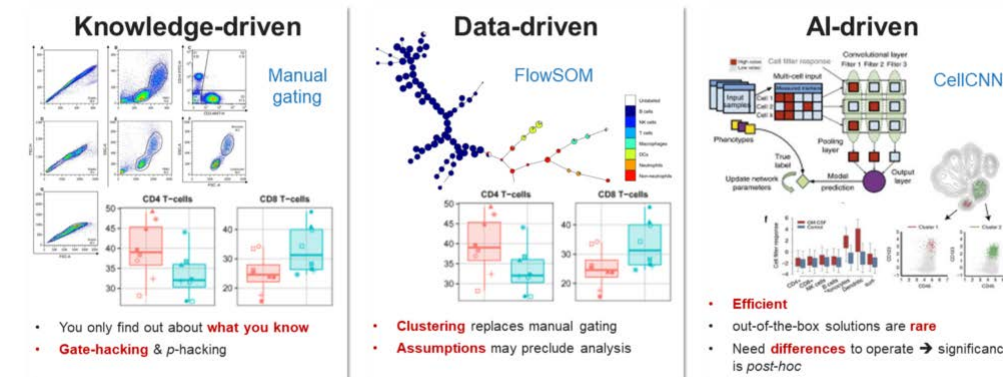
The other advancement is the field of AI-driven analysis. Abraham notes that "more and more people are moving towards data-driven solutions where one looks for differences at the single cell and not population level. Now there is a catch to this approach: it works under the assumptions that the difference you're looking for is in the data you collected, and there is no guarantee of that. So, while I see AI-driven analysis as a major evolution in the future of this field, I think we will see it combined with descriptive views."

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Key Question: One thing that makes Single Cell Proteomics difficult is the cost. Do you think this will change in the future?

"I think that there are there two drivers to the costs. One of these is the price of the antibodies you're putting in the mixture. For many of them, they are commodities, but then they are not necessarily cheap commodities. And for particular questions, companies can build to scale, so the price decreases. But sometimes, you want to add your own therapeutic antibody, and you want to know whether it's binding the target and measure receptor occupancy. That can amp up the cost of your experiment. The second is reagents, be it sequencing reagents, in the case of cyclic or simply the heavy metals in the antibodies. So, unless we see a significant

Single cell analysis



Janssen R&D Data Science

janssen

▲ Diagram of Single Cell Proteomics Analysis Styles

evolution in instrumentation, the limitations will be precisely that: the price of the antibodies and the price of the reagents."

What methods do you use for protein preservation?

"So what we are dealing with nowadays is two solutions. Either we are using fresh frozen samples, or we are using fixated samples. In the latter case we are using custom panels where we have to do a lot of optimisation to make sure that all the antibodies we are interested in work. One of the big promises of CITE-seq was the possibility to monitor both surface protein as well as cytoplasmic proteins. Then it turned out that many antibodies didn't work anymore after formalin-fixation.

"Most available samples are paraffin-embedded. And that's the killer question I usually ask any technology provider; is your technology compatible with paraffin-embedded tissue? A couple of years ago, the answer was no. You have some positive responses nowadays, but you may run into issues with some epitopes disappearing in the procedure. But it is much better than five years ago."

For more on single-cell proteomics sample preparation, read about [Ryan Kelly's work on sample miniaturisation and NanoPOTS](#).

Final Thoughts & Conclusion

At Oxford Global, we could not be more pleased with the turnout and feedback from this discussion. This meeting provided the perfect setting for exchanging ideas, sharing innovations, and discussing the ever-evolving digital therapeutics landscape.

MICROFLUIDIC DROPLET-BASED PCR: A TOOL TO SOLVE CHALLENGES IN PERSONALISED MEDICINE AND CANCER DETECTION?

In this commentary article, we present Dr Valerie Taly's insights on the latest research into digital droplet PCR and the potential applications of liquid biopsy in cancer detection.

Presented by: Dr Valerie Taly, Group Leader & CNRS Research Director, Cordeliers Research Center, INSERM, University Paris Cité

Transcribed by: Ben Norris

Dr Valerie Taly, Group Leader & CNRS Research Director, gave an excellent speech addressing the potential applications leading research in Droplet-Based Digital PCR For Cancer Patients at Oxford Global's 2021 Omics UK conference. This article will further explore the applications of ddPCR in the early detection of mutated or methylated tumour circulating DNA in cancer patients and what this could mean for future screening through liquid biopsy.

Droplet-Based PCR and Mutation Detections Through Liquid Biopsy

Although DNA sequencing techniques have come on in leaps and bounds since their inception in the 1970s, significant challenges remain in the blanket detection of localised and metastatic colorectal cancers. Headed by Dr Taly, the Translational Research and Microfluidics (TRAM) Research Group at the University of Paris Cité had identified an avenue for applying quantitative approaches to biomarker detection, which could potentially increase the reliability of cancer screening.

The approach worked on by Dr Taly's team involved looking for genetic or epigenetic alterations associated with the development of cancer, which serve as acutely specific biomarkers. One of the primary methods for this is liquid biopsy, where fluid samples are investigated for tumour-related elements (including nucleic acids or cells) that may be circulating in the blood. Specific DNA sequences are commonly detected using conventional polymerase chain reaction (PCR methods); improvements in detection methods such as droplet-based digital PCR (ddPCR) for detecting rare events

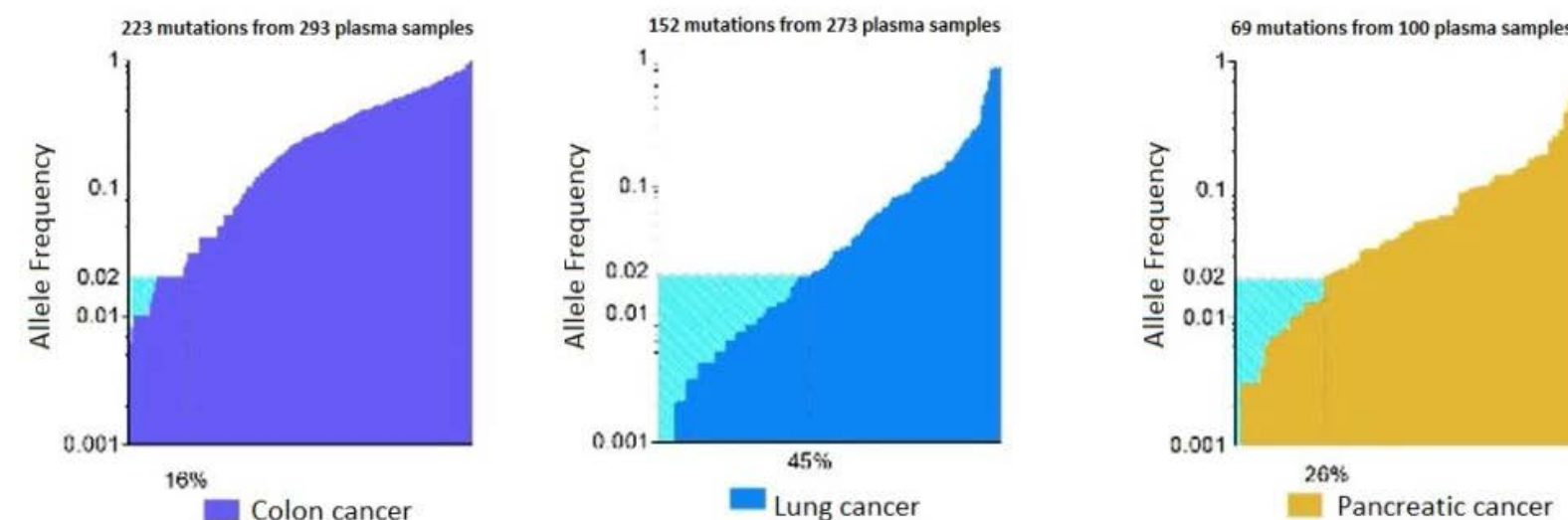
like the early onset of cancers have enabled their use in clinical settings. The prompt detection of cancers through circulating tumour DNA (ctDNA) analysis could have a significant impact on the morbidity and mortality of localised and metastatic colorectal cancer patients.

Complications of Scale Hindering PCR Analysis

However, bloodborne ctDNA concentrations are often fractional relative to concentrations of normal DNA coming from non-tumour cells. "Here comes the need for sensitivity," Dr Taly explained. "You're really at the single-molecule level – ctDNA can be detected within blood samples, but since it presides in very low amounts it may only represent a tiny fraction of the total circulating DNA." Conventional PCR-based procedures presented significant limitations to research when the project began, back in 2008, while the highly sensitive procedures necessary to detect trace ctDNA cannot do so at very low quantities.

"Here comes the need for sensitivity... ctDNA can be detected in blood samples, but since it presides in very low amounts it may only represent a tiny fraction of the total circulating DNA."

Data collected by the research team on colorectal cancer, lung cancer and pancreatic cancer suggested the typical concentrations of ctDNA that may be anticipated in patients with advanced cases of these cancers. "We looked into how many patients will have less than 2% of ctDNA, and this fraction is quite high," continued Dr Taly. "It's 16% in colon cancer, 45% in lung cancer and 26% in pancreatic cancer." Essentially, a high proportion of patients will demonstrate a low amount of ctDNA even in cases of advanced cancer (as seen in Figure 1), so the challenge for Dr Taly and her team was the development of a reliable method for ctDNA screening that detected as many mutations as possible.



▲ Figure 1. Proportion of advanced cancer patients with less than 2% of ctDNA in plasma. (Postel et al., 2017)

Applications of ddPCR for Localised and Metastatic Colorectal and Cancer Patients

The solution to the TRAM Research Group's Gordian Knot concerned the use of digital droplet PCR (ddPCR) to enable the high-resolution identification of mutated DNA, as well as the determination of mutant allelic fractions (mA%). The process involves water in oil droplet technology: a standard sample is fractionated into five to ten million droplets, with PCR amplification of the template molecules occurring in each individual droplet. This approach enabled a far higher degree of accuracy across a much greater sample size than previous studies, circumventing the obstacle of high-resolution sampling which slowed down conventional PCR techniques.

Dr Taly's laboratory assembled what she affectionately described as "homemade microfluidic stations" for development and platform design. Dedicated improvements included a customised optical setup and microfluidic chips; a clinical analysis of samples was carried out in collaboration with Raindance Technologies, using the company's RainDrop system for assay development and clinical validation. Screening for universal colorectal cancer markers was also assessed in droplets with the use of methylated markers, enabling researchers to discriminate malign tumour tissues from healthy adjacent tissues. An analysis of 56 tumour tissues revealed that all were positive, with 94.3% positive for one marker.

Dr Taly told the audience that ddPCR allowed for the performance of quantitative and highly sensitive measurements at a resolution of 1/200,000 mutant DNA in wild-type DNA, as opposed to 1/10 in bulk experiments. Even at high dilutions, the detection of target sequences through this procedure is quantitative, making it an

efficient means for identifying mutant sequences and a potential tool for addressing challenges in personalised medicine. Highly sensitive methods are required for ctDNA detection and quantification in order to detect cancerous or otherwise malign DNA. Even advanced cancer patients often registered less than 2% ctDNA in their plasma; earlier cancer developmental stages aligned with the lower patient detection through ctDNA monitoring.

Future Applications of Liquid Biopsy for Cancer Detection

The main conclusions from this research were that a ctDNA follow-up could be a pertinent marker of cancer progression, since diagnostics suggest a correlation with conventional markers. Moreover, a ctDNA follow-up could be the only molecular marker of cancer progression for CEA-negative patients. One of the many practical applications of this procedure is that healthcare providers could determine if a particular chemotherapy treatment is effective after one cycle, instead of administering several rounds of unsuccessful therapy. Dr Taly concluded her presentation by asserting that liquid biopsy could be used to track tumour DNA in advanced and early colorectal cancer patients, with optimism for its use in clinical settings in years to come.

References

Postel, M., Roosen, A., Laurent-Puig, P., Taly, V. and Wang-Renault, S., 2017. Droplet-based digital PCR and next generation sequencing for monitoring circulating tumor DNA: a cancer diagnostic perspective. *Expert Review of Molecular Diagnostics*, 18(1), pp.7-17. presented significant limitations to research when the project began, back in 2008, while the highly sensitive procedures necessary to detect trace ctDNA cannot do so at very low quantities.



LONG AND SHORT READ SEQUENCING: REVOLUTIONS IN GENOMICS DATA

In this Discussion Group Report, we investigate some recent applications of long- and short-read sequencing technologies. Continued improvements and advancements in the field mean sequencing errors are increasingly a thing of the past, while the portability of some new units makes them ideal for work in the field.

Edited by: Ben Norris

Many of the opportunities and sequencing techniques available to researchers today feel like products of science fiction, but all of the latest methods discussed in this NextGen Omics Discussion Group are very much science fact. New developments in efficiency and portability make it cheaper than ever to sequence a genome partially or fully, and the speed at which this can be achieved is constantly increasing.

Discussion was led by Patrick Descombes, Senior Expert in Genomics and Genomics Group Leader at Nestle Research, with Edward Oakeley, Associate Director of Genomics at Novartis and Massimo Delledonne, Professor of Genetics at the University of Verona there to co-lead. Read on to learn about some of the changes that have taken place in the field of long- and short-read sequencing since the turn of the millennium.

Advances in Genomics Technologies

"I think we all know there's been a fantastic revolution in genomics technologies over the past 15-20 years," Patrick Descombes said as he opened the discussion. Advances in the field are consistently hitting new milestones, with tremendous increases in throughput and reductions in cost. "Some companies are claiming they can go down to less than 100 dollars per genome, which of course is just fantastic. And we

know that these types of technologies can be applied to any species with DNA, be they humans, bacteria, or plants."

According to Descombes, this has led to a 'fantastic development' of very large-scale studies, driven by the development of these methods and a reduction in cost. "Still, genomics is a field which is quite complex, and mastering these technologies and their application requires some expertise."

"Genomics is a field which is quite complex, and mastering these technologies and their application requires some expertise."

When it comes to specific technological developments, long-read sequencing technologies are particularly useful for resolving complex genomic regions. Synthetic long-reads use 'tricks' to assemble long-read proteins from short-read proteins from short-read segments. "When people ask 'what should I do', clearly the choice of the approach and technology is driven by the goal of the study," said Descombes. As such, a major focus for researchers is on the complementarity of long- and short-read sequencing technologies and applications.

Portable Short-Reads

After Descombes had introduced the range of topics for discussion, Massimo Delledonne kicked off the conversation by recounting a recent field expedition to the Mongolian Gobi Desert. He explained that his team had been using Oxford Nanopore Data Reads for the project due to their high portability. "We were keeping material frozen during the night by using the Oxford Nanopore box container, which was fantastic, and during the day we were using a portable freezer to keep the sample frozen."

Descombes asked Delledonne what the benefits were to doing sequencing work on-site as opposed to collecting the samples in the field and working on them in the laboratory. "If you think about Mongolia, they have no sequencer," Delledonne replied. "In a hotspot for biodiversity, they can analyse the sample there and it's a very limited cost, because Oxford Nanopore is very cheap." Delledonne added that many species in Mongolia were on the red list and at risk of disappearing, which made the ability to monitor them in situ very valuable.

Obstacles to Future Sequencing in Genomics

Hywel Williams, Senior Lecturer in Bioinformatics at Cardiff University, explained that he worked on ways of looking at rare diseases and identifying causative variants in patients who are diagnostically negative. "One of the things I'm interested in with long-read sequencing is whether it can identify pathogenic variants in these kinds of patients."

Williams continued that in terms of barriers to developing this understanding further, expense and irreplicable historical samples are two prime issues. "Third, when you find something, how can you understand whether that is potentially pathogenic, or whether it's just a rare variant in the genome that we haven't sequenced enough people to understand?" He made the case for a 'gnomAD': a Genome Aggregation Database for long-read structural variants to improve the genomic resolution.

Descombes added that the field was, broadly speaking, still in its infancy. "In order to understand the consequence of having phased variants as opposed to unphased, most of the field has been evolving with this artificial notion of the human haploid genome with short-read sequencing, so it's only now starting to go into this. A lot of the new development will have to come from sequencing, not only family tree yields, but also larger cohorts with long reads."

Potential Applications of the Pan-Genome

Discussion then turned to the concept of the pan-genome: a family tree of genomes for sequencing, with a graph based on having all the relevant genomes and splits represented. The goal of developing the pan-genome is to simplify the ways in which analysis can be applied and executed. "When you want to map your own genome on a graph of the pan-genome, you

should have a high-quality genome," said Delledonne. "You cannot simply use short-reads to build up a graph pangenome because they don't know where to be inserted or added to the pan-genome when they don't map on the graph."

Williams mentioned that he had worked extensively with the UK's 100,000 Genomes Project, and that the next stage would involve the sequencing of roughly 2,000 people with PacBio. "In terms of strategy, that is probably the optimum way to go forward at the moment. At this level, a couple of thousand genomes is going to be the minimum amount we need, but if you can do that first couple of thousand and potentially get a handful of diagnoses thrown into that as well then that is going to be a really good use of that money."

"The 100,000 Genomes Project in the UK is fantastic," Delledonne agreed. "It's a great example of what you can do now when you mix the genomics genetic data with the phenotype. So nowadays, genotyping is as important as phenotyping. At the end, you will have a lot of high quality genomics data, but don't forget you need to do something with this data. You need to associate them to phenotypes."

— Part II —

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Accelerating translational research through advanced biomarker technologies & strategies

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HIGH DIMENSIONAL FLOW CYTOMETRY IN CLINICAL TRIALS

How can the biomarkers industry best implement multi-parameter flow cytometry in clinical drug development? Vilma Decman of GlaxoSmithKline takes us through the advantages of using spectral flow cytometry, the importance of viability dyes, and high-parameter panel design.

*Presented by Vilma Decman,
Director and Head of the Cellular Biomarkers Group, Bioanalysis,
Immunogenicity and Biomarkers at GlaxoSmithKline*

Transcribed by Tia Byer

The Cellular Biomarkers Group at GlaxoSmithKline supports clinical biomarker testing through immunohistochemistry, flow cytometry, genomics, imaging mass cytometry, spatial profiling and other emerging technologies. In recent years, multi-parameter technologies have dominated biomarkers research to help streamline the drug development process. As part of Biomarkers Week: Online held in May 2021, this article will examine how utilising viability dyes and implementing a multi-parameter approach to flow cytometry can radically optimise biomarker discovery.

Implementation of Multi-Parameter Flow Cytometry in Clinical Trials:

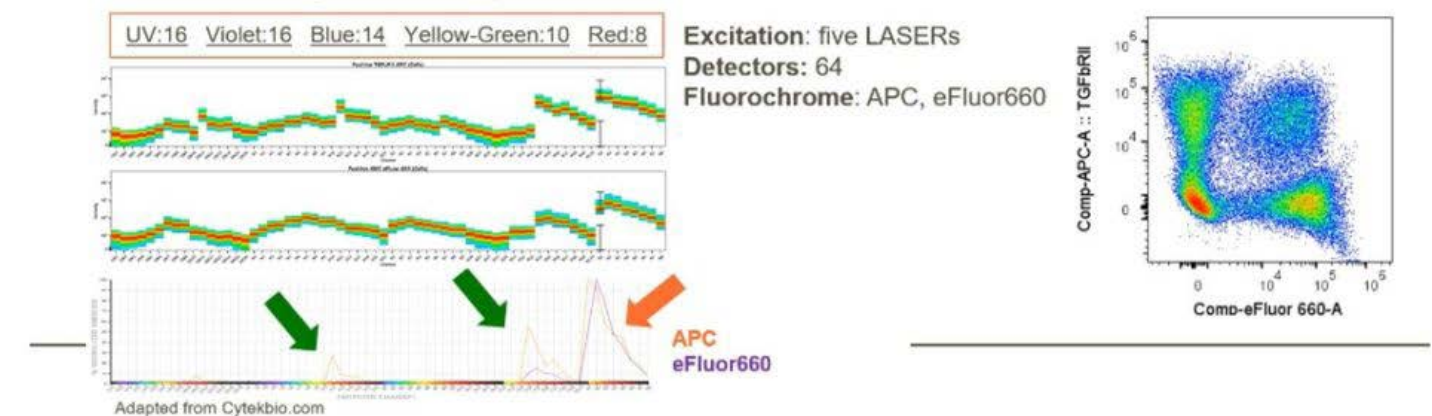
Implementation of multi-parameter flow cytometry in clinical trials enables simultaneous examination of multiple targets on various cell subsets from a relatively limited sample size. Multi-parameter flow cytometry can identify, enumerate, and track cellular markers (both intracellular and intranuclear) during treatment. The technology provides valuable information on both the target engagement and the mechanism of action of new therapies. Furthermore, it aids in understanding disease pathology, toxicological assessment of new drugs, and their efficacy.

The initial strategy for assay development during clinical sample testing requires understanding the biomarkers needs for a particular drug and its targets. Among the most prevalent biomarkers are the pharmacodynamic biomarkers that show a biological response has occurred in an individual following treatment. Biomarker testing aims to individualise treatment by targeting the right patients with the right therapy at the right time. However, the presence or absence of any single biomarker may not provide a complete understanding of the diverse interactions occurring within the disease microenvironment. Therefore, a toolbox approach is required; evaluating multiple biomarkers in combination by flow cytometry provides a more accurate and comprehensive assessment.

Spectral Flow Cytometry: The Evolution and Advantages

Spectral flow cytometry brought new possibilities of deep profiling of many populations, including more precise tracking of rare events. Conventional flow cytometry uses one detector to capture partial emission of individual fluorochrome that has been excited by one laser. Spectral flow cytometry uses multiple detectors to capture full-spectrum emission of every single fluorochrome extracted from multiple lasers. Spectral flow cytometry, therefore, generates individual fingerprints for each fluorochrome.

There are several advantages of spectral flow cytometry over conventional flow cytometry, such as allowing for more

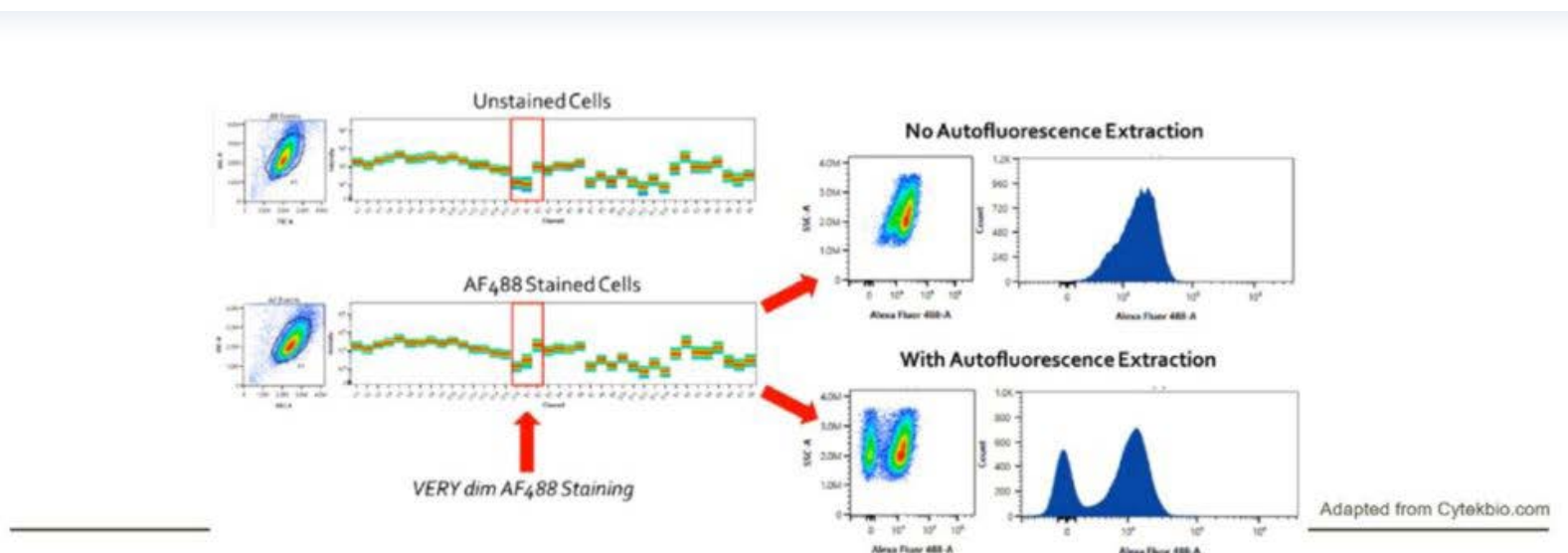


▲ Figure 1. The advantages of spectral flow cytometry as evidenced by simultaneous staining with APC and eFluor660 showing that fluorochromes with identical peaks of emission but different off-peak spectra can be efficiently differentiated using unmixing algorithms. This was not possible with conventional flow cytometry approach. Spectral flow cytometry also has a more straightforward set-up process that requires no filter changes.

fluorochrome choices and improved flexibility in reagent selection. For instance, fluorochromes with identical peaks of emission but different off-peak spectra can now be efficiently differentiated using unmixing algorithms. This was not possible with conventional flow cytometry approach. Spectral flow cytometry also has a more straightforward set-up process that requires no filter changes.

One of the most significant advantages of spectral flow cytometry is that it allows for the removal of autofluorescence which aids in improved and more accurate cell and marker separation. Certain cell types such as monocytes, neutrophils, and different tissue cells can have a very strong autofluorescence signals. These signals can interfere with precise marker detection and separation. Spectral flow cytometry can overcome this issue. In particular, it can detect full spectrum of autofluorescence of unstained cells. By extracting each autofluorescence signature and then separating it from fluorescent data, spectral flow cytometry improves resolution.

▼ Figure 2. Autofluorescence extraction in spectral flow cytometry. Autofluorescence is compromising resolution of dim population. Upon autofluorescence extraction, resolution of the positive population is improved significantly.





Vilma Decman

Senior Director and Head of Cellular Biomarkers at GSK

As Senior Director and Head of Cellular Biomarkers at GSK, Decman leads several teams that support clinical biomarker testing through immunohistochemistry, flow cytometry, genomics, imaging mass cytometry, spatial profiling, and other emerging technologies. Previously she has worked for companies such as Bristol-Myers Squibb, where she was appointed Associate Director of Clinical Flow Cytometry, and Flow Metric, Inc., and Regeneron. Decman completed her PhD in Immunology at the University of Pittsburgh and holds a Master of Science degree in Molecular Biology from the University of Zagreb.



The Importance of Viability Dyes:

Clinical flow cytometry assay development process can be divided into three steps; feasibility, assay development and validation. The assay development is perhaps the most critical stage of the process. This step involves both antibodies titration and performing additional panel optimisation. In addition, developing appropriate controls for the panels requires careful consideration. The last step of the process is fit for purpose assay validation tailored to the intended use of data.

An important step in panel development includes the use of viability dyes. Viability dyes for flow cytometry can efficiently and reliably analyse both live and dead cells. Dead cells bind antibodies non-specifically which can result in background staining and false positives. Therefore, excluding dead cells via viability dyes allows for a cleaner separation and detection of cell populations. This approach produces reliable, reproducible, and high-quality data.

The Importance of High-Parameter Panel Design:

GSK recently introduced two Cytex Aurora flow cytometry panels to accelerate flow cytometry biomarker testing, one being a 27-colour panel and the other a 23-colour panel. These panels were created for clinical sample testing and consisted of 8 cross-over backbone markers. Although generating a high-parameter panel can be a tedious and lengthy process, it is important to do careful antibody titrations to ensure high quality data. Indeed, titration of antibodies makes it possible to generate 20+ panels as it allows the determination of the amount of antibody that enables the best separations and the optimal measurement of expression levels of individual markers.

Multi-Parameter Flow Cytometry: Key Take-Aways

High dimensional flow cytometry in clinical trials is essential to improving data quality and optimising drug development. While highly powered designs cannot always predict all possible scenarios and drawbacks associated with a clinical trial setting, recognition of the need for power acceleration can significantly improve efficacies and provide comprehensive biological insights. The utilisation of automated data analysis holds many promises for the biomarker industry. At Oxford Global, we look forward to seeing the next advancements made by this technology over the coming years.

WHAT'S NEXT FOR PREDICTIVE BIOMARKERS IN IMMUNOTHERAPY?

From maximising immune response with combination biomarkers to conducting diagnostic innovation in immunotherapy prognosis, the future of predictive biomarkers is fast approaching. We speak to senior representatives from industry and academia to find out more.

Edited by Tia Byer

Predicting immunotherapy response is a predominant and increasingly important concern for oncology. In fact, the use of predictive biomarkers over the last ten years has steadily grown and now accounts for approximately three-quarters of the oncology pipeline.

As of 2021, the global oncology market was valued at 85 billion USD and is expected to reach 277.2 billion USD by 2030 at a CAGR of 12.6%. Key market drivers include the prevalence of cancer, the trending adoption of immunotherapy, and the availability of advanced healthcare facilities. Rising consumer expenditure on healthcare is also causing a noticeable impact on the field.

As the biggest and fastest-growing engine for biopharma R&D, oncology requires predictive biomarkers to better direct and improve patient response to immunotherapy treatments. The demand for cancer immunotherapy can largely be attributed to long-term treatment effects and convenience. Immunotherapy offers an improved treatment option devoid of unfavourable side effects such as organ failure, cancer reoccurrence, and the high costs commonly associated with conventional chemotherapy.

Featuring exclusive insights from key opinion leaders at Oxford Global's Biomarkers Week 2021, this article explores the future projections of the predictive biomarker market for improving immunotherapy response.

Maximising Immune Response with a Combination Biomarker Approach:

Using a composite of biomarkers can facilitate a precision approach to immunotherapy treatment. A combination approach is preferable to single biomarker use due to its ability to provide a doubly accurate form of measurement for immunotherapy. Whilst there are still some areas for development, harnessing multiple biomarker technologies in the clinical setting is an exciting prospect.

Richard Hughes, Director of Strategic Marketing at NeoGenomics Laboratories, claims that "there is a lot more work that needs to be done for combination biomarkers, especially on the patient end of the response." However, efforts are being made to address such difficulties, and The Institute of Cancer Research, London, is especially working towards combining clinical findings with computational biology. The organisation opted to use composite biomarkers to treat colorectal cancer after discovering issues with heterogeneity with single biomarker use.

Anguraj Sadanandam, Director of the Centre for Global Oncology and Head of Systems and Precision Cancer Medicine at The Institute of Cancer Research (ICR), explains how "instead of a single indication, we decided to use a multiple gene concept as a biomarker. For example, we have defined four to five subtypes in colorectal cancer, which can be identified using

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800 genes." The ICR experimented with different machine learning approaches to determine how much it could shrink the biomarkers. "We found that on average, eight genes per subtype with higher sensitivity are required for the patient to respond to," Sadanandam continued.

The ICR concluded that gene expression alone could not fully capture treatment response for colorectal cancer. "The potential for biomarker use does, of course vary depending on what the clinical endpoints are; factors that are very important to the predictive outcome," Sadanandam highlighted.

"Realistically speaking, the more sophisticated the biomarker technology and techniques employed, the more difficult data extraction is"

Data Analysis – The Challenges:

Predictive determination for immunotherapy response relies heavily on digital pathology and data analysis. Ed Schuur, Head of the Laboratory for Molecular Pathology at the University Medical Center Groningen, explains how "if trials were only to rely on biopsies, then we would not be able to answer everything." Systematic technologies such as PET scanning and in vivo imaging provide an objective view of the entire body, and with it, extensive information. Other example technologies include serum testing, virtual microscopy, and tumour immune microenvironment phenotypes.

However, it is not always possible to feed the diversity of data collected from patients through a singular platform. There needs to be a collaboration of different disciplines to measure and interpret static biomarker findings accurately.

"Realistically speaking, the more sophisticated the biomarker technology and techniques employed, the more difficult data extraction is," Schuur continued. In particular, novel platforms such as advanced PET scanning, liquid biopsies, and microbiome biomarkers still require development before they can efficiently lend themselves to concise data analysis. "They are not yet able to combine all of these data factors to perform simplified statistical readings," Schuur said.

Diagnostic Innovation for Predictive Biomarkers:

One strategy to overcome such issues is combining results through computational analysis. Achieving the next level of data acquisition from patients requires digital methods, nevertheless, ethical issues persist around the necessity of patient record access. "When it comes to capturing data on an electronic medical record, whether for ctDNA or microbiome analysis, we must be particular in determining who will gain access," Hughes stated.

It is essential to decide and disclose the exact data handling parameters in clinical research. For instance, pharma companies should adequately communicate whether data records will be passed on to all interventionists and physicians along the care pathway for that patient. Moreover, ensuring transparency about record handling can address these ethical considerations.

Prioritising Multiple Data Analysis with Limited Patient Samples:

Limited patient samples often take the form of restricted blood volume or difficulty accessing fresh tumour samples. When this occurs, data analysis is made more difficult. In particular, multiple data analysis becomes challenging and is, as Nadia Hassounah, Principal Scientist at Novartis explains, "a problem we face constantly in clinical trials for biomarker development."

Hassounah spoke of Novartis's experience with limited patient samples during Phase I trials. "We have to think about how to most efficiently collect the necessary readouts from a minimal data set or tissue sample," she continued. Opportunities for overcoming this hurdle include relying on RNA and DNA isolation to multiple slides from a single biopsy.

"Prioritising biomarkers to determine which one has shown the optimal pharmacodynamics (PD) readout during preclinical trials is critical," Hassounah claims. Selecting an adequate platform to provide a broader set of readouts is also beneficial. Hassounah identified RNA sequencing as a top priority for tissue biomarker analysis.

It is essential to decide and disclose the exact data handling parameters in clinical research.

Future Outlook:

Predictive biomarkers for immunotherapy response are an important development area for the pharmaceutical industry. Research exploration continues to interrogate the complex interaction between the immune system's regulation and the comprehensive diversity of the effects of immunotherapy. As the development of basic technology enters the landscape, we can expect to see improved accuracy in biomarker evaluation and monitoring techniques.

Advanced measurement technologies will continue to maximise the patient benefits of immunotherapy. This will, in turn, facilitate the next generation of the transformative treatment. At Oxford Global, we look forward to welcoming the future of immunotherapy and its predictive biomarkers.

TRANSFORMING CLINICAL DEVELOPMENT WITH BIOMARKERS

How can the industry transform itself with improved biomarkers for clinical development? And what hurdles stand in the way? We sat down with senior representatives from Galapagos, Ohio State University, and Genentech to find out more.

Edited by Tia Byer

Biomarkers are vital for all phases of drug development. The global market for these predictive technologies is vast and is set to grow from 43.3 billion USD in 2020 to 92.1 billion USD by 2025, at a CAGR of 16.3%. Key market drivers for increased attention in clinical biomarkers include the prevalence of terminal diseases, expanding diagnostic applications, and increased R&D funding for pharma and biotech companies. Other factors include the implementation of new initiatives for biomarker research and an enhanced number of CROs. Key players in the field include Roche, Abbot Laboratories, Epigenomics AG, Johnson & Johnson, Thermo Fisher Scientific, Rio-Rad Laboratories, Siemens Healthineers, and Qiagen.

Clinical Biomarkers: The Rationale

From pre-clinical assessment to the clinical regulatory use, biomarkers, as diagnostic, predictive, prognostic, or response tools, can accelerate drug development and ultimately benefit patients. When implementing biomarkers in pre-clinical experiments and clinical studies, it's critical to consider may operational factors including sample type (biopsy, serum, plasma etc.), biomarker stability, as well as assay type and

assay quality. From a patient selection or stratification perspective, according to Matthew Randall, Associate Clinical Biomarker Leader at Galapagos, with respect to a particular genotype, "knowing if my patient does or does not have this [genetic] marker, [may influence whether] my drug [candidate] is useful in that patient population." Together these considerations are required to appropriately design a robust biomarker plan for your clinical study.

Screening technologies can be advantageous to discover biomarkers that support translation between pre-clinical models and human disease, and vice versa. That being said, focussed and strategic biomarker assessment is suggested for late-stage clinical studies to support the study design and drug development. This does not of course limit the implementation of exploratory readouts in late-stage. Randall simply advises that "the samples that you collect [should] bring value" and to weight the value of the possible results compared to the burden on the patients for repeat sampling or invasive procedures.

Moreover, there are still significant limitations in biomarker accuracy and standardisation remain across all technologies and fields. In this article, we look at some of the advances

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being made in the biomarker field in neurological and oncology and assess their potential to transform clinical development.

Challenges of Clinical Biomarkers For Neurological Disorders:

Biomarkers for neurological disorders is a fast-growing market. However, their diagnostic and predictive value for frontotemporal dementia and Alzheimer's disease requires further development. According to Rawan Tarawneh, Assistant Professor of Neurology at The Ohio State University (now Associate Professor of Neurology at the University of New Mexico), "biomarkers for Alzheimer's disease especially have not been very well utilised in clinical trials thus far". Currently, the "stratification of patients and diagnosis is mostly based on the clinical picture, supported by MRI and only supported by CSF markers of amyloid and tau proteins in some but not all clinical trials which has been a major limitation", she continued. "Many trials did not include measures to confirm the presence of amyloid or tau pathology and therefore, did not include measures to confirm the presence of the pathologies being targeted by investigational treatments or measures to exclude cognitively impaired individuals who may have other underlying pathologies contributing to their clinical symptoms."

Looking ahead, there is a need to better incorporate amyloid and tau markers into the stratification of patients into clinical trials.

Another major problem is that since clinical trials include patients who are already cognitively impaired, it may be too late to intervene. "Alzheimer's is more than just amyloid and tau, and it may be already too late at that point to reverse the diseases process", Tarawneh added. This means the patient would have potentially already accumulated significant degrees of neurodegeneration and irreversible neuronal synaptic loss. Therefore, looking ahead, there is a need to better incorporate amyloid and tau markers into the stratification of patients into clinical trials.

The Promise of Diagnostic Biomarkers For Neurological Disorders:

Over the past couple of years, industry strives have been made to incorporate and translate biomarkers into the diagnostic scheme. This is particularly the case with the Amyloid Tau Neurogenerative (ATN) framework proposed by the National Institute of Aging and the Alzheimer's Association, which makes it possible to predict the presence of Alzheimer's using CSF and imaging biomarkers even before the onset of cognitive impairment. Those 'cognitively normal' individuals

who have biological evidence of precursory traces of amyloid and tau, must be targeted prior to the development of cognitive impairment. "These are the people you want to analyse to ensure diseases modifying treatment trials have a higher chance of success", Tarawneh confirmed.

Further efforts to address challenges include introducing ADCs into clinical development to overcome the lack of standardisation of the different assays and cohorts being produced between laboratories. This is because A-beta can be highly sensitive to the test tube's physical properties and freeze-thaw processes. ADCs offer a more reliable alternative, and the blood-based immunoassay for p-tau and a-beta has so far demonstrated compelling results as easier to standardise.

Challenges of Clinical Biomarkers For Oncology:

From an operational perspective, biomarkers for personalised medicine in oncology require some improvements. Comprehensive biomarker research is needed to prepare for the growing demand for tailor-made treatment. "We don't have enough biomarker data points yet to prepare for that imminent future," Alekhya Pochiraju, Clinical Product Development at Genentech, claimed. She continued, "a catch 22 in the industry right now is that as we move into adaptive and enrichment-based clinical trials, we need more biomarkers, meaning the more specific biomarker signature patients we are looking for, the more screening has to be done."

Inevitable consequences include higher costs due to lengthier and more frequent clinical trials. The industry must reach an equilibrium between streamlining clinical testing in precision medicine and offsetting increased costs. Furthermore, in the absence of rapid clinical assessment, the accuracy of the assay may become compromised and the turnaround time delayed. Biomarker assays demand a quick turnaround time to determine the patient eligibility for the clinical trial design.

Improving Standardisation and Stratification of Clinical Biomarkers For Oncology:

Nevertheless, the past couple of years has seen an increase in clinical trials designed to collect biomarker information. This is a trend we can expect to see continue. "We see this increase across all categories such as predictive biomarkers to determine treatment outcome and diagnostic biomarkers to help screen, identify, diagnose, and even measure the severity of disease," Pochiraju elaborated. Other strategies for improvement include greater patient selection ranges and stratification with the help of machine learning and AI tools. Combination biomarkers that combine panels whilst integrating patient data will likewise improve disease monitoring and treatment efficacy. Randall commented, "when



it comes to biomarkers, not just looking at one biomarker on its own, but using a composite [of biomarkers and clinical tools] may be the way forward.”

The industry must reach an equilibrium between streamlining clinical testing in precision medicine and offsetting increased costs.

To overcome standardisation issues for biomarkers in oncology, fostering collaborations across industry and academia will also be beneficial. Implementing a consortium will produce an authoritative standard of care and assessment. Advances in liquid biopsy are gaining sizeable importance within oncology research and diagnostics. The market itself is market rapidly expanding area of the field and is expected to increase at a CAGR of 13.5% to reach \$19.35 billion by 2028. As a pioneering technique harnessing Next Generation Sequencing technology, liquid biopsy testing presents an opportunity for non-invasive testing for cancer detection and monitoring response to treatment.

Future Outlooks:

The future of biomarkers remains an area of intensive research and sustained investigation. Despite the hurdles that still require addressing, there is evident momentum surrounding the latest market expansion. With developments in targeted patient selection, combination strategies, and efforts in standardisation, it seems only a matter of time before the biomarker industry in neurology and oncology reaches its full potential. It may even be that neurology and oncology will end up broadening the learning potential across other fields and diseases regarding the transformative power of biomarkers for clinical development. If one thing is certain, here at Oxford Global, we firmly believe the biomarkers industry will only continue to go from strength to strength.

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LIQUID BIOPSY FOR EASY AND DIFFICULT CANCER

Liquid biopsy has the potential to become a transformative cancer management and therapeutic strategy within the next 5 to 10 years. However, its capability across all cancer stages largely depends upon assay sensitivity. In this commentary piece, the University of Edinburgh's Tim Aitman takes us through a novel approach set to pioneer the landscape of liquid biopsy.

By Tim Aitman, Professor of Molecular Pathology and Genetics at University of Edinburgh

Transcribed by Tia Byer

The liquid biopsy market is a rapidly expanding area of the biomarkers field and is expected to increase at a CAGR of 13.5% to reach \$19.35 billion by 2028. It is a pioneering technique which is anticipated to gain sizeable importance within oncology research and diagnostics. The rapid market growth is attributed to cutting-edge advancements in Next Generation Sequencing technology, the evolving pipeline of commercialised products such as circulating DNA-based liquid biopsy tests, and the increasing opportunities for personalised cancer management. Liquid biopsy testing also presents an opportunity for non-invasive testing for cancer detection and monitoring of response to treatment.

Liquid Biopsy Background and Applications:

Liquid biopsies are comprised of more than just cell-free DNA and contain an entire host of components, including circulating tumour cells, exosomes, RNA, red blood cells, and platelets. While these are interesting in the research scenario, cell-free DNA assays have shown far greater clinical applicability than other assays. Clinical applications of liquid biopsy in cancer management can pertain to all stages of the patients' journey, from early cancer detection in healthy people and treatment selection in defined cases, to determining the level of minimal residual disease. Predicting the treatment outcome and early indications of disease recurrence and progression are additional benefits.

Opportunities for Easy and Difficult Cancers:

While research attests to the clinical applications of liquid biopsies, the varying complexity of cancer types remains a bottleneck for their wide-spread use. Within cancer management

there are easy and difficult scenarios. Some cancers secrete a lot of DNA, such as bladder, colorectal, gastroesophageal, and ovarian and there are those that secrete very little, such as prostate, renal cell carcinoma, thyroid, and glioma. We do not know precisely why this is, but glioma is undoubtedly the most difficult to detect circulating tumour DNA. It is also recognised that early-stage cancer detection is more difficult than late-stage cancer as this is partly due to the multiple sites and partly due to the tumour burden in the individual. Screening for circulating tumour DNA in the aftermath of initial treatment can be highly predictive of the outcome 2 to 5 years later.

Easy and Difficult Cancers: Two Exemplar Projects

At the Genome Medicine Research Group our researchers discussed two projects to exemplify liquid biopsy for easy and difficult cancers. The first, and the easier scenario, was the use of human papillomavirus (HPV) as a biomarker for head and neck cancer. We report a novel method to tackle the more difficult cancers using cell-free DNA capture by apheresis, conducted by the institution's spinout company, BioCaptiva.

1.) HPV Detection in Head and Neck Cancer: Researchers deemed HPV biomarker as the easier of the two because it detects the viral DNA that gets incorporated into the cancer cells. There are often multiple copies of the viral genome in the cell, and it is secreted at a relatively high proportion into the circulation. Approximately 70% of head and neck cancers are associated with HPV, and in 2020, the research group conducted an experiment with 104 patients with oropharyngeal squamous cell cancer. During this, plasma cell-free DNA was extracted from 10 ml blood, and then a follow-up was conducted with 48 patients for 20 months. Researchers established ddPCR assays for the five following HPV subtypes: 16, 18, 31, 33, and 35. Pre-treatment results were then compared to conventional p16 immunohistochemistry and qualitative PCR of tumour tissue.

70% of these cancers were HPV positive for one or more HPV

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subtypes. Overall results were 93% concordant with immunohistochemistry findings and the conventional PCR of solid tumour tissue, testifying to the assay's accuracy. The longitudinal follow-up showed that 40 out of the 48 patients fell to zero and remained undetectable. In 8 out of 48, levels either never fell to zero or did fall to zero and then rose subsequently. A rise in HPV copy number in more than one blood sample was associated with or predicted disease progression with these patients. It was concluded that routine pre-and post-treatment measurement of cell-free DNA HPV copy numbers can assist with challenging management decisions and may improve patient outcomes.

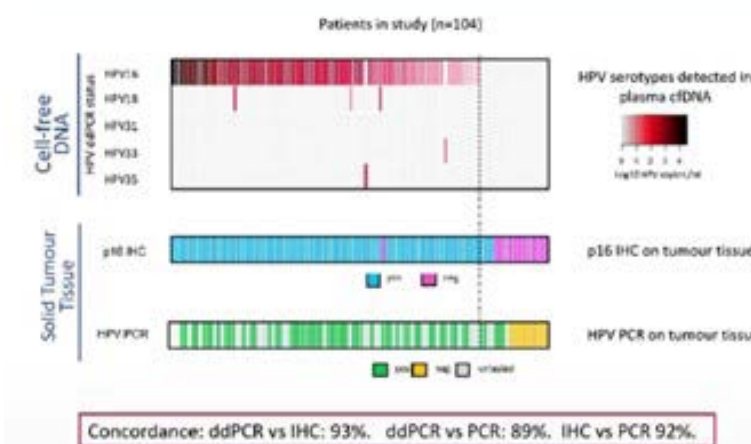
2.) Cell-free DNA Capture by Apheresis (BioCaptiva):

The second example comes from the university's spinout company, where the team has developed a method to increase the sensitivity of liquid biopsies by apheresis-based capture of cell-free DNA. BioCaptiva aims to markedly increase assay sensitivity for liquid biopsy as a diagnostic tool with a novel blood-based biopsy device. Because of low circulating tumour DNA concentration, most cell-free DNA studies and assays have had the greatest success with high circulating tumour DNA-secreting cancers or late-stage cancers. However, BioCaptiva found themselves asking whether it would be possible to use apheresis to capture up to 500 ng of cell-free DNA, 50-100-fold greater than usually extracted from a blood draw. The solution was to devise and patent the DNA BioCaptor, a hemocompatible DNA-binding polymer device to transform liquid biopsy testing and enhance cancer detection.

Apheresis functions in a similar way to dialysis or plasmapheresis in that a needle is injected into a vein of one arm, and then the blood gets channelled through a cartridge, which filters and binds DNA from either whole-blood or separately from the plasma. After separating the desired component then you return the rest of the blood to the patient.

Head and Neck Cancer - HPV liquid biopsy: Detection of HPV in cfDNA by ddPCR

Thomson et al, medRxiv, 2020

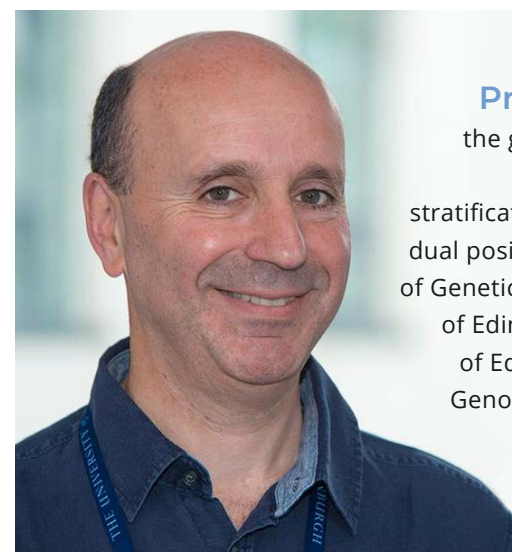


▲ Pre-treatment results for HPV detection in head and neck cancer. Taken from Thomson et al, medRxiv, 2020.

Apheresis is helpful in procedures such as hemodialysis for removing toxic chemicals and in plasmapheresis for removing cholesterol or antibodies that are causing damage to the body. In an early experiment, a functional prototype of the DNA BioCaptor, 83cm3 capacity could bind upwards of 5,500 ng cell-free DNA, approximately 1000-fold more than what is usually obtained from a blood draw.

Forward Outlook:

The DNA BioCaptor provides a biocompatible environment that can safely and effectively deliver high input levels of cell-free DNA for liquid biopsy analysis. The DNA binding technology will undergo clinical trials next year and is expected to drive significant commercial growth. Whilst, current cell-free DNA protocols have demonstrated efficacy for detecting minimal residual disease and have potential in population screening, low cell-free DNA concentration remains a challenge. However, HPV cell-free DNA is an available biomarker in head and neck cancer that defines HPV status and predicts recurrence. Its promise to significantly improve clinical management has us at BioCaptiva very excited and we look forward to seeing its impact.



Professor Tim Aitman's research uses genome technology and information to elucidate the genetic basis of both common and rare human disorders and to develop biomarkers for cancer diagnostics and management by liquid biopsy. He is committed to advancing the diagnosis and stratification of human disease, and to move such advances towards routine healthcare. Aitman holds a dual position as Director of the Centre for Genomic and Experimental Medicine within the MRC Institute of Genetics and Molecular Medicine and Professor of Molecular Pathology and Genetics at the University of Edinburgh. He also works as a Consultant Physician in NHS Lothian and Fellow of the Royal Society of Edinburgh, of the Royal Colleges of Physicians of London and Edinburgh and co-PI of the Scottish Genomes Partnership. He was the Specialist Advisor for the 2009 House of Lords Inquiry in Genomic Medicine. Professor Aitman is a founder and Director of the Company BioCaptiva Ltd.

DIGITAL BIOMARKERS – AN INDUSTRY GAME CHANGER

This commentary article looks at the latest applications in digital and wearable biomarkers, with industry and academic insights from GlaxoSmithKline, Roche, and University College London.

Transcribed by Tia Byer

Digital biomarkers refer to the quantifiable, physiological, and behavioural data collected and measured by means of digital devices. These devices include sensors, wearables, implantables, and mobile phones. Digital biomarkers offer a patient-centric approach to therapeutics with the attitude that no one device is applicable to all cases. In particular, sensor technology is selected on a case-to-case basis during a data analytics phase where the goal is to extract objective measures reflecting a typical disease manifestation. This information is used to align with targeted concepts of interest and enhance the value of medicine through objective or real-world patient data.

Developing Digital Biomarkers for Rheumatoid Arthritis: a GSK Case Study

While there are some broad cross therapy applications, each disease area has its own unique set of challenges and requirements, resulting in a diversity of applications across the GSK portfolio. The digital biomarkers team at GSK focuses on assessing efficacy, safety, and patient stratification.

In his presentation, Valentin Hamy, Data Analytics Leader – Digital Biomarker at GSK focused on efficacy with a case study on rheumatoid arthritis where GSK aim to capture patient function for the generation of patient-centric endpoints.

Rheumatoid arthritis is a chronic autoimmune disease characterised by persistent inflammation of joints with chronic pain, stiffness, and swelling, leading to deformities and limited ability to perform daily activities. The joints most commonly affected by the disease are the wrists, the hands, the knees, the ankles, and the feet. Data collected in clinical trials presents an incomplete view of disease activity due to fluctuation and heterogeneity of daily symptoms. “Instead, digital technology such as wearable devices and mobile applications can enable continuous monitoring and objective assessments of the impact of rheumatoid arthritis”, Hamy claimed. Objective measures of physical function include sit-to-stand transition times, and joints range of motion, which are commonly used in clinical assessments of the condition although with high intra and inter tester variability.

BIOMARKERS

Applications for Rheumatoid Arthritis:

In response, GSK has conducted several projects using real-world data to assess disease impact for rheumatoid arthritis. Conducted a few years ago, the PARADE study included the collection of real-world data from patients through an iPhone app. The Watch and mobile devices in the Assessment of Rheumatoid Arthritis Burden on daily Life compared to Patient Reported Outcomes (wearABLE-PRO) is a pilot study building upon PARADE and using an iPhone app in combination with an Apple Watch allowing for continuous collection of accelerometer and a heart rate data. These digital devices enabled the supply of high-quality data to evaluate symptoms that matter to patients, such as morning stiffness, fatigue, pain, and sleep quality. The latest project leverages the UK Biobank actigraphy data to conduct a large-scale assessment of physical activity patterns.

According to Hamy, “the work conducted at GSK for the development of novel digital biomarkers in rheumatoid arthritis has led to a wealth of information found in data that can be accessed through advanced analytics tools”. He explained how digital biomarkers can benefit drug development in a number of disease areas by predicting and demonstrating changes in health outcomes. However, digital sensors data are different from any other type of data typically collected in clinical trials. Potential challenges include the fact that multiple source data although rich is also very complex. Hamy explained, “this requires specific considerations in terms of fitness for analysis, pre-processing and quality assessment which may vary depending on the study setting and the selected sensor or device”. Nevertheless, digital biomarker technology, science, and application continue to evolve rapidly. Projects such as PARADE and WearABLE-PRO enable the transition from potential to validation and application of digital biomarkers in clinical trials.

Digital Biomarkers: Clinical Utility

David Nobbs, Digital Biomarker Disease Area Lead at Roche, observes that “while there are many opportunities in drug development, the industry still has a long way to go”. Currently, approximately 79% of drugs fail to launch not only due to safety and efficacy but also an inability to measure effectiveness. Accordingly, improved biomarkers are required. The latter can also significantly improve the average time it takes to progress a target identification to the market, which is approximately 14 years and increasing. According to Nobbs, “if treatment outcomes could be determined faster and in smaller sample sizes, this would reduce development time, bringing treatments to patients faster and at lower cost”. This, in turn, will have a significant impact on lowering the price of pharmaceuticals.

The advantages of measuring with digital health technology are numerous. As Derek Hill, Professor of Digital Health at the University College London, points out that “digital health technologies such as wearables have the potential to provide

new drug development tools such as home-based ecological validity”. The generalisability of measurement to real-life improves validity because patients may present to the clinician or perform differently in the clinic compared to real life. Daily measurement capture fluctuating symptoms, and the high precision sensor measurement can reveal clinically meaningful differences. Sensors can also directly measure the behaviours and physiological characteristics of interest, allowing greater accuracy and reliability.

Regulatory Improvements:

“Regulators have yet to provide guidance on evidentiary requirements specific to digital endpoints”, Nobbs explains. This means the industry remains uncertain about how best to implement multimodal endpoints. For instance, the acceptable methods for combining data and measurements from different modalities, whilst promising, currently remain out of reach. Furthermore, the cross-disease context of use requires guidance. As certain diseases share symptoms and pathophysiological characteristics, it is within the regulators’ interest to identify the relevant evidence necessary to validate measures across multiple disease areas and thus usher in the next generation of digital biomarkers.

Finally, one of the main priorities moving forward for facilitating the improvement and emergence of digital biomarkers includes implementing regulatory guidelines for missing data. When captured remotely, data can go missing. This can be due to technical problems, non-adherence or corruptibility. In such instances, it will be imperative to receive guidance on how best to demonstrate that data is not missing due to a disease-related reason.

Future Outlook:

With increased resources and efforts in applying the latest technology to further the drug development process, wearable biomarkers are fast becoming a priority for the pharmaceutical industry. While the regulatory landscape for digital biomarkers requires revision and refined specification, their clinical utility is undeniable. At Oxford Global, we look forward to seeing just how the digital biomarker is set to revolutionise the pharmaceutical industry.

— Part III —

IMMUNO

Accelerating the design & delivery of innovative immunotherapies to transform cancer care.

BIOMARKERS IN IMMUNO-ONCOLOGY

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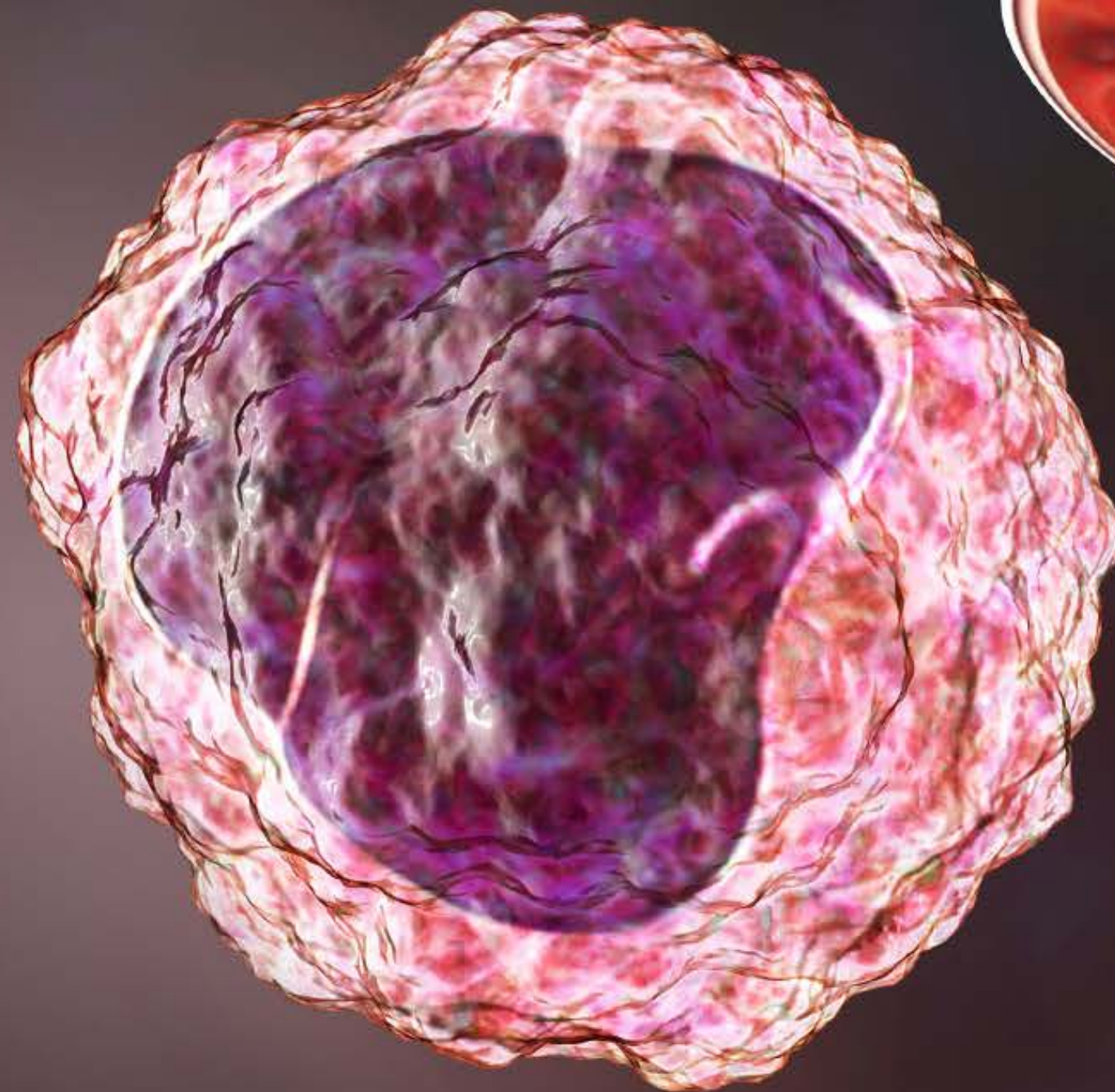
The Future of CAR T for Solid Tumours: Antigen Discovery, Persistence, and Fitness

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THREE LEADING PERSPECTIVES ON BIOMARKER DIAGNOSTIC DISCOVERY IN IMMUNO-ONCOLOGY

Early and effective treatment of cancers could mean the difference between life and death. Therefore, companion diagnostics and biomarker discovery could be considered one of the most important avenues to explore in immuno-oncology.

Edited by Tom Cohen

Oxford Global's 2021 Biomarkers UK conference played host to some influential names working in biomarker research. Our expert panel discussed companion diagnostics and biomarker discovery: their challenges in the field and where they think it is going.

Our moderator, Philip M. Arlen, has been associated with the National Cancer Institute for over two decades and is also President and Chief Executive Officer of Precision Biologics. Precision Biologics develop immuno-therapeutics for oncology and research their corresponding companion diagnostics and biomarkers.

Alexandra Sevko is the Director of Translational Research at Prokarium, a bioscience company researching the use of genetically modified bacteria to kill cancer. Prokarium is currently investigating the effect of *Salmonella Typhi* on non-muscle invasive bladder cancer and has a therapeutic in preclinical stages of development.

T. Scott Reid is the Vice President of Companion Diagnostics and Alliances at NeoGenomics, which works in oncology testing. NeoGenomics offer laboratory testing and assay

development services for pharma and biotech companies.

Commercialisation of Companion Biomarker Diagnostics: Transitioning from Research to Commercial

Reid kicked off the discussion by pointing out that transitioning a companion diagnostic from research into its commercial phase is most challenging in newer and less established approaches. "PCR, IHC, FISH, and even NGS can now be considered fairly well-established technologies," he said.

There has been an especially high recent interest in multiplexing in newer approaches. Multiplex approaches can look at more complex gene expression or protein expression profiles, not just the expression of one protein or gene. Here, multiple markers are interrogated simultaneously, which brings great new potential.

However, Reid explained that the problem was that these innovations are so new that there is "not yet a well-established regulatory pathway to approval." Sevko also added to this point by stressing the need to be wise in selecting the discovery biomarkers, leading to the successful selection of

companion diagnostics later down the road.

Arlen strongly concurred with Sevko on this point. He says, "you take the wrong marker, and you deep-six your whole program; that's because of your choice of predictive marker and how you go about developing it."

Arlen's company is currently developing antibodies for clinical trials and working with biomarkers in the process. He says they are taking a simplified approach by developing an IHC assay, as it is post-translationally modified and is unsuited for gene to protein sequencing methods.

"You take the wrong marker, and you deep-six your whole program; that's because of your choice of predictive marker and how you go about developing it."

IHC is tried and trusted in assay development: "it works, it is reliable, and it provides a nice spatial context for the very specific biomarker that you are looking for," explained Reid. That spatial context allows you to determine whether the biomarker is expressed in the tumour versus the regular tissue environment. But still, Reid cautioned that there should still be a lot of thought put into carrying out IHC assays to make them reliable enough to be commercially viable.

Biopsy and Tumour Tissue Samples for Biomarker Diagnostics

However, Sevko pointed out that often tissue biopsies are not available to investigate biomarkers. Scientists may only have a limited amount of material to study, meaning that the biopsy will be biased to the "time snapshot" from which the tissue was taken.

The risk for Sevko is that there will subsequently be "huge gaps between when the biopsy is taken and when the treatment is applied, meaning you won't see what is going on in the tumour." There is also the surprisingly high possibility that the tissue taken does not contain any tumour tissue, which frustratingly exacerbates the problem with biomarker research.

"Now, there are so many different methods allowing us to gather information about what is happening in and around the tumour," Sevko mentioned, "but they are not yet approved as official tests for diagnostics." Sevko thinks these methods should be considered when planning a biomarker discovery strategy.

However, there are more considerations for biomarker discovery strategy. Among them are the type and location of the cancer and the research budget. The wide variety of modern methods available for use can make it difficult for

scientists to decide which best suits their research.

Making the Most of Biomarker Diagnostics

Arlen then posed the question: how do researchers translate newly discovered biomarkers into valuable clinical tools? It's a question that touches the heart of why scientists go looking for biomarkers; turning their discovery into something that could potentially make it through the regulatory process and become approved.

In Sevko's experience of working in small biotech companies, although those small companies have an enviable creative environment, their resources are lacking compared to larger organisations. This means that a lot of their research gets outsourced, so it is essential for them to select a contract research organisation (CRO) that can provide various resources.

It is also essential to carry out thorough audits on the CROs before working with them. Sevko warned against getting transfixed by the salesmanship of these companies and recommended that biotechnology companies ask themselves what their ideal criteria for an appropriate CRO might be.

Arlen stressed the importance of the timing of the task at hand. "What specific needs are there at the time?" Arlen asked, continuing: "but I think it's important to engage in discussions even before you're ready to embark on that project."

This reduces the risk of making missteps in developing the project and having to circle back and fix those mistakes. In Arlen's experience, there can be so many hurdles to jump during development that thinking about what is required from a CRO early on can help deal with future challenges.

GPR65 IS A CRITICAL INNATE IMMUNE CHECKPOINT IN TUMOUR ASSOCIATED MACROPHAGES:

HUMAN GENETIC VALIDATION AND DISCOVERY OF POTENT AND SELECTIVE GPR65 ANTAGONISTS

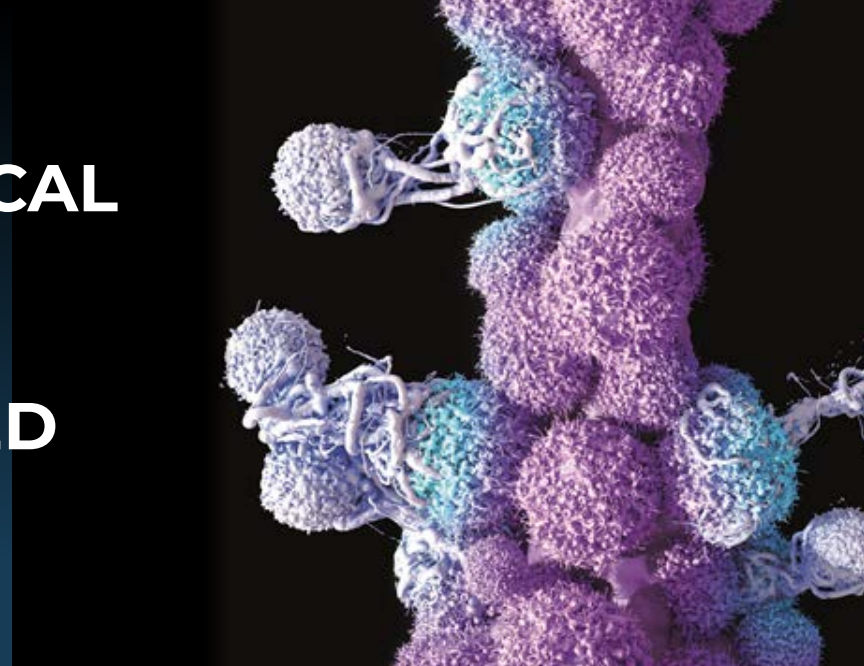
The activation of GPR65 on immune cells by the acidic tumour microenvironment and the resultant immunosuppression may be one of the principal reasons why many cancers do not respond to current T Cell checkpoint therapies. Chief Executive Officer, Stuart Hughes delves into some of Pathios Therapeutics' recent developments in macrophage conditioning and its promise to enable a successful anti-tumour immune response.

Presented by Stuart Hughes, Chief Executive Officer at Pathios Therapeutics

Transcribed by Tia Byer

Pathios Therapeutics is a cutting-edge biotech company that does not use the term 'Innate Immune Checkpoint' lightly. Instead, it is a qualification that they are incredibly compelled by; with recent research into the validation of GPR65, Pathios is excited to propose GPR65 as a critical innate immune checkpoint in human cancers. Findings show that GPR65 activation by an acidic microenvironment is a key causative factor in creating a 'cold' tumour which therefore may explain why many cancers do not currently respond adequately to T cell checkpoint inhibitors.

Based in Oxford, UK, Pathios Therapeutics has a sole interest in pursuing small molecule inhibitors of the pH-sensing GPR65 for applications in cancer immunotherapy. In 2021, Pathios announced the discovery of potent, selective, and orally bioavailable small molecule inhibitors of this immune



checkpoint. Proprietary understanding of GPR65 derives from human genetics, single-cell transcriptomics, and computational modelling. Pathios's 'Macrophage Conditioning' approach holds significant promise as a strategy for targeting the innate immune system in cancers and, as such, holds substantial clinical promise.

GPR65 – What is it?

GPR65 is a pH-activated G-protein-coupled receptor (GPCR) on immune cells. It belongs to the pH-sensing GPCR family and is highly enriched on cells of the immune system, especially on macrophages. GPR65 is somewhat unusual because its natural ligand is a proton, so an acidic environment activates it. In macrophages, GPR65 non-redundantly transduces an extracellular low pH signal into an intracellular elevation in cyclic adenosine monophosphate (cAMP). While this mechanism is also found in other immune cells, particularly T cells and NK cells, Pathios believes the pathway is particularly important and prominent in macrophages. GPR65 and its downstream signalling partner, ICER (CREM gene), are highly expressed in tumour-associated macrophages in a range of human tumours. Examples include renal cell carcinoma (RCC), ovarian cancer, melanoma, glioblastoma, and breast cancer.

Impact on the Tumour Microenvironment:

The presence of the GPR65 receptor in macrophages is highly relevant when contemplating the tumour microenvironment. Most immune cells that are present in solid tumours are macrophages. These are primarily immunosuppressive pro-tumourigenic cells that are essentially locked in a wound repair state, suppressing inflammation, supporting vascularisation, and encouraging tissue growth. Unsurprisingly, a higher content of these cells is typically associated with a worse prognosis or a worse response to a range of treatments. Many tumours also exhibit a low pH microenvironment which negatively impacts response to immunotherapy. Tumour pH is typically in the range of 6.5 to 7.2 due to the high expression

of glycolytic and acidifying enzymes. Acidic tumours tend to have a worse response to immunotherapy, as highly glycolytic acidic tumours present an additional barrier to achieving a successful immune response.

Low pH causes a pronounced and widespread alteration in gene expression including the upregulation of growth factors, neutrophil chemoattractants and angiogenic factors, and the downregulation of key cytokines. Other alterations include a suppression of both chemokines that attract T cells and NK cells, and type I/II interferon (IFN) response genes. The capacity of GPR65 signalling to suppress the expression of pro-inflammatory genes involves the transcriptional repressor, ICER. The activation of GPR65 by low pH leads to an elevation in ICER, a shortened isoform of the CREM gene that lacks a transactivation domain, but which binds with high affinity to the promoters of a host of pro-inflammatory genes to inhibit their expression.

The Solution: Small Molecule GPR65 Inhibitors

By blocking GPR65, Pathios propose that pH-induced immunosuppressive pro-tumourigenic polarisation of macrophages in the tumour microenvironment can be reversed, and in doing so, support a successful immune response to be used either as a monotherapy or in combination with currently approved checkpoints. One of the key molecules to emerge from their active drug discovery programme is PTT-3196 (see Figure 1). PTT-3196 is a selective small-molecule inhibitor that can inhibit GPR65 in multiple contexts such as in human macrophages and peripheral blood mononuclear cells (PBMCs).

Most significantly, this molecule can dose-dependently counteract low pH-induced suppression of anti-tumour immunostimulatory pathways and reduce expression of pro-

tumourigenic immunosuppressive and tissue repair pathways. It also robustly activates the innate immune system *in vivo* as evidenced by an increase in key chemokines and Type I/II IFN genes following oral dosing to mice that have been reconstituted with stem cell-derived human immune cells and that have been implanted with RCC patient-derived xenograft (PDX) tumours.

Forward Outlook:

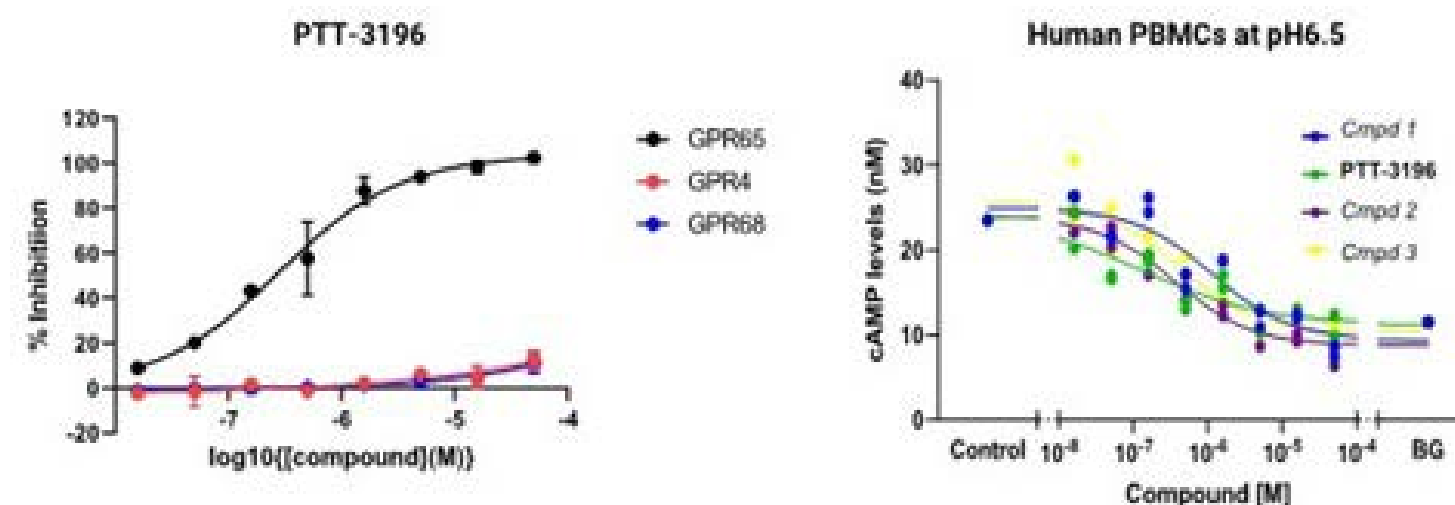
Human genetics supports the assertion of GPR65 as an innate immune checkpoint since homozygosity for a reduced function coding variant of GPR65 (I231L) provides a significant survival benefit across multiple cancers. A host of evidence and data shows that suppressing the signalling of this receptor will be clinically important, enabling a successful immune response to the disease. Pathios's macrophage condition strategy holds significant promise for targeting the innate immune system, and here at Oxford Global, we look forward to seeing its impact on the field.

Speaker Biographies

Stuart Hughes, Chief Executive Officer at Pathios Therapeutics – Hughes is Chief Executive Officer of Pathios and brings over 20 years experience in scientific leadership and drug discovery expertise. Hughes has worked across several therapeutic areas including CNS disorders, immunology and oncology, and has deep expertise in guiding early stage drug discovery programs from inception toward clinical development. He has gained wide-ranging experience from positions in both academic and industrial settings. Previous positions include Research Fellow and Senior Lecturer at Cardiff University, Principal Research Scientist at Eli Lilly and most recently, Senior Director at Vertex Pharmaceuticals. He holds a PhD in Neuroscience from Cardiff University.

▼ Characterisation of the Selective GPR65 Inhibitor:

Effects of PTT-3196 in HEK cells stably overexpressing either GPR65, GPR4 or GPR68 (left) and in human PBMCs.



USING LIVE GUT BACTERIA AS A CO-THERAPY FOR CHECKPOINT INHIBITORS

Microbiotica's live bacterial therapeutic is currently manufacturing for phase Ib trials in 2023.

Edited by Tom Cohen

At Preclinical Oncology: Online, Ghaith Bakdash, Director of Cell Biology at Microbiotica gave a fascinating presentation which outlined the development of his company's therapeutic. MB097 is a co-therapy for anti-PD1 checkpoint inhibitors and is set to enter phase Ib clinical trials.

Microbiotica develops bacterial based therapeutics by linking changes in the human microbiome to clinical outcomes. Dr. Bakdash explained that the company uses its bacterial genomic database to define microbiome composition in relevant patient groups. Microbiotica's in-house bioinformatics platform can then identify microbiome signatures linked to patient outcomes, allowing for the development of live bacterial therapies.

"All this made possible by our own unique microbiome discovery platform," said Dr. Bakdash. Not only does the company define the microbiome make-up of patients, but it

also isolates the patients' bacteria which contributes to their personalised bacteria bank.

Microbiotica is in possession of an enormous proprietary collection of bacterial strains: "all of these are potential therapeutics," added Dr. Bakdash. The company has also constructed its own reference genome database which allows for "deep and precise determination of microbiomes." Microbiotica's database is ever-growing and based on global samples.

Melanoma IO Program: MB097

The therapeutic candidate developed by Microbiotica is intended to fill the gap in efficacy of immune-checkpoint-inhibitors (ICIs) when it comes to melanoma indications. ICIs have revolutionised treatments of multiple malignancies by increasing long-term survival rates. For melanoma patients, this increase in the survival rate is high, but still only 40 to 50% benefit.

"There is a major need for a co-therapy to increase the response rate to ICIs," described Dr. Bakdash. Mouse models have shown that faecal microbiota transplants (FMT) from responsive patients to a tumor-bearing mice can increase the efficacy of ICIs in those mice. Dr. Bakdash added that further evidence has corroborated this effect in humans, rendering non-responding patients responsive via FMT from responding patients.

The challenging factor, according to Dr. Bakdash, is that there has been no consensus on the bacterial species that cause this effect. "There have been five clinical studies that report different species that could be involved in boosting response to ICIs," explained Dr. Bakdash. He added that combining the analysis of these five studies has also proven ineffective at narrowing down the effective species.

To resolve this conundrum, Microbiotica launched its own study named MELRESIST in collaboration with Cambridge University Hospitals. Dr. Bakdash specified that MELRESIST had the largest number of patients in comparison to the previous studies: 69. Stool samples were collected from melanoma patients before and throughout ICI therapy, those samples were all processed and analysed using Microbiotica's in-house bioinformatics tool.

By combining the data from the MELRESIST study with three other published studies, Microbiotica managed to generate a common signature that can predict patient response to ICIs with 91% accuracy. This common signature can be developed into a biomarker and was used to develop a live bacterial therapeutic.

MB097: A Live Bacterial Therapeutic to Induce Tumour Killing by Activated CTLs

The results of the melanoma studies returned nine bacterial species identified as being associated with response to ICIs and consistently more abundant in responsive patient. These were developed into their therapeutic: MB097. Dr. Bakdash detailed Microbiotica's attempts to validate the anti-tumour efficacy of MB097 by addressing dendritic cells/cytotoxic T lymphocytes (CTLs) axis.

Dr. Bakdash outlined the mechanism by which MB097 can support the effect of ICIs. MB097 can activate dendritic cells, in doing so, it skews the cytokine production from the immune inhibiting IL-10, toward the activating cytokine IL-12, "which has an important role in activating CTLs."

Microbiotica then wanted to verify whether dendritic cells treated with MB097 could give rise to activated CTLs. The researchers assessed this by investigating the production of interferon- γ and granzyme B by activated CTLs, which Dr. Bakdash reported as a success. Finally, the company verified whether the activated CTLs were functionally capable of killing tumour cells, which was also a success.

The final part of validating the therapeutic was to assess

the capability of MB097 to have anti-tumour effects *in vivo*. Microbiotica used a MCA205 syngeneic model in SPF mice to do this. The mice were treated with antibiotics and then MB097. Dr. Bakdash reported that the results of the study showed the combination of MB097 and anti-PD1 ICI managed to slow tumour growth far more than control groups.

Running a New Observational Study Called MITRE

Microbiotica is now running a new observational study called MITRE, which is similar to MELRESIST but operates on a much larger scale. MITRE is being run in collaboration with Cancer Research UK, involving 12 centres in the UK. Enrolment started in July 2020, aiming at recruiting 1,800 patients undergoing ICI therapy and this time will be looking at non-small cell carcinoma and renal cell carcinoma in addition to melanoma.



Speaker Biographies

Ghaith Bakdash is an immunologist with broad experience ranging from immune oncology to autoimmune disease and mucosal immunology. Following his PhD at the University of Amsterdam, his interest in translational research led him to a post-doc position at Radboud University Medical Center in Nijmegen, the Netherlands, where he contributed to the development of dendritic cell-based cancer vaccines. Later, he joined GSK as an investigator in the Respiratory Therapy Area, where he was participating in the development of new modalities to induce immune tolerance in the context of allergy and asthma. Currently, he is an Associate Director at Microbiotica, where he is leading the efforts of the Cell Biology team in live bacterial therapies development by elucidating the interactions between these therapeutic bacteria and host cells in the context of cancer and ulcerative colitis.

behaviour, could be suitable as a “consistent backbone” for new and interesting CAR T therapeutics in solid tumours.

Algorithms designed to identify targetable neoantigens are one such technology that Zaiac referenced. Furthermore, Zaiac mentioned ‘armouring technology’ that would be able to make T cells better sustained in the tumour microenvironment.

Still, Zaiac reiterated that Novartis are currently only “dipping their toes in the water,” due to the lack of major breakthroughs in the field. Novartis has not made any R&D decisions on any single technology for the time being, but the company is investing having in the diagnostics in place to have access to emerging neoantigens.

Aleta Biotherapeutics: Using CAR T Engagers

Rennert said of breaking into the solid tumour space that “it takes a village to raise a child.” Although Aleta is a small biotech company, the work they do for CAR T technology is making impressive breakthroughs.

Rennert explained that Aleta debuted in CAR T with their engager platform. Aleta-001, their inaugural biologic, will be entering the clinic in 2022 with Cancer Research UK funding. Aleta-001 links CD19 extracellular domain to anti-CD20. The very small protein solves a simple problem: the fact that CAR T tends to lead to relapses due to loss or down-regulation of CD19.

The biologic coats every molecule of CD20 with CD19. “Therefore, the CAR doesn’t care, it just sees CD19,” Rennert added. Herein, it doesn’t matter if CD19 is downregulated as the cell is coated with it. “The CAR continues to kill even if the tumour is trying to escape.”

There are two benefits to this. The first is the obvious fact that the antigen density will substantially increase: “There is about a five-fold CD20 on most lymphomas compared to CD19,” said Rennert. Secondly, if the patient loses native expression of CD19, the biologic will still coat the CD20, effectively acting as a substitute for that native expression.

Aleta On Translating to Solid Tumours

Rennert then went on to discuss how Aleta is working to translate this capability into the solid tumour space. He highlighted the research he had seen which suggested that

targeting solid tumours was possible with CARs. “The issue with those studies is the persistence of those CARs is limited,” said Rennert.

So, Aleta encoded a CD19–anti-HER2 CAR T engager inside their CD19 CAR, and then saw whether it was capable of attacking solid tumour cells. This meant that the engagers were encoded to be secreted extracellularly by the CAR T itself. “It works really well,” affirmed Rennert; “the potency is incredible.”

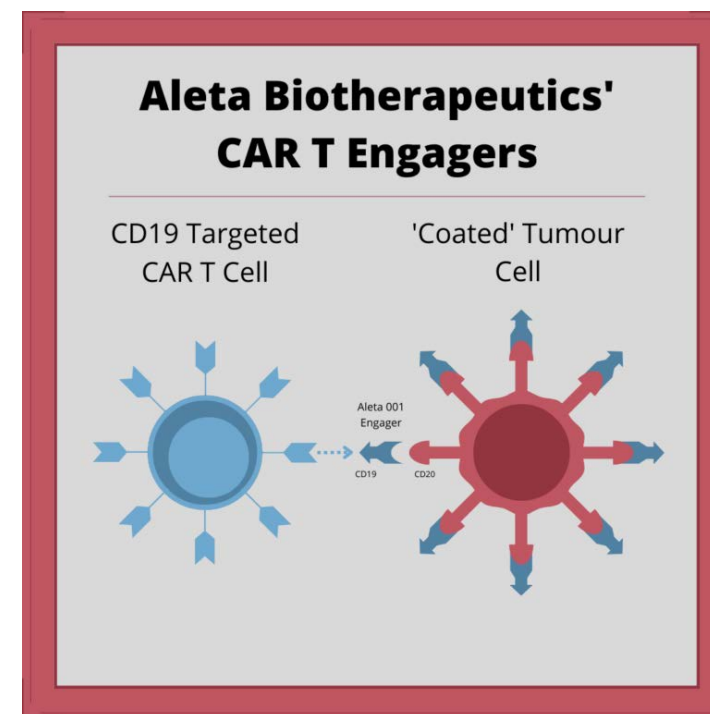
“We’re going to let those CARs wipe out the normal B cells in order to build a fit and highly persistent CAR T cell pool.”

The antigen density is so high because Aleta makes sure to always build multi-antigen targeting CAR T engagers. For example, for HER2 central nervous system (CNS) metastases, their engager targets HER2 and B7-H3 simultaneously. Further still, for paediatric CNS solid tumours, Aleta is targeting three antigens, all encoded in the same CAR T engager.

Rennert said that the problems that Aleta is currently trying to solve are countering tumour heterogeneity and improving both CAR T persistence and fitness. The prospect of using engagers for these challenges looks very promising for Rennert and the rest of Aleta.

Furthermore, the close watch of innovative Pharma companies over developments in the CAR T space (especially in neoantigen discovery, e.g., NEON and KITE neoantigen TCR technology acquired by BioNTech) seems to indicate that it is being taken seriously across the pharmaceutical

industry, with plenty more developments to come.



THE FUTURE OF CAR T FOR SOLID TUMOURS: ANTIGEN DISCOVERY, PERSISTENCE, AND FITNESS

CAR T, the rockstar therapy of immuno-oncology, finds its next big challenge in translating research for use in solid tumours.

Edited by Tom Cohen

The development of chimeric antigen receptor (CAR) T cell therapy has been ground-breaking in the treatment of haematological cancers. As such, the successes that the therapy has brought to the clinic and beyond has driven interest and excitement in translating it to solid tumour indications.

Solid tumours are more difficult, however. Solid cancer cells present far more multivariance in the antigens that they express: as a result, finding tumour associated antigens with the right degree of specificity is a significant challenge. In addition, reaching the tumour cell population and effective action within the tumour is made more difficult by the microenvironment as well as immune evading properties of solid tumours.

Discussing the challenges, developments, and strategies for using CAR T on solid tumours, Oxford Global hosted an

Immuno series discussion group titled “Cell Therapies and Solid Tumour Therapy Development.” Leading the discussion were Michael Zaiac, Head of Medical Affairs Oncology Region Europe at Novartis, and Paul Rennert, CEO of Aleta Biotherapeutics.

Novartis Keeping their Finger on the Pulse

Zaiac explained that Novartis has been increasingly interested in the solid tumour space for CAR T technology. This has been due to the growth in capacity and understanding of tumour genomics. Furthermore, the ability to crunch large amounts of data, in turn predicting neoantigen targets and their ideal combinations, has piqued the company’s interest.

As such, according to Zaiac, Novartis has been keeping a keen eye on the emerging technologies in this field. Zaiac hopes that Novartis’s capability to create CAR T cells with a quick turnaround and hence a potentially more “natural” T cell-like

MACROPHAGES: A NEW FRONTIER FOR TREATING SOLID TUMOURS

Tumour associated macrophages are critical drivers in cancer progression and metastasis. Clinical studies now suggest that changing polarisation of macrophages can significantly improve immuno-oncology response. Speaking with senior representatives from Verseau Therapeutics, Pathios Therapeutics, and Faron Pharmaceuticals, we delve into some of the latest techniques in macrophage targeting.

Edited by Tia Byer

Despite all the advances in immuno-oncology over the past 10 years, the treatment of cancer remains a significant challenge. In particular, even though close to half of all cancer patients are eligible for T cell based IO therapies, only about 12% of eligible patients derive benefit from T cell checkpoint inhibitors. Recent research has led experts in the field to turn their attention to the role of macrophages in immunosuppression and tumour promotion. Macrophages are one of the most abundant immune cells in the tumour microenvironment and are found in over 75% of cancer patients. Because macrophages play a key role in suppressing the immune system, they have long been associated with poor patient prognosis.

The Challenge:

Potential therapeutic strategies to target tumour-associated macrophages are the new frontier in solid tumour treatment. Juho Jalkanen, Chief Development Officer of Faron Pharmaceuticals, describes the growing interest in macrophages as “a poorly understood, but promising area of immuno-oncology”. The need for better solid tumour treatment is in many ways due to the restrictions of other leading immunotherapy techniques. “For select tumours, there have been significant advances with T cell checkpoint inhibitors, specifically PD-1 inhibitors”, says Igor Feldman, Co-founder and Chief Analytics Officer at Verseau Therapeutics. “However, less than 6% of cancer patients benefit from CPI treatments, so there is a clear need for better medications for the vast majority currently not being served.”



The New Frontier: 3 Industry Case-Studies

So how is the industry addressing the need for therapeutic strategies targeting macrophages? From polarisation and infiltration to conditioning, the possibilities are numerous. We sat down with 3 leading pharmaceutical companies on the cusp of macrophage treatment to find out more.

1.) Verseau Therapeutics

Founded in 2017, Verseau Therapeutics' discovery engine has identified and validated over 20 druggable macrophage targets from human tumour tissue analysis. Currently, the company is prioritising two main targets for clinical development. These programs are based on encouraging *ex vivo* human studies and preclinical data in humanised and syngeneic murine models. The first program is expected to initiate clinical trials in early 2022 with a second program to reach the clinic about a year later.

Verseau's 'Macrophage Repolarisation Target Discovery Engine' implements a patient-focused discovery process; leveraging human biology to respond to unmet clinical needs. The engine involves the identification of the macrophage modulating targets from cancer patient tumour data and is followed by validation of those targets whose knockdown most potently repolarises human macrophages. Lead selection for the two prioritised, first-in-class targets identified the most promising monoclonal antibodies across a range of functional characteristics and preclinical studies, including repolarisation of pro-tumorigenic human macrophages, *ex vivo* assessment of human TME remodelling, and *in vivo* assessment of antitumour biology. Clinical testing of both novel and differentiated programs will be underway within the next 18 months. According to Feldman, Verseau's two programs “are monoclonal antibodies that have the potential to reduce tumour growth by transforming the tumor microenvironment from an immunosuppressive state to an immune-activating state where the macrophages can unleash an immune attack on tumour cells.”

2.) Pathios Therapeutics

Also founded in 2017, Pathios Therapeutics is a cutting-edge biotech company that solely pursue small molecule inhibitors of the pH-sensing GPR65 for applications in cancer immunotherapy. The activation of GPR65 in immune cells by the acidic tumour microenvironment and its resultant immunosuppression may be one of the principal reasons many cancers do not respond to current T Cell checkpoint therapies. With its recent research into the validation of GPR65, Pathios found that GPR65 is a critical innate immune checkpoint in human cancers. GPR65 activation by an acidic microenvironment is a key causative factor in creating a 'cold' tumour, which explains the lack of patient response to treatment. To address this, Pathios propose their 'Macrophage Conditioning' approach, which holds significant promise as a strategy for targeting the innate immune systems in cancers

and, as such, holds substantial clinical promise.

The active drug discovery programme has resulted in the discovery of PTT-3196, a selective small-molecule inhibitor that can inhibit GPR65 in multiple contexts such as human PBMCs. Most significantly, this molecule can dose-dependently counteract low pH-induced suppression of anti-tumour, immunostimulatory pathways and decrease expression of pro-tumourigenic immunosuppressive and tissue repair pathways. “It robustly activates the innate immune system *in vivo* by inhibiting proximal signalling and rescues the suppression of key chemokines”, explains Chief Executive Officer Stuart Hughes.

3.) Faron Pharmaceuticals

Founded in 2003, Faron Pharmaceuticals is a Finnish clinical-stage biopharmaceutical company developing novel treatments in immuno-oncology. Currently, Faron is focusing on targeting macrophage scavenger receptors. Juho Jalkanen, Chief Development Officer, summarises Faron's approach as “aiming to work with and reprogram macrophages instead of depleting them.” Scavenger receptors are classical M2 markers associated with cancer growth and metastases. Reprogramming macrophages by targeting scavenger receptors in TAMs enhances innate and adaptive antitumour immune response.

Faron proposes a precision immunotherapy called Bexmarilimab, which is an anti-CLEVER-1 mAb. Jalkanen terms CLEVER-1 (also called Stabilin1) as “the biggest, baddest scavenger receptor of them all” and is closely associated with survival, therapeutic response, and T-cell dysfunction. Bexmarilimab reprograms the CLEVER-1 by converting immunosuppressive M2 macrophages to immune-stimulating M1 macrophages in the tumour microenvironment. The therapeutic is currently in phase I/II clinical development for patients with untreatable solid tumours, and data shows that Bexmarilimab can be the first-ever macrophage immune checkpoint therapy.

Future Outlook:

The future of macrophage treatment has the potential to revolutionise the treatment of cancer. The pioneering efforts of industry leaders such as Verseau Therapeutics, Pathios Therapeutics, and Faron Pharmaceuticals attests to the promise of macrophage targeting. At Oxford Global, we believe that this is the field of immuno-oncology to watch, and we have our eyes firmly peeled.

— Part IV —

PHARMATEC

Accelerating digitalisation and automation in pharma R&D & enhancing mobile robotics and digital medicine approaches.

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MERCK KGaA ON TRAINING A DATA- CAPABLE WORKFORCE

Dr Boris Adryan, Digital & Data Capability Building Lead at Merck Healthcare KGaA, teaches the company's workforce the importance of data.



Edited by Tom Cohen

Dr Boris Adryan kicked off Oxford Global's 2021 Pharma Data & Smart Labs digital day with a stirring presentation on the importance of digital and data literacy in the pharma workforce. Dr Adryan wants his company, Merck KGaA, to make the most of today's technology opportunities. He says that central to this task will be to initiate a 'cultural shift' and mindset change in how employees think about and relate to data. As well as trying to foster this change of culture, Dr Adryan wants to provide the right tools for employees to learn about data and help the management areas of the company see the benefits in this learning.

Where and How to Target Digital and Data Strategy

There are three factors, says Dr Adryan, to consider when promoting data literacy. Those are technology, process, and people. The infrastructure needs to be present to provide workers with the right tools, which means everything from the electronic laboratory notebooks (ELNs) that scientists use to ensuring their labs have secure and stable internet access. Scientists also need to be trained to make data FAIR and reusable. Therefore, data-orienting the workforce aims to anticipate the metadata they need to provide with their experiment data.

But training an entire faculty on digital and data literacy is

no small task, especially a workforce made up of a variety of cohorts, skills, and skill levels. Determining the groups and skill level granularity of data literacy strategy is vital. Dr Adryan explains another question: whether to train a workforce via in-person classrooms or remotely. The benefit of classroom training is that it is human, traditional, and people 'get it'. However, it requires a lot of organisation and a lot of overhead. Dr Adryan opted to recommend remote training for Merck KGaA due to the large number of trainees it would have to take on.

Merck KGaA set out to train their workforce via LinkedIn's Learning platform, allowing the company to target each trainee with a course suited to the amount of detail they need which fixes the granularity problem. However, LinkedIn provides a variety of courses at varying levels of skill, so how do you decide which course is best for whom?

Merck KGaA's Data Literacy Solution

Essential to Merck KGaA's training plan is sending employees for a 'job-ready assessment' to outline the gaps in training each trainee needs to fill. The company trialled the Workera platform and offers it now to data and analytics practitioners, who benefit from spot-on bite-sized content fitting their skill level. Courses offered will be tailored based on their current level of skill, and after employee maturation, they will become a part of Merck KGaA's 'communities of practice' to share their learned skills amongst colleagues with similar interests.

Dr Adryan insists that the overall goal is to have 'the entire workforce understand data to a reasonable degree'. The goal is to allow employees to talk about data, understand how data decision making works, and have the necessary tooling skills where appropriate.

Dr Adryan wants to ensure that the workforce properly internalises Merck KGaA's training sessions. He introduces Bloom's Taxonomy as a way of achieving optimal teaching strategy, explaining it is only worth commencing the training if the information can be retained and applied to obtain a return on investment.

Bloom's taxonomy shows that efficacious implementation of taught skills comes only at the top of the pyramid. Practical skills, therefore, are what it takes for a better learning strategy, but the problem is how to implement this in a remote setting. Merck KGaA settled on Coursera, who offer 'Guided Projects', a practical online sandbox for remote training.

What's Next?

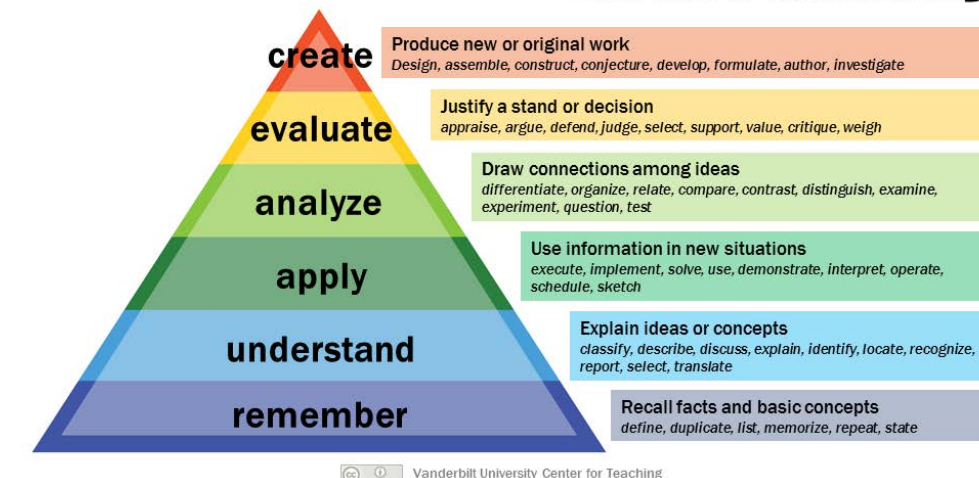
With all that Merck KGaA has learned from embarking on training for data literacy, Dr Adryan asks what's next and how far can you go with this? Dr Adryan explains that the managerial sector needs to see the benefits of data literacy training, part of the cultural shift necessary to procuring 'data-driven decision makers' in the workforce. Dr Adryan contemplates that perhaps circulating the training amongst managers first would go some way toward stepping across this barrier.

Merck KGaA must also decide whether the implementation of training is voluntary or mandatory; they need to prove its efficacy outweighs its cost. The online platforms used, Workera, LinkedIn Learning, and Coursera, are not free tools and should provide long term benefit to both the staff and the company. Therefore, Dr Adryan says that the workforce's ambitions should be reflected to nurture a digital mindset that is palatable to their goals.

Does Culture Eat Strategy for Breakfast?

Dr Adryan says that 'culture eats strategy for breakfast' is a cliché phrase. He says that by implementing the right strategy, the seeds can be sown for a new culture to grow, a culture of data literacy. Merck KGaA has therefore implemented this new culture by sowing what it sees as the right strategic seeds. Before training an entire workforce in data literacy, you first need to meet people where they are. Merck KGaA must tailor the learning experience of each worker to jump off from their current level of competency. It also means making learning exercises as practical as possible to promote the application of the knowledge they acquire, which will increase retention.

Bloom's Taxonomy



All these factors are challenging to achieve on a purely online platform. However, Merck KGaA has found some success by providing Workera's 'job-ready assessments' to its workforce and using modular and practical learning tools like LinkedIn and Coursera; this creates an applied and adaptive way to teach data literacy.



Speaker Biographies

Dr. Boris Adryan spent several years as academic group leader in genome research and machine learning method development. He was lecturer for genome bioinformatics and course director for an interdisciplinary programme in computational biology at the University of Cambridge. As entrepreneur and data science freelancer, he worked with various startups in the IoT space, notably smart workplace company OpenSensors Ltd. Following a short stint with international consultancy Zuhlke Engineering GmbH in Frankfurt, he joined Merck KGaA as data scientist and data strategist in their Chief Digital Office at the Darmstadt HQ. Following a transition into Merck's Healthcare business, in his recent role as Digital Academy Lead, he is responsible for planning and organising 'digital and data upskilling' for Healthcare.

DISCUSSION GROUP REPORT: MOBILE ROBOTICS

challenges | usage | potential



Edited by Oliver Picken

In November 2021, our PharmaTec Series discussion group focused on mobile robotics. We explored their current uses, challenges and potential. Oxford Global's discussion groups bring together a select collective of 15-20 key industry leaders for approximately one hour for in-depth knowledge sharing and conversation.

Leading this group was Mike Ouren (OEM Sales Manager, Lab Automation at Thermo Fisher Scientific). Mike has spent 20 years engineering, supporting, and selling robotic systems in the life sciences, electronics, and pharmaceutical industries. Joining him as panellists were David Wolton (Engineering technology lead at Takeda) and Charly Coulon (Head of Future Manufacturing Concepts at INVITE GmbH), as well as attendees from several major pharmaceutical and manufacturing companies.

Exploring Use Cases: Logistics

While robots were once seen as a threat to jobs or as expensive to implement, the past few years have proven that robots can be valuable colleagues, working together with scientists and manufacturers to make their jobs easier and more productive. Robots have rapidly become integral in the logistics sector, and work is underway to expand their capabilities and efficiency.

David Wolton introduces the topic by saying, "one thing worth mentioning about logistics is that you don't bring in a robot for just one thing. You utilise it 24/7 for as much as you can. They don't mind which department they're in. You can get them delivering spare parts; you can get them delivering consumables. You can get them delivering gowning, you can get them taking out the waste bags; they don't really care. So, what you start to do with these small mobile robots is to look

at what can they be used for and how can they be integrated. On-demand delivery of consumables is perfectly possible. Also, delivery of kits, we're already delivering kits for production areas, totally autonomously. There's no human being involved in delivering these kits at all. All perfectly possible, but it's maximising the use of them when you've got them.

"What we're seeing is that there's a strong desire to automate logistics. And not only just automate the warehouse but automate beyond; I think the vision that we are aiming for is that you can order certain things from your iPad, or your phone or your desktop, and they will be delivered to where you are in the factory.

You don't bring in a robot for just one thing. You utilise it 24/7 for as much as you can.

"There are still a number of challenges that we see at the moment. One is how you store things in the warehouse for them to be then sent over on demand. But then it's also how do you get the product from the warehouse to the production area because often that's a change of grade. There are different classifications in pharma. So, you start with what they call CNC, which is controlled unclassified, and the highest grade is Grade A – and the difficulty is getting through control points.

"We've started looking at how you get it from the warehouse to, say, the first airlock. In effect, it's picked from the warehouse automatically, it's put on the back of a robot, and it gets as far as it can before it stops in the grade change area. A lot of the big biologic's facilities, certainly the ones producing COVID vaccines, are using closed processes. I'm really

interested in solving that problem."

Changing Practices: Towards Integration and Industry 4.0

When mobile robotics were first introduced, they were mostly independent and constrained to one area. Integrating robotics usage with existing and future workflows can be challenging and expensive, but this is now beginning to change. David Wolton explains that "the integration costs can be considerably more than the robot itself. But there's a real shift. A good example is the Andrew Alliance plus robot made by Waters. There is no integration; there is no integrator, you get it out of the box, you put it on the surface, you open up the computer, and it will work with minimal training.

"What that means for the pharma industry is a significant reduction in cost. Because, in effect, you're not paying for the integrators. You take it out of the box; it learns the environment. And this is the I think the cosmic shift in what's happening is you're going away from the classical way robots are put into production lines, to much more flexible, ready-to-use robots that can go into laboratories, that can go into biological safety cabinets that go in cell and gene therapy, where you're not having to do a huge amount of programming upfront."

Mike Ouren expanded on this, connecting the issue to IT and safety challenges. He noted that in the past, integration "wasn't really a concern because it was all disconnected from the main network, and people were bringing USB sticks to pull off Excel CSV files to transfer to the next island. You had this scientist-driven workflow of moving things around the lab, now with industry 4.0, from labs, to automotive and making cell phones, integration is everywhere, in manufacturing. And data management optimisation is certainly something that is involved making it easier to bring all this together.

"But it also creates a bigger IT problem because now you're involving more of the infrastructure, more subsystems that might be in different sites entirely. It must go through the IT infrastructure to get there, it does make the life of the scientists easier because the data is managed in a way that is automated, just like the system that generated it. That's great, but it presents huge IT and safety problems."

Safety and Regulation

Safety has always been a critical concern in the pharmaceutical industry, and regulatory agencies often have strict restrictions and processes that must be followed. An attendee explained that "with complex workflows, that workflow might quite rightly change with a degree of regularity. You're going to be forever updating the regulatory paperwork and getting the assessment done before you can use it. And by the time it's time to use it, you need to make another change. So, you're walking around, and round and round, and you never get there. Charlie mentioned integration software, and it's good, but it doesn't always fit nicely into what the IT department would call a secure tidy landscape.

"If a bad actor or somebody can get onto that network and make a command, either intentionally or by accident, and for example, an arm performs unpredictably, you've potentially got bad things happening. So, you need a security barrier in the IT systems that isn't just an IT worry; it's a laboratory safety worry. And that fits into a whole change control landscape."

Final Thoughts & Conclusion

At Oxford Global, we couldn't have been more pleased with the turnout for our first mobile robotics discussion group. The conversation was engaging, the debate stimulating, and the event provided the perfect setting for exchanging ideas. Attendees were very positive about the group, with one stating that "this is the first time I've heard this discussed in this form. Maybe I've been attending the wrong groups. But these discussions need to be more widely had."

David Wolton agreed and made the point that "big pharma companies are slow, and it can be difficult to get money in budgets and lots of silos. However, if we continue to have these open discussions with large companies, small companies, being free to share ideas. Smaller companies might be able to react faster benefits the patient. It benefits the industry. I would say open discussions like this, and open conferences help pharmaceutical companies share ideas and concepts with smaller companies that can move faster. In the end, it benefits the patient."

Mike Ouren concluded the meeting by saying, "we're at a great point to push the mobile robotics industry in a new direction. And you're right; these discussions are a big part of that."



SMART LABS & AUTOMATION: DIGITAL LITERACY USER FRIENDLINESS

The pharmaceutical industry is working on improving the usability of automation platforms but challenges remain.

Edited by Oliver Picken

Automatisation and digitalisation of pharmaceutical labs have increased significantly in recent years. Despite this, there remains a level of resistance from many researchers and scientists. One of the more considerable hurdles is ensuring that each new system introduced is easy to use and comprehensible for lab workers.

Automation: User Friendliness and Talent Recruitment

A great deal of work has been put into improving the usability of automation platforms. Evidence has shown that some of the more advanced multi-rate monitoring technologies and real-time monitoring technologies developed over the last decade no longer require specialised knowledge to use. Once you package these technologies, provide proper training, and make them available in the cGMP environment, they are easy to use. The skillset required is quite low, similar to a smartphone. At the same time, it is still critical to have staff or onboard experts for troubleshooting. If anything goes wrong, you need to be able to address the issue quickly.

Despite the progress made, many pharmaceutical companies struggle to find staff with the right skills within the current workforce. In addition, recruiting talent is also problematic as pharmaceutical companies must compete with every other industry sector for high demand experts.

The Coalition of State Bioscience Institutes (CSBI) report found that competition for STEM talent, particularly “secondary”

industry talent (for example, in engineering, IT, or data sciences), was fierce. Additionally, the life science industry requires a higher percentage of highly skilled staff than any other sector.

Digital Literacy & Change Management

Digital literacy is the capability to use digital platforms and understand, assess, and communicate through them. In the UK, the Association of the British Pharmaceutical Industry surveyed people working in the pharma industry as part of their report on skill gaps in the biopharmaceutical sector. They found that responders rated digital literacy as the highest priority problem to be addressed.

There are two main ways of tackling this issue: decreasing the complexity of equipment and increasing the digital literacy of staff. These approaches can also help pharmaceutical companies reduce their need to compete with other industries for skilled workers.

Improving the digital literacy of managers is one top-down method aimed at increasing digital literacy. One industry insider explains that “for managers, the ideal case is that they are digitally literate enough to ask the right questions. And the second part of that is change management adoption. A lot of people are used to operating through conventional means, but now we are talking about changing that with a digital workflow. So that requires a bottom-up, top-down alignment to encourage change. IT solutions should be enabling and simple. User interfaces and training are important, but adoption challenges should not be underestimated. It is not

just a technology problem; it is also a human resource and skillset challenge.”

Augmented Reality

Many industries have also begun to use Augmented Reality (AR) to boost productivity and simplify complex tasks. Augmented reality differs from virtual reality; instead of an entirely virtual display, the real-life environment is mixed with computer-generated elements. As a result, it can be used to guide scientists through new procedures and equipment operations, reducing the likelihood of human error.

Some argue that AR can be used to change opinions on technology and make training more straightforward for scientists. One of the simpler ways to convince people about the advantage of technology is to use it for training. Augmented reality enables users to look at something and to understand it visually, which to many would be preferable than having to read lengthy SOPs (standard operating procedures).

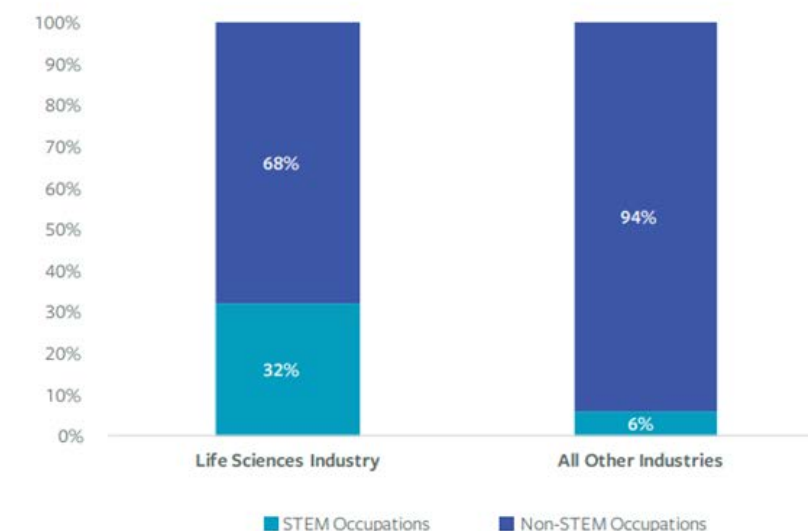
Andrew Garrood, Global Service Delivery Manager at Novartis, thinks that AR will become “one of the ways we interact with technology and data, just as the keyboard, mouse and touch screen did. I think we’re going to see a surge in interest in the ability to interface with technology without physical contact. So, whilst now it seems cool, exciting, and futuristic, it won’t be long before we just expect it as part of our devices we work with.”

While AR has the potential to transform the lab, it is not yet widely used in the industry. Andrew believes that this will change and argues that “nobody thinks twice about having a mouse or using a touch interface on their smartphone. Just

▼ Association of the British Pharmaceutical Industry chart showing that digital literacy is an area of concern for scientists

Source: ABPI (Association of the British Pharmaceutical Industry), Bridging the skills gap in the biopharmaceutical industry, 2022

Figure 9: Share of Employment in STEM Occupations, Life Sciences vs. All Industries, 2020



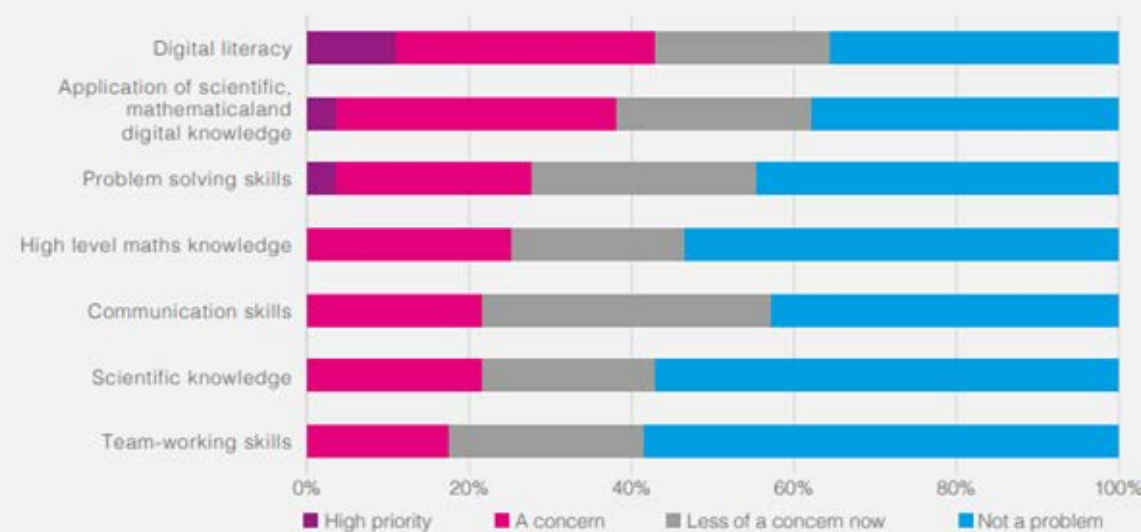
Source: TEconomy Partners' analysis of Emsi 2021.2 data set.

▲ Coalition of State Bioscience Institute chart displaying demand for STEM talent

like both of these inventions, it will revolutionise how we work, and we'll wonder how we ever lived without them. I think AR will be the technology that really has the most impact as it will integrate into our physical and virtual worlds.”

Final Thoughts:

As the industry moves towards smart labs and automation, addressing existing skill gaps and ensuring equipment is easier to use and maintain becomes ever more crucial.



FAIR DATA IMPLEMENTATION: A WORTHWHILE INVESTMENT

The amount of data created by pharmaceutical companies has increased exponentially over the last few decades. Managing these enormous volumes of data can be challenging, an issue compounded by a lack of standardisation. Data is often gathered from numerous sources and devices and stored separately, making it difficult to retrieve, query, share and analyse.

Edited by Oliver Picken

The amount of data created by pharmaceutical companies has increased exponentially over the last few decades. Managing these enormous volumes of data can be challenging, an issue compounded by a lack of standardisation. Data is often gathered from numerous sources and devices and stored separately, making it difficult to retrieve, query, share and analyse.

FAIR data is data that meet principles of findability, accessibility, interoperability, and reusability. FAIR data principles were created to increase standardisation and harmonisation across the industry and allow more efficient management, processing and analysis. Supporters argue that FAIR data has the power to transform the industry and accelerate faster drug discovery and development, but the pharma industry has been slow to adopt it. Implementing FAIR principles can be a daunting task; it is rarely a quick and straightforward process, as it requires a shift in how data is created and used within an organisation.

Where Are We Now?

There are significant technical and cultural barriers to FAIR data implementation for pharmaceutical companies.

Despite this, the benefits far outweigh the problems for most organisations and make data optimisation worthwhile. Three of the biggest obstacles to FAIR data implementation are initial costs, attitudes towards data management, and resistance to changing workflows.

Misinterpretations: What is FAIR Data?

As FAIR data is a relatively new term, it has been the subject of many discussions. To understand its concept, we should examine some misconceptions associated with it. FAIR is not a standard but instead a set of principles. Standards are prescriptive, while principles allow room for interpretation and customisation. For data to be FAIR, it merely needs to follow the fundamental principles of being Findable, Accessible, Interoperable, and Reusable. Companies can and should create standards that make sense for their data assets.

FAIR is also not solely based on making data more manageable for humans. Instead, one of the critical drivers for FAIRification is to increase how effectively AI and ML applications can analyse data.

Finally, and perhaps most importantly, FAIR data does not have to be open access. There are many reasons that data may need to be kept private, including personal details, national

security, and competitiveness. The 'A' in FAIR stands for "Accessible under well-defined conditions", and these conditions can vary depending on the source and use cases of data.

Examining the Cost: Implementation and Returns

FAIRification can be a costly task, requiring updated infrastructure time, effort, and training. Applying FAIR data principles to retrospective data can be a particularly daunting task. Despite this, research from the European Commission has estimated the minimum annual cost of not having FAIR data as €10.2bn across the European Union despite the initial costs. In addition, the benefits of improved data management have long-term financial benefits. Aligning data with FAIR principles can positively impact pharmaceutical organisations by increasing the value and insights gained from their data assets. Additionally, FAIRification helps improve the effectiveness of AI and machine learning. In turn, this can lead to benefits across the entire value chain, from discovery and development to patient outcomes.

Adjusting Attitudes: Data Management and Workflows

While FAIRification of data is generally agreed to be beneficial, it has met resistance from some scientists. Updating data is often viewed as an additional task that reduces productivity and takes away the time scientists can spend on furthering scientific research. Alharbi et al. (2021) surveyed scientists to improve their understanding of attitudes towards FAIR data. They found that "respondents asserted that the time spent aligning data with FAIR principles might affect an individual's productivity and thereby significantly influence a company's day-to-day business of drug discovery. They stated that the FAIRness of the data is not their top priority, but rather a priority secondary to the scientific progression of the project."

Attitudes towards FAIR data are only likely to change when the



▲ FAIR Data Diagram providing an overview of the four FAIR principles

benefits become more apparent. The acronym and principles were very recently defined in a March 2016 paper in the journal Scientific Data. It will take some time before insights uncovered through the FAIRification of data become more widespread. Additionally, once workflows have shifted to a FAIR data pipeline, most research scientists will find that they spend less time managing data.

Final Thoughts

The pharmaceutical industry is becoming ever more data driven as regulatory requirements increase and trial data becomes more complex. FAIR data principles provide guidelines on organising and managing data and increasing the value and insights gained from data assets. The implementation of FAIR also has the potential to enable greater collaboration and streamlined data management, opening doors to new innovations and insights.

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PHARMA MANUFACTURING: DIGITALISATION & INDUSTRY 4.0

Digital tools to boost productivity and optimise pharma manufacturing are becoming increasingly prevalent through smart sensors, AI, ML, virtual reality, and cloud computing. Digital transformation is here for Pharma, and it is changing companies' priorities towards digital solutions.

Edited by Oliver Picken

Digital tools to boost productivity and optimise pharma manufacturing are becoming increasingly prevalent through smart sensors, AI, ML, virtual reality, and cloud computing. Digital transformation is here for Pharma, and it is changing companies' priorities towards digital solutions.

Pharma Manufacturing: Falling Behind?

Research has shown that pharmaceutical companies have been reluctant to adopt digitalisation and new manufacturing technologies. As a result, the pharmaceutical industry is falling behind compared to other industries. While significant advancements have been made, defect rates, quality control and waste reduction remain relatively poor. This leads to increased manufacturing costs, which leads to reduced profits or higher prices for patients.

Despite this, there is a clear trend of manufacturing supply chains moving away from traditional paper-and-technology models towards digitalisation and automation. The drive for this comes from research, such as that from McKinsey, which shows that companies that embrace digitalisation can expect an average earnings increase of around 3.25%, a significant sum when considering the revenue of large pharmaceutical

companies. Moreover, the benefits of digitalisation and industry 4.0 technologies have become increasingly apparent in recent years and, for most, hold potential too great to ignore.

The Next Revolution: Industry 4.0 and Digitalization

Industry 4.0, also known as the fourth industrial revolution, is centred around digitalisation and connected technology. Artificial intelligence, machine learning, the Internet of Things (IoT), and blockchain are emerging technologies that are becoming increasingly important to stay competitive in the rapidly changing manufacturing industry. These all can potentially advance the way we manufacture pharmaceuticals, particularly biopharmaceuticals. The key feature of an Industry 4.0 workflow is the integration of connected devices, artificial intelligence, and robotics, which allows the automation of complex or repetitive processes as well as minimising or removing the need for human intervention.

Understanding The Challenges: Implementation, Data and Regulation

Most of the critical challenges of adopting Industry 4.0

technologies for pharma manufacturing are technical in nature and will require extensive investment and time to adjust and refine new practices, which is off-putting to many manufacturers. Companies will also need to improve their abilities to handle and optimise Big Data. Widespread adoption of Industry 4.0 will require cultural changes and innovations by manufacturers. Regulatory authorities will also need to adjust their practices.

While implementing many of the advanced technologies and manufacturing approaches for industry 4.0 will be difficult, the potential benefits are too great to ignore.

Current regulatory frameworks will not be suitable for processing industry 4.0 environments. Slow regulatory updates can hinder advancement and encourage manufacturers to continue using conventional methods. The risk of a product not being authorised for sale due to regulatory framework incompatibilities with innovative new technology is too significant a risk for many companies, particularly in pharmaceuticals where the development process is both expensive and time-consuming. Existing industry 3.0 technologies such as continuous manufacturing and adaptive control systems can still be an issue today.

While implementing many of the advanced technologies and manufacturing approaches for industry 4.0 will be difficult, the potential benefits are too great to ignore. Early research and testing have shown the possibilities of decreased costs, higher output, increased manufacturing safety and improved product

quality.

Pharma Manufacturing: Looking to the Future

There is no doubt that pharmaceutical companies are taking the challenges of Industry 4.0 seriously. Digitalisation has become important enough that the FDA has begun to adapt their frameworks to fit the changing requirements. Last year, the FDA published a report that they are currently developing new rules for the use of artificial intelligence in medicine. While the digital transformation of the pharmaceutical industry is in the early stages, both industry and regulators are working hard to prepare for the next generation of manufacturing systems, data management, and computing technology. As the early adopters prove their value, these processes will become more refined and affordable, benefiting not just the companies but also patients who will benefit from safe, higher-quality drugs delivered sustainably and with a lower risk of shortages and defects.



FORMULATION & DELIVERY

Advancing drug formulation, delivery and product development strategies and optimise therapeutic modalities.

INHALED DRUG DELIVERY

How Are Inhaled Drugs Absorbed and Distributed in the Lungs?

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RNA FORMULATION & DELIVERY

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RNA FORMULATION & DELIVERY

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HOW ARE INHALED DRUGS ABSORBED AND DISTRIBUTED IN THE LUNGS?

Inhaled drugs are a viable means of targeting tobacco smoke-related diseases in the lungs: permeability is key to their successful delivery. This Commentary article explores the mechanisms by which drugs are delivered across cell membranes, as well as pertinent areas for present and future research.

Presented by Carsten Ehrhardt, Professor in Pharmaceutics and Biopharmaceutics at Trinity College Dublin

Edited by Ben Norris

As a druggable target for inhalation therapies, the lung presents a unique challenge for those working in pharmaceutics. Different tissue and cell populations of the lung have distinct transporter expression patterns; to complicate things further, particles have to dissolve before they are removed by mucociliary clearance or phagocytosis. However, for Carsten Ehrhardt, Professor in Pharmaceutics at Trinity College Dublin the focus is on drug absorption into the lung tissue. As he explained to the audience at our Formulation and Delivery UK: In-Person event in May 2022, membrane transporters may also be novel drug targets in tobacco smoke-related diseases.

Action and Uptake of Inhaled Biologics

Ehrhardt kicked off his presentation by explaining that the focus of his research was not so much on getting material inside the lung. “We’re more interested in what happens when the particle or droplet is deposited,” Ehrhardt said. “Depending where you are in the central lung, the particle is probably sitting on the mucous layer, and needs to dissolve first before it’s removed by mucociliary clearance.” From here, the drug penetrates across the epithelial layer and into the lung tissue and bloodstream.

Inhaled drugs can then either stay in the epithelial cell or diffuse out into the tissue or the bloodstream. “As long as there’s a concentration gradient, this is a passive process that can go in both directions,” said Ehrhardt. “However, due to the pKa of the molecule many of these drugs have a net positive



charge,” Ehrhardt continued. When interacting with a positively charged cell membrane, this creates trouble. In addition, charged hydrophilic molecules don’t like crossing the lipid bilayer. “So, while there’s a small percentage of molecules that might be net neutral and permeate across, it’s probably not the main absorption mechanism.”

So, how do molecules permeate across these membranes? Some molecules move across via passive diffusion, but this is not a main contributor to absorption. More significantly, molecules may be substrates for transporters protein localised in the cell membrane. “We have transporters in many biological barriers that help molecules across, and in some cases, they also recognise therapeutic molecules.” Ehrhardt gave the example of carbohydrates: sugars are hydrophilic molecules which are superficially unable to cross the cell membrane but are nonetheless absorbed after a meal.

Transport of Inhaled Biologics: Membrane Transporters

One issue with drug uptake in the body is that different drugs may target the same transporter. “Currently, about half of newly FDA-approved molecular entities have patient information warnings about drug–drug interactions caused by transporters,” said Ehrhardt. In these interactions, two drugs will compete for the same transporter, which may impact on their pharmacokinetics, which are governed by drug absorption, distribution, metabolism and excretion.

As Ehrhardt explained, many researchers have not yet focused on transporters in the lungs, making them a relatively novel target in pulmonary drug development. He told the audience that his research had investigated ATP-binding cassette (ABC) transporters. “They have a binding site for ATP, so they can transport a substrate against the concentration gradient,” he said. These transporters efflux molecules out of the cell, which is a deleterious process in the gut as substrates are kicked back out into the lumen.

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However, in the lung the story is different. “If you have this transporter sitting in the luminal side of the cell, it will recycle the drug back into the lung,” said Ehrhardt. “That could be a potentially interesting mechanism to achieve a higher lung residence time.” The first transporter investigated for this was P-glycoprotein (P-gp/MDR1), which Ehrhardt introduced as “the best-known efflux transporter we have”.

“If you have this transporter sitting in the luminal side of the cell, it will recycle the drug back into the lung – that could be a potentially interesting mechanism to achieve a higher lung-residence time.”

Drug Transporter Studies

There are particular trends in inhaled drug permeability that can be monitored for, as Ehrhardt explained. “When you do a transport study – which means you grow your cells to a confluent layer on a permeable insert, you measure the bi-directional flux of the drug – if you have a passively-diffusing drug the permeability coefficients in absorptive and secretive directions should be the same.” In scenarios such as these where absorption is much slower than secretion, there is an indication that a luminal efflux transporter is present. In this instance, the transporter acts to hinder absorption.

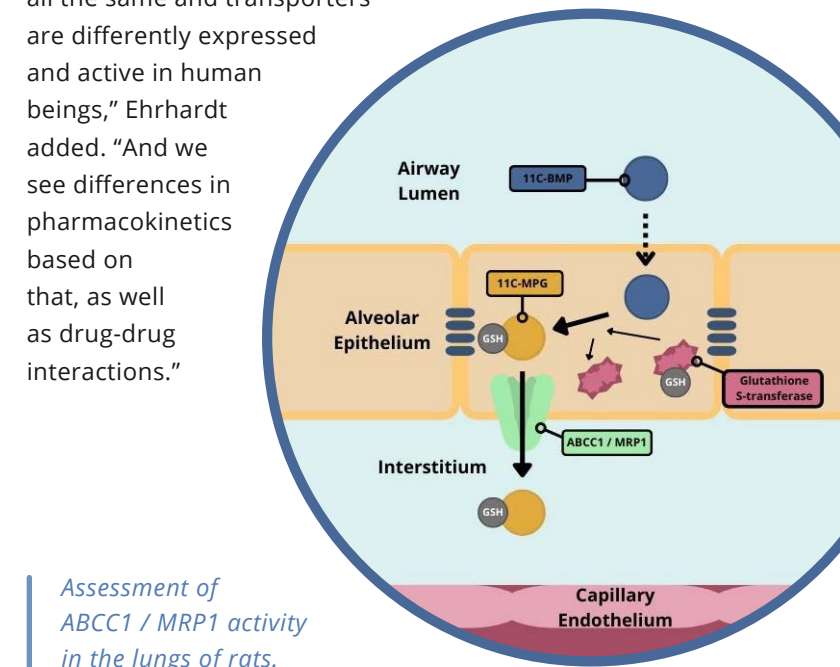
Ehrhardt then moved on to discussing the results from a mouse model, which investigated the adsorption of P-gp substrates from isolated perfused mouse lungs. In wildtype animals P-gp substrate drugs exhibit higher dwell times. “When you inhibit the transporter genetically or pharmacologically you have a quicker absorption and clearance, which clearly shows that the transporter is involved in pharmacokinetics.”

The other transporter Ehrhardt discussed was MRP1, which is a physiologically-relevant transporter because it excretes many endogenous substrates in addition to phase 2 metabolites from the cell. MRP1 is abundantly expressed at the lung epithelial barrier, where it may influence the pulmonary deposition of inhaled drugs and contribute to variability in the therapeutic response. Observations on lung kinetics in living rats found that the wild-type animals exhibited a much faster clearance of MRP1 substrates from the lung, and when the transporter is attenuated either by knockout or pharmacological inhibition, residence time in the lung is increased.

Future Implications for Inhalation Biopharmaceutical Research

Ehrhardt wrapped up his presentation by discussing the implications for the expression of efflux transporters in human lung epithelial cells. “A number of inhaled drugs have been found to interact with membrane transporters *in vitro*, *ex vivo*, and *in vivo*.” P-gp increases the pulmonary retention time of inhaled drugs, while MRP1 facilitates lung drug clearance, meaning inhaled drugs stay in the lung for longer. Transporters might be novel drug targets in chronic obstructive pulmonary disease (COPD) and other smoke-related diseases, because MRP1 expression is associated with COPD severity.

This is a pertinent area of research to focus on as similar studies may be applicable in humans to assess the effects of disease, genetic polymorphisms, or concomitant drug intake on pulmonary transporter activity. “I think we should consider these inter-individual variations, because we are not all the same and transporters are differently expressed and active in human beings,” Ehrhardt added. “And we see differences in pharmacokinetics based on that, as well as drug-drug interactions.”



Assessment of ABCB1 / MRP1 activity in the lungs of rats.

Speaker Biographies

Carsten is Professor in Pharmaceutics and Biopharmaceutics and Fellow at Trinity College Dublin. He also holds an Adjunct Professorship at the University of Southern California. His research is focused on lung drug disposition and pulmonary epithelial transport. Moreover; he is interested in molecular origins of airways disease. Carsten has edited 1 book and (co-) authored 102 peer-reviewed publications and over 250 abstracts. He is the recipient of honours and awards from German Pharmaceutical Society (DPhG); American Physiological Society and Galenus Foundation. Carsten serves as Associate Editor of Journal of Aerosol Medicine and Pulmonary Drug Delivery and Section Editor of European Journal of Pharmaceuticals Sciences.

PROMINENT NAMES IN RNA DELIVERY DISCUSS...



WHAT'S AROUND THE CORNER?

Beyond the blood-brain barrier, the pulmonary power of nebulisers, and RNA science in the post-COVID era

Edited by Tom Cohen

Oxford Global kicked off another exciting discussion group all about RNA therapeutics and emerging technologies for their delivery. Those attending were experts from the leading authorities in the field. Leading the conversation was Susan Rosenbaum, Founder, Chairman, and CEO of Lauren Sciences, a company that has been the recipient of twelve foundation grant awards and won first prize for Global Healthcare Innovation at BIO 2019. Also in the group were key experts and representatives from Johnson & Johnson, BioNTech, AstraZeneca, and Boehringer-Ingelheim, to name a few.

During the discussion, we heard about Lauren Sciences' innovative technology, V-Smart®: a global solution for RNA targeting the central nervous system. We also heard from Dr Cees van Rijn, Professor of Nanotechnology and Microfluidics at the University of Amsterdam, about his team's work on Vibrating Mesh Nebulisers and their opportunities for pulmonary delivery.

The central dogma of molecular biology is that DNA gets transcribed into RNA, which then synthesise proteins. There are also forms of RNA (e.g., shRNA, siRNA, miRNA) that affect production/activity of proteins and, thus, have therapeutic potential. RNA, therefore, should be seen as an invaluable tool

for pharmaceutical scientists across the board. Although there is so much potential in RNA, a therapeutic is ineffectual if not able to make it to the right cells, that's why drug delivery is such a necessary pursuit.

V-Smart® and Overcoming the BBB Challenge

Ms Rosenbaum started the discussion by outlining how her company is solving what is perhaps the most notorious challenge of delivering RNA therapeutics. This is, of course, the challenge posed by the blood-brain barrier (BBB) when delivering to the central nervous system (CNS); many therapies simply refuse to cross the BBB or, when directly injected into the brain, do not diffuse, and reach their target cells.

However, Lauren Sciences's V-Smart® technology uses nanovesicle encapsulation in order to provide non-invasive, oral and IV delivery to the brain. The V-Smart® platform is able to encapsulate any oligonucleotide therapy and transmit them to the brain crossing the dreaded BBB; whether it is an mRNA, siRNA, or DNA therapeutic, the system works the same. Furthermore, once these encapsulated therapeutics have made it across the barrier, the V-Smart® system is able to target specific brain cells, further specialising the therapeutic approach.

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Their technology uses what they have coined a "brain delivery trifecta": Macro brain-targeted delivery pre-release, micro brain cell-specific targeting, plus internal cell effects, yielding many advantages. Chief among them being the ability to sustain a selected, targeted release. The V-Smart® therapeutic (containing an encapsulated drug) spreads throughout the brain first before, then, targeting the specific cells it needs to. Along with this, Lauren Sciences promises no need for agent modification, high stability, and no degradation.

"The many possibilities for this technology are almost overwhelming."

Ms Rosenbaum says that Lauren Sciences' "competitive edge" is the fact that it has been shown to be safe and effective in preclinical animal efficacy trials. The many possibilities for this technology are almost overwhelming. Lauren Sciences is developing V-Smart® therapeutics where macro-targeting is used for neuro-AIDS and chronic pain applications; while other V-Smart® therapeutics are, in addition, micro-targeted to deteriorating motor neurons, dying brain cells, and dopaminergic neurons in ALS, Alzheimer's, and Parkinson's applications, respectively. With V-Smart® drugs for ALS and Parkinson's already proven themselves in animal efficacy models, who knows what new modalities could be around the corner?

Tackling Degradation in Pulmonary RNA Delivery

Dr Cees van Rijn then chimed in to discuss his team's work on reducing the particle degradation of vibrating mesh nebulisers to achieve pulmonary RNA delivery. Inhalation of RNA is a very worthwhile field to develop. One reason for this is the fact that when vaccines are delivered directly to the lungs, a smaller dose is required to trigger the same immune response as injection, around 20%. Furthermore, it has been suggested that up to 20% of people have an aversion to needles and would prefer to have drugs delivered via an inhaled method, this makes inhalation prosperous for uptake in vaccine and other RNA therapeutic compliance.

Vibrating mesh nebulisers for the delivery of RNA therapeutics work by pushing RNA molecules through nanometre-scale holes in a mesh. Positively charged lipids then bind to the negatively charged RNA and form the inner core of the lipid nanoparticle. The problem that Dr van Rijn and his team at the University of Amsterdam are working to tackle is the fact that the force that is required to push the nanoparticles through the mesh can often deform their nanoparticle structure and even the chain length of the mRNA molecules. When the structure of these particles becomes stressed like this, there is a tendency for the nanoparticles to break down into many sub-particles, possibly ruining their cell permeation efficacy.

It was this understanding of nanoparticle degradation that led Dr van Rijn and his team to test the efficacy of a novel syringe-based nebuliser that uses constant pressure instead of aggravating vibrations. They ultimately found that the standard vibrating mesh devices produced particles with a degradation of 20–40%, whereas the novel syringe-based nebuliser produced particles with only a 2–5% level of degradation: a huge improvement. Dr van Rijn concluded that "the more energy you need to make your nebule, the more degradation of nanoparticles you will find."

More Modalities, More Advances

The excitement in the delivery of therapeutics lies in the endless new syntheses and approaches to be tried. Dr van Rijn and Ms Rosenbaum both were decided on some of the special difficulties they would face. Flirting with the subject of nasal delivery, Dr van Rijn commented that: "of course, it's always problematic to get the right dose into the system". Perhaps more optimistically, he suggested that Lauren Sciences consider how V-Smart® might fare for pulmonary delivery; "provided, of course, that you make a nebulisation with the carriers, and they stay intact."

"We've been approached by companies about targeting the ear barrier or the eye barrier and these would all be by non-invasive administration."

Ms Rosenbaum did reply that she "firmly believed" V-Smart® could be used to target organs other than the brain, such as liver or lungs: "We've even been approached by companies about targeting the ear barrier or the eye barrier and these would all be by non-invasive administration." However, Ms Rosenbaum made clear that this prediction relates to the distant future. For now, they stick to the CNS due to its uniqueness and lack of current delivery modalities.

Final Thoughts on the Importance of mRNA Therapeutics in the COVID Era

The COVID era has seen mRNA go from fairly niche scientific jargon to a near household name, thanks to Pfizer-BioNTech and Moderna's latest vaccine achievements. Ms Rosenbaum emphasises that it is exactly these accomplishments that prove the importance of the field. Pondering an op-ed written in The Wall Street Journal about mRNA, Ms Rosenbaum said it was always curious to read about pharmaceutical science when it breaks out of the realm of pure science and into public discourse, calling it "very positive and very interesting". Thanks to developments in mRNA, especially in vaccines, Ms Rosenbaum proclaimed, "I think humans on earth might well live into their hundreds."

TECHNOLOGIES ADVANCING DRUG DELIVERY THROUGH THE BLOOD-BRAIN BARRIER

In this Insight Article, we delve into the complex intricacies of the Blood-Brain Barrier, explore its challenge for drug delivery, and look at 3 of the most exciting solution technologies.

Edited by Tia Byer

The Blood-Brain Barrier (BBB) presents a significant challenge for effective drug delivery. The BBB is a diffusion barrier, a selective semipermeable border that regulates the transit of pathogens and toxins between the bloodstream and the brain. It comprises of tightly packed layers formed of endothelial cells, astrocytic endfeet, and a basement membrane. In a healthy brain, the BBB plays a vital role in maintaining an optimal environment for neuronal functioning and acts as an interface between the circulation and central nervous system (CNS). In a diseased brain, however, the BBB becomes radically disrupted.

Brain diseases such as brain cancers and central nervous system disorders are among the most prevalent and poorly treated conditions. The BBB hinders the treatment of these diseases, by preventing the entry of conventional methods of drug delivery and other therapeutics to the brain. Given the complexity of the brain and its impermeable barrier, the time taken to develop CNS drugs is a considerably longer and more arduous process. Nevertheless, there are approximately 76 pharmaceutical and biotechnology companies currently developing cutting edge technologies to advance drug delivery methods through the BBB. The market is even projected to reach an impressive 4.6 billion USD by 2026. Some of the most prolific innovations are coming from companies such as CarThera and Bristol-Myers Squibb, universities including Northwestern, MIT, Harvard, and research

initiatives such as the European Union's Horizon project.

The Challenge of Delivery to the Blood-Brain Barrier:

So, what about the BBB is so challenging? A physiological hurdle, the BBB obstructs around 95% of molecules during the drug development stage. Most macromolecules are unable to penetrate the brain's endothelium. Only small and lipid-soluble molecules that weigh less than 400 Da can successfully cross the BBB, significantly restricting the number of molecular candidates available for delivery. In other words, therapeutic molecules that may prove effective in diagnosis and treatment, fail to obtain clinical approval because they cannot reach their cerebral target in adequate amounts. Integral to overcoming the resistance of the BBB is a thorough understanding of the delicate intricacies of the brain as an interface and how its function becomes rapidly compromised under pathological conditions such as multiple sclerosis, Parkinson's disease, seizures, strokes, and Alzheimer's.

Improving the success rates of neuropharmacology requires advanced examination of the body's neurovascular units and their role in the BBB's resistance. Neurovascular units describe the relationship between brain cells and their blood vessels. Peter Westenskow, Senior Principal Scientist and Section Head at F. Hoffmann-La Roche Ltd, claims that "scientists must find out how to restore normal

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homeostasis to a neuron so that it stops sending signals that tell the vasculature to misbehave". He continues, "it is vital to learn as much as possible about every single neuron in these neurovascular units, learn what they like to eat, how they respond to stress, learn what other supporting cells are present, and how they contribute to the issue". Therefore, it is vital to think of the neurovascular unit as a whole: an interconnected and dependent interface instead of a unitary and independent construct.

Advances in Technology: Overcoming the Blood-Brain Barrier

Developing optimised BBB models and technologies is a key area of research for many scientists working within the field and there are a number of exciting developments being made in the advancement of neuropathological drug transporters. We look at the top 3 most promising of these technologies and devices:

1. Ultrasound Implant: An ultrasound implant is currently under development with the aim to facilitate an on-demand opening of the BBB during drug delivery. In particular, the intracranial implant opens the blood-brain barrier before or after the injection of therapeutic agents. By emitting low-intensity pulsed ultrasound waves, this device will temporarily increase the permeability of the blood vessels in the brain and allow for the advancement of treatments for severe brain disorders including Alzheimer's diseases, brain metastasis, and glioblastoma. It will also reduce the side effects caused by chemotherapy and facilitate a 7-time higher rate of targeted delivery to the brain.

One such company to develop this highly anticipated implant is the French MedTech corporation, CarThera. A clinical-stage company, CarThera are currently initiating their ultrasound implant, called the SonoCloud. In 2020 they teamed up with Northwestern University and Bristol-Myers Squibb to run a phase I/II clinical trial to test the device's effectiveness when used in combination with the drug Abraxane on patients with recurrent glioblastoma. The product is expected to launch by 2023.

2. Nanomedicine: The delivery of nanoparticles is an increasing area of development in BBB engagement. Nanomedicine applies the tools and understanding of nanotechnologies to both prevent and treat diseases. Nanotechnologies are devices that use nanoscale materials (particles that are 1 to 100 nanometres in size) to interact with cells and their tissues on a molecular level and have the potential to carry out multiple specific functions simultaneously. Cutting-edge research has now found that nanomedicine can successfully facilitate siRNA delivery across the BBB to treat traumatic brain injury (TBI).

In January 2021, researchers at MIT and Harvard Medical School used engineered nanoparticles that contained siRNA

fabrications to inhibit neurodegeneration. By modulating surface chemistry and coating density, scientists were able to adjust the entry of nanoparticles across the BBB through tight junctions. Results showed that the number of nanoparticles present in the TBI mouse model was three times higher than in those using traditional methods. Other key players in the use of nanotechnologies include the biotech company CNS Pharmaceuticals, Inc who with MD Anderson, is in the process of investigating a new class of subnanomolar DNA-binding agents (WP1244). Pre-clinical tests have found the agents' successful penetration of the BBB with no observable toxicity in animal models.

3. Nose-to-Brain Patch: Funded by the European Union's Horizon 2020 program, the N2B-patch project or nose-to-brain patch is a practical and minimally invasive drug delivery system. It transports biologics drugs directly from the nasal cavity to the brain via a medical device in order to bypass the BBB. The carrier consists of a hydrogel-formulated application and offers a painless and more efficient administration. An innovative procedure, the N2B patch aims to treat multiple sclerosis and as of 2021, ranked fifth on the technology readiness level.

The field of potential applications for the N2B patch is vast, with researchers confident in its prospective to treat further CNS conditions. In particular, the European Union hopes that the program will impact social sustainability by ensuring improved drug delivery for hundreds of thousands of people. The patch also promotes the use of personalised medicines, which has the potential to make the required interventions more efficient. By bringing a new medical device to the market, the N2B project predicts its ability to create a manifold of new work opportunities for European distribution and manufacturing companies.

Despite its notorious difficulty, the BBB is an ever-evolving area of industry development. The strides taken to overcome the technological and delivery challenges posed by the barrier and its near-inviolable diffusion is encouraging. Ultrasound techniques, nanomedicine, and the nose-to-brain patch bring scientists one step closer to achieving the optimal delivery of therapeutics to the BBB every single day. But it is not only the technological advances in the industry that are proving ground-breaking. Research discoveries are also gaining momentum. Only in April this year, scientists at the University of Pennsylvania found a link between the BBB and the onset of psychological disorders such as schizophrenia. At Oxford Global, we believe that these exciting developments and breakthroughs mean it will only be a matter of time before scientific innovation acquires the foresight to defeat the BBB once and for all.

mRNA MANUFACTURING: SCALABILITY & PROCESS ANALYTICAL TECHNIQUES

mRNA delivery techniques took a long time to come to fruition, but recent advancements have enabled production at a scale never seen before.

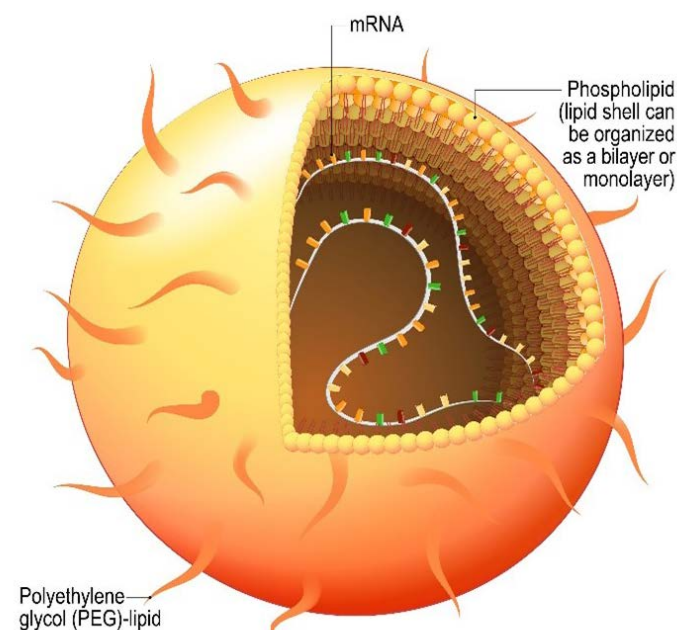
Edited by Oliver Picken

The clinical application of mRNA remained out of reach for nearly two decades due to issues with instability, immunogenicity-related toxicity concerns and scalability. Another issue that prevented the widespread adoption of mRNA technology was the need for a cold storage delivery chain. Fortunately, chemical modification, innovative carriers, and new manufacturing methods are overcoming these issues.

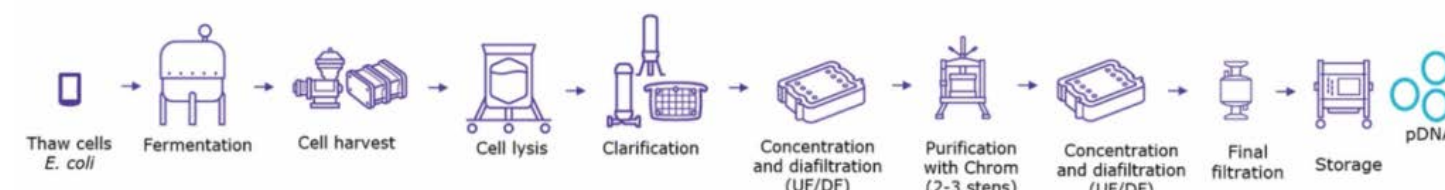
Delivery: Lipid Nanoparticles

Various materials have been developed for mRNA delivery, including lipids, lipid-like materials, polymers, and protein derivatives. Lipid nanoparticles have been thoroughly investigated and successfully entered the clinic for mRNA. Notably, two COVID-19 vaccines, mRNA-1273 and BNT162b21, use lipid nanoparticles to deliver antigen mRNA. In addition, many other lipid nanoparticle mRNA formulations have been developed and are under clinical evaluation to prevent and treat virus infections, cancer, and genetic diseases.

To be successful, lipid nanoparticle carriers for mRNA need to overcome many obstacles. Challenges include nuclease degradation, interception, clearance, and the accuracy of to target issue and cells. Finally, mRNA molecules must escape endosomes to reach the cytoplasm, where translation occurs. Until recently, the demand for lipid nanoparticles was low, and there were few facilities and established supply chains.



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▲ mRNA Manufacturing: Purification & Scale Up

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Fortunately, most excipients for nanoparticles are well understood and are relatively simple to produce. However, in some cases (for example, the Pfizer/BioNTech and Moderna mRNA vaccines) production requires crucial excipients that are much more difficult to make. Therefore, these companies need specialised manufacturing expertise, equipment, and facilities to create under good manufacturing practice (GMP) conditions at the high-quality level necessary.

Manufacturing Advances

In the past, lipid nanoparticle formulations were produced by thin-film hydration and reverse-phase evaporation. Extrusion techniques were then applied to limit variability in size.

Today, Lipid nanoparticle-mRNA formulations are mainly produced using rapid mixing techniques. This involves mixing lipid components and mRNA molecules using tightly controlled flow rate and pH levels. Crucially, this allows lipid nanoparticle-mRNA formulations to be reproduced with high encapsulation efficiency and homogeneous size distribution, improving scalability. Furthermore, due to the COVID-19 pandemic, many CMOs (contract manufacturing organizations) rapidly expanded their lipid nanoparticle manufacturing capacity.

mRNA Manufacturing: Purification & Scale Up

mRNA manufacturing is a complex process split into many stages. The creation of plasmid DNA templates is a crucial first step. Based on these DNA templates, the mRNA is then transcribed *in vitro* in the presence of an RNA polymerase and ribonucleoside triphosphates. The residual DNA template is removed by DNase digestion, and chemical or enzymatic methods cap the mRNA molecules. Unfortunately, these DNA

templates are easily contaminated with *E. coli*, RNA, proteins, and endotoxins, necessitating purification steps through the manufacturing process.

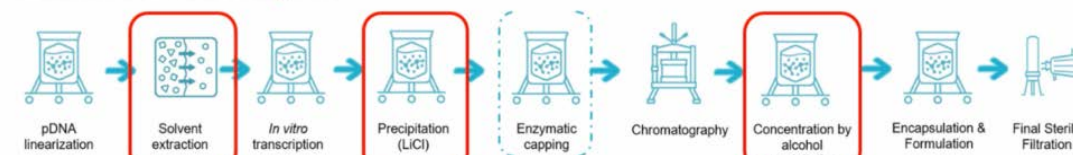
Process Control Analytics

Purification and formulation consist of a series of unit operations, each with its own operating requirements and performance parameters. Establishing a benchmark or expected result for each unit operation and real-time measurement of the unit's performance can reduce the frequency of failed batches. In addition, critical process failures can be traced back to the exact unit operation and batch of material where a deviation occurred. Product stability, purity and quality can be assessed using several analytical techniques, such as gel electrophoresis and high-performance liquid chromatography (HPLC).

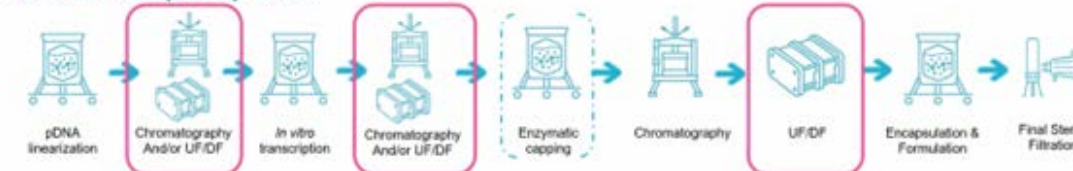
Chromatography & Scalability

David Gemmel, MSAT Biomanufacturing Engineer at Merck, explains that “solvent extraction or precipitation steps are problematic at lab scale. They utilise solvents, which is not ideal once you scale up to commercial volumes and operate within a GMP environment. They also have significant scalability issues. The solvent extraction and precipitation steps can be swapped with chromatography or tangential potential flow filtration (TFF). These technologies scale incredibly well, so once you've done your proof-of-concept step using solvent extraction and other techniques, you should start utilising devices for chromatography and TFF because it provides much better predictability at larger volumes.”

• Common lab-scale template



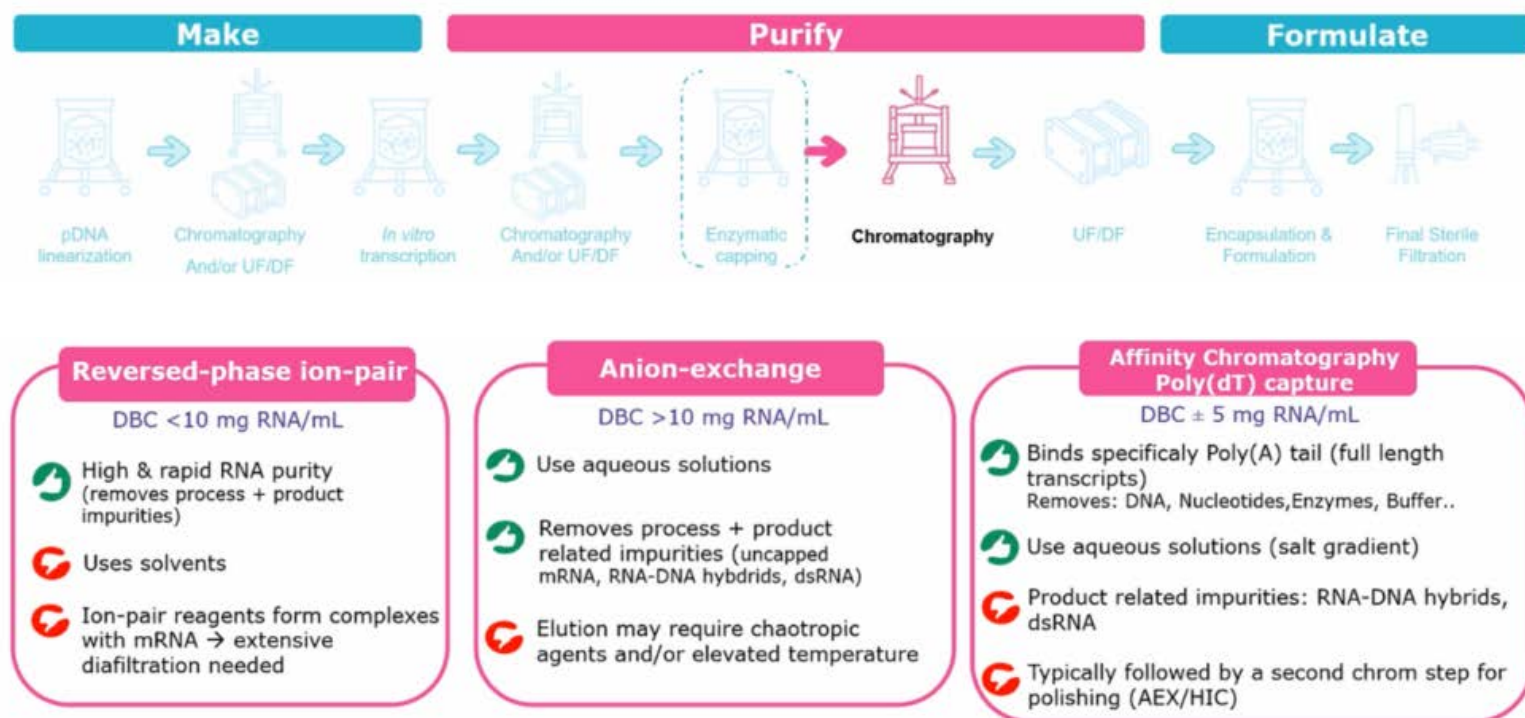
• Scalable template process



Extraction and precipitation steps replaced by chromatography and/or TFF

◀ Chromatography & Scalability

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mRNA Purification

One crucial step in mRNA manufacturing is RNA purification. A common first step is to use DNase digestion that separates DNA strands into much smaller fragments, which can then be purified using TFF, with cut-offs of between 30 and 200 kilodaltons. This is required because of the risk of DNA fragments hybridising with mRNA, causing a significant impurity that must be removed.

At this stage, the aim is to remove DNA fragments, endotoxins, wholesale proteins and restriction enzymes before moving downstream. Chromatography is typically used as a final purification step. There are three main options, each with benefits and downsides. It is vital to understand your impurity profiles and select a strategy that minimises time and financial requirements. Chaotropic agents are toxic and can cause harmful side effects so their removal must be thoroughly validated.

Reversed-Phase Ion-Pair

Reverse Phase Ion-Pair provides an excellent level of impurity removal quickly. However, it does use solvents. At GMP scale this is problematic, and potentially pair reagents may form complexes with the mRNA. This ultimately means that extensive diafiltrating is needed, increasing your process cycle time and the amount of buffer you need. In turn, this can lead to much higher costs.

Anion Exchange

Anion exchange utilises aqueous solutions, which is much easier to work with than solvents. It can remove process and product impurities, such as uncapped mRNA, DNA hybrids, and double-strand RNA. When using anion exchange, you may require chaotropic agents and elevated temperatures. Unfortunately, chaotropic agents can cause changes to the properties of the proteins in your solution, while elevated temperatures carry the risk of degrading mRNA and other delicate biologics.

Affinity Chromatography Poly(dT) capture

The final option is using affinity chromatography step with a poly DT capture resin that will bind specifically to your poly (A) tail. This will remove any DNA, nucleotides, enzymes, and buffer impurities. It uses aqueous solutions, but it will not renew or remove product-related impurities. The main problem this causes is that you will need a second chromatography step to remove RNA and DNA hybrids and double-strand RNA.

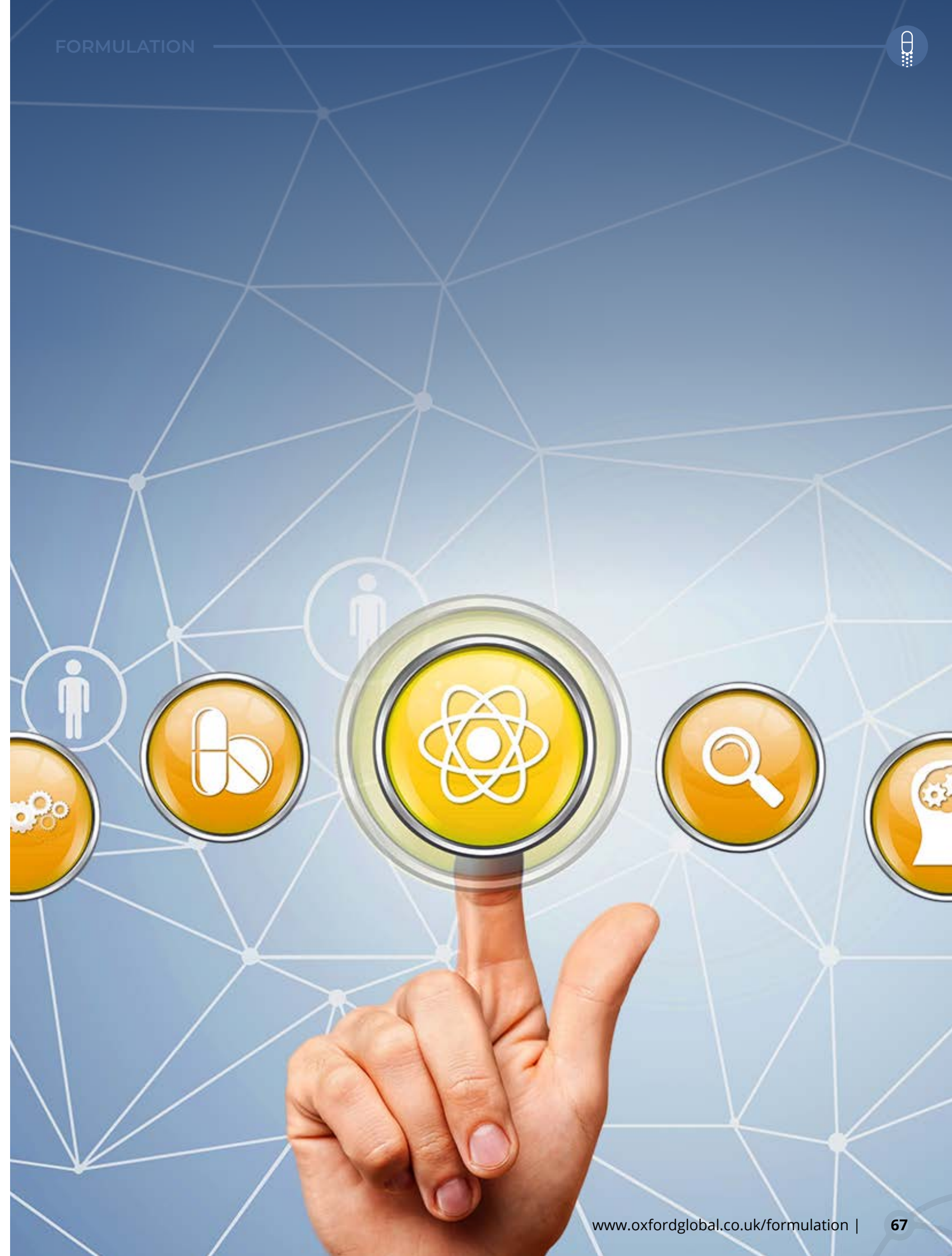
Final Thoughts & Conclusion

mRNA took a long time to come to fruition, but recent advancements has enabled mRNA manufacturing at a never seen before scale. Now, more than ever, researchers and pharmaceutical companies have a multitude of options to trial and test. The success of mRNA COVID-19 vaccines has finally bought mRNA into the limelight and opened new doors to future vaccines and therapeutics.

Speaker Biographies

David Gemmell – MSAT Biomanufacturing Engineer at Merck

David Gemmell has been working for Merck for over six years. Based in Glasgow, Scotland, he is employed within the Technical & Scientific Solutions MSAT (Manufacturing Sciences & Technology) department. David's primary focus is on optimising or troubleshooting downstream purification unit operations for European customers, focusing on both novel and traditional modalities, across all purification technologies. He also has a focus on upstream viral risk mitigation, specifically around pasteurization systems. At University David obtained a BEng (Hons) in Chemical Engineering and an MSc in Advanced Chemical & Process Engineering. His early career was based in the nuclear and power generation sectors before moving into biopharmaceutical and life sciences.



A NEW FRONTIER: RNA-BASED VACCINES & CANCER TREATMENTS

RNA based therapeutics, particularly mRNA, have been experiencing a period of rapid growth, partly due to the influx of funding for mRNA Covid-19 Vaccines. One area that has the potential to benefit from mRNA vaccines is cancer. There are various techniques that have potential to combat the deadly disease.

Edited by Oliver Picken

The two major classes of nucleic acids are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). DNA is the genetic material found in all living organisms. RNA is primarily engaged in protein synthesis. In eukaryotes, the DNA molecules never leave the nucleus but instead use an intermediary to communicate with the rest of the cell. This intermediary is the messenger RNA (mRNA). Other types of RNA—like rRNA, tRNA, and microRNA—are involved in protein synthesis and its regulation. Harnessing the power of RNA is currently a key area across the pharmaceutical industry.

A New Frontier: mRNA Cancer Vaccines

mRNA vaccines are central in the drive to establish more effective cancer treatments. mRNA vaccine cancer vaccines alert the immune system, making the immune system attack tumours. Several approaches to mRNA cancer vaccines are currently in development. Examples include dendritic cell vaccines and numerous types of directly injectable mRNA. Clinical trials have shown promising results showing antigen-specific T-cell responses and prolonged disease-free survival. Some

experts believe that an mRNA vaccine for cancer could be approved in the US by the FDA within five years.

Many mRNA vaccines for cancer take a mass-production approach: Ready-made vaccines are created to target proteins found on the surface of specific cancer tumours. Sadly, the scalability and efficacy of mRNA cancer vaccines are currently still speculation, but some experts expect challenges. Unfortunately, with cancer, there is no single target, and DNA mutations in cancer cells differ from patient to patient. This makes the creation of a widely applicable vaccine difficult. This is where personalised or precision medicine may provide the answer.

Precision Medicine: Personalized RNA Cancer Vaccines

RNA presents many new opportunities for precision medicine. For example, when fighting cancer, a tissue sample can be taken from a patient's tumour. Their DNA is then analysed to identify mutations that distinguish the cancer cells from normal, healthy cells. Next, computers compare the two DNA samples to identify the unique mutations in a tumour. Finally, the results are used to create a molecule of mRNA that will be delivered through the vaccine. Personalised cancer

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vaccines wake up specialized T-cells that recognize abnormal cancer cells and trigger them to attack.

Phase I clinical trials were recently completed at Mount Sinai Hospital in New York. Patients were given ten personalised vaccines over six months after having undergone standard cancer treatment such as surgery. Thirteen patients with a high chance of tumour recurrence participated in the trial. After 880 days, four had no evident cancer, four were receiving other cancer treatments, four had died, and one person had dropped out. While the trial used only a small number of patients, researchers are hailing it as a success and a vital first step to alleviating safety concerns.

The cost of personalised medicine has long been its most significant hurdle. There is reason to be optimistic, however. The first time the human genome was sequenced, it took 13 years and a billion dollars. Now it can be done for a few thousand dollars in a day or two.

RNA Interference: Controlling Protein Production

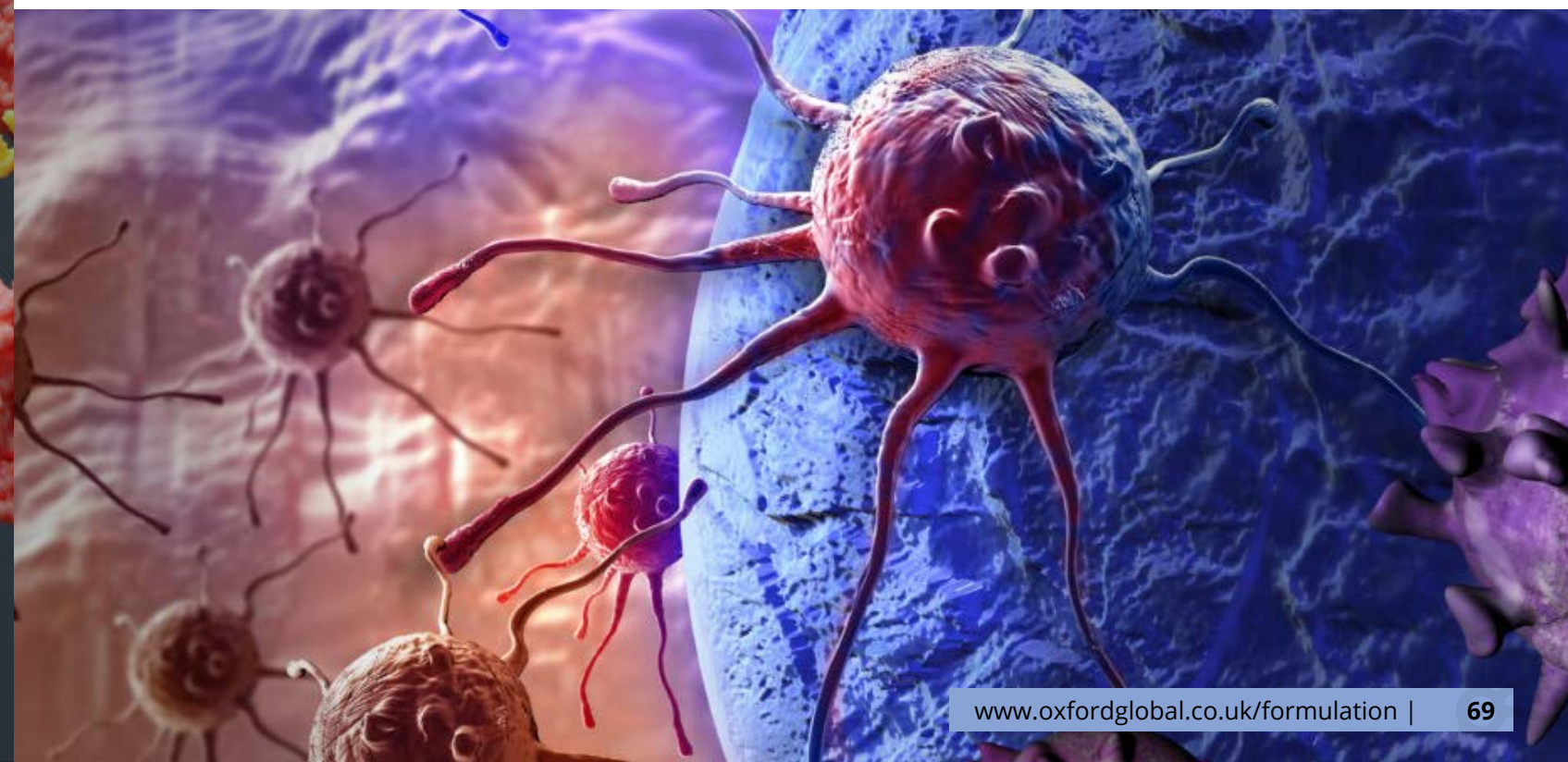
A growth area for RNA cancer treatment is RNA interference (RNAi), which is the process by which RNA stops or inhibits protein creation by the body's genetic code. It is in essence, a stop-sign for our DNA, regulating protein production and ensuring that the body does not overproduce. In 2006, Fire and Mello were awarded the Nobel Prize for their discovery of RNAi.

Researchers are exploring what happens small-interfering RNA (siRNA) is inserted into cells. In theory this would provide an effective way to control genes and, in turn, protein production. RNAi therapy is a potential game-changer for cancer treatment however, there are still challenges to overcome. siRNAs have not yet been widely adopted due to

their low stability and difficulty to deliver. Efficacy, however, is only half of the battle as the market will only tolerate certain routes of administration. Unprotected siRNAs are attacked by the immune system and are easily removed by the kidneys, eliminating many common delivery routes. In addition, siRNA therapeutics do not work until entering the cytoplasm.

Conclusion

Cancer is currently the leading cause of death in humans. Many researchers have dedicated their lives to battling the disease and while advances have been made, many forms of cancer still do not have an effective treatment. However, RNA-based approaches are providing new options for advancing cancer immunotherapy. The next few years will be a proving ground for RNA-based therapies against cancer as more companies progress to clinical trials. But, for now, the outlook appears promising.



— Part VI —

CELL

Transforming regenerative medicine through
advancing cell & gene research and therapies.

GENE THERAPY DEVELOPMENT

Challenges and Opportunities in Viral Vector-
Based Gene Therapy

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GENE THERAPY DEVELOPMENT

Inciting Rapid Standardisation in Cell and
Gene Therapy Manufacturing

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CHALLENGES AND OPPORTUNITIES IN VIRAL VECTOR-BASED GENE THERAPY

Oxford Global's Discussion Groups provide a focused networking opportunity for a handful of like-minded experts on a monthly basis. Our October Discussion Group addressing 'Challenges and Opportunities in Viral Vector-Based Gene Therapy' was a tremendous success. The discussion brought together an exclusive panel of key opinion leaders to address the key factors behind the market's growth, its priorities, future outlook, and challenges.

Edited by Tia Byer

Our October group came together for an hour of specialist discussion about the current challenges and opportunities in viral vector-based gene therapy. This discussion group was a select group of approximately 15-20 key industry leaders working to overcome challenges in the technical development of vectors, including safety, scalability, and novel manufacturing approaches from pharmaceutical and biotech companies. John Moscariello, Executive Director Viral Vector and Gene Editing Process Development at Bristol Myers Squibb, moderated the discussion, and panellists included Kyle Grant, Director of Vector Production at Voyager Therapeutics, and Blair Madison, Executive Director at Poseida Therapeutics.

John Moscariello kicked off the discussion with a brief overview of the gene therapy landscape and the recent advances in technological strategies for viral vector-based treatments. During this, Moscariello delved into the current state of the field and touched upon alternative technologies to deliver genes besides viral vectors. In particular, Moscariello started by sharing a 2017 article by The New York Times, entitled 'Gene Therapy Hits a Peculiar Roadblock: A Virus Shortage'.

The article documented how "custom-made viruses required to insert good genes into cells, are in short supply". The purpose of drawing attention to this news coverage was to show, according to Moscariello, how "over the past few years there has been an increasing amount of national and international press around the challenges of viral vectors and how the industry must optimise this technology to treat targeted indications". Overall, he identified increasing yield, purification, addressing immunogenicity concerns, and advancing characterisation as the key market trends driving the viral vector field.

After Moscariello's introduction concluded, the debate began. Kyle Grant opened the floor to discuss the current high interest in accelerating timelines and yields during viral vector commercialisation. He explained how "most of the excitement about viral vectors came from pursuits in academia". "Unfortunately, those methods of material provision did not advance until recently into a scalable process that makes sense commercially", he continued. Grant identified space and supply chain limitations as a major hurdle for viral vector-based commercialisation. To overcome this, the industry must aim to implement a scalable manufacturing platform

to meet current market needs. Blair Madison agreed, claiming "there is huge space for potential reinvention of the platforms for manufacturing specifically". Grant noted that while "early methods were rudimentary, new developments such as suspension-based culture may prove an exciting prospect".

The panel continued with Moscariello inquiring about the best practices for implementing technological advances for large scale DNA processing. In response, Grant identified the need for improved upstream processing and referenced Pfizer's AAV production as an exemplar in its ability to purportedly reach up to 1000 litres. This is an impressive feat considering most laboratories are limited to 500 litre batches of AAV, making the upstream process difficult. The debate also covered the importance of downstream processing. Madison highlighted material uniformity and toxicity as primary concerns in bridging the gap between manufacturing viral vectors and viral vector-based gene therapy. He claimed that CHO cells particularly suffer from genome instability and thus require improved manufacturing. Madison explained how "CHO maintenance is a challenge because over time they lose copies and suffer dwindling production." The panel also pinpointed cell line engineering as a helpful way to edit and restore DNA pathways and cell lines. Other opportunities and current market trends in large-scale DNA processing include ensuring batch-to-batch uniformity and reducing toxicity.

In response to the various hurdles posed by commercialisation and manufacturing, the discussion turned to future outlooks and opportunities for increasing the yield of viral vectors. For Moscariello, there seems to be within the cell and gene therapy industry "an undeniable push to get larger and larger indications, whilst trying to make more and more therapeutic products". He identified this driver as resulting from product demands and suggested using genomic analysis and stable cell line engineering to enable several of these different indications. According to audience member and Chief Scientific Officer of Ultragenyx Pharmaceutical, Sam Wadsworth, employing a helper cell line is a compelling strategy to produce infectious particles for transgenesis. At Ultragenyx, Wadsworth found that "this producer cell line which happens to be based on a healer cell, scales very nicely beyond 2000 litres". He added by saying that "the so-called trigger for AAV production and its clonal system is to infect the lines with wild type adenovirus," which then enables genetic modification of the cell-substrate and healer cell to help the virus post-interaction. Conducting genomic and informatics analysis on the selected deletion provides a valuable understanding of how to boost the yield further.

The discussion group concluded with some final thoughts on the future outlook of viral vector-based gene therapy. With ongoing research and development and numerous exciting pipelines emerging in the field, the future looks bright for the gene therapy industry. At Oxford Global, we couldn't have been more pleased with the turnout for our October cell discussion group. The conversation was engaging, the debate stimulating, and the industry insights invaluable.

INCITING RAPID STANDARDISATION IN CELL & GENE THERAPY MANUFACTURING

Can the cell and gene therapy field create industry consensus on a more detailed level? In this Insight Article, Jorgen Magnus, Strategic Program Lead of Cell and Gene Therapy at Bayer, highlights the promise of process mapping for reaching a harmonised approach to development and manufacturing standards.

Edited by Tia Byer

With the potential to revolutionise modern medicine, cell and gene-based therapeutics face a frantic pace of development and manufacturing. In fact, the global cell and gene therapy market is estimated to grow at a CAGR of 27.8%, increasing from 3.8 billion USD in 2019 to 13.0 billion USD by 2024.

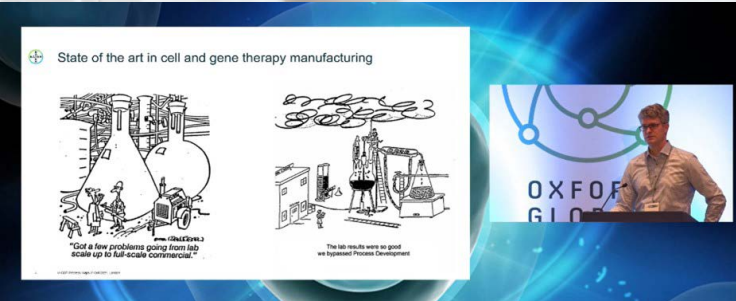
The untapped opportunities for improvement in the efficiency and robustness of gene pharmaceuticals mean the industry now turns its attention to establishing a synergistic form of process mapping. With the advent of next generation of cell and gene-based manufacturing technologies, exacting standards will be needed for regulators to keep up.

By mobilising a harmonised journey to manufacturing, with the help of distinct development strategies, streamlined quality control testing, and advanced scale-up processing measures, the gene therapy field can take a much-needed step towards industry standardisation. The foremost key players in the arena include Novartis, bluebird bio, Roche (Spark), Sangamo, RegenexBio, Bayer (AskBio and BlueRock), UniQure, Astellas to name a few.

Key Market Drivers:

In 2020 alone, the number of phase III clinical trials reached over 360, a figure that is set to rapidly increase over the coming few years. The exponential growth witnessed by the cell and gene therapy market can be attributed to several prominent drivers. In particular, the introduction of advanced therapies into the biopharmaceutical industry has helped reshape the treatment paradigm of numerous rare and life-threatening diseases.

The expanding cell and gene therapy arena has also seen the introduction of several new players and an increase in strategic alliances between pharmaceutical companies and contract manufacturers. In addition to this, the prevalence of



▲ Figure. 1
The Inadequacies of Current Technology for Cell Therapy

Research and Development centres is forecast to further drive market growth, especially in North America.

But how exactly can regulators and developers alike adopt a standardised approach to delivering streamlined manufacturing to meet current market demand? And what can we expect from the future of gene-based therapy manufacturing?

Process mapping is integral to ensuring compliance at every phase of the development and commercialisation process.

Process Mapping: A Step Towards Industry Standardisation

Process mapping is integral to ensuring compliance at every phase of the development and commercialisation process. According to Jorgen Magnus, Strategic Program Lead of Cell and Gene Therapy at Bayer, “the industry standard in production technology for cell and gene therapy is still very immature.” The proper development of production technology has been side-lined due to the field’s rapid growth.

“Because there has been no time, attention has consequently focused on jumping immediately to large scale manufacturing without adapting the process,” he continued. However, the problem with this approach is that developers only increase the volume and yield of drug candidates without actively implementing any changes. Concertation and focus on full-scale commercialisation often bypass process development

(see figure. 1).

In particular, current technology for cell therapy is not suitable for large scale production. Due to the demand to, as Magnus puts it, “the supply for hundreds of patients with cell therapy, the solution is to number up the plates that are used for cell growth.” There are enormous disadvantages to this approach.

Such technologies are laborious, involving several manual steps. They can also be very expensive. Furthermore, insufficient development processes lead to variability and a poorly controlled process. “As such, we are eagerly awaiting for this leap in innovation to happen in producing perhaps suspension culture or an encapsulation system,” Magnus highlighted.

Why Create Process Mapping?

Technological innovations aside, the implementation of process maps offer a powerful management tool and strategy for the development and eventual rapid manufacturing of cell and gene therapies. Process maps represent example processes and unit operations to accelerate collaboration projects.

The rapid expansion and technological advances in the cell and gene therapy arena mean achieving a harmonised and thorough standard of practice requires a dynamic effort.

Magnus then spoke about his involvement in BioPhorum, an organisation that brings biopharmaceutical companies together to work on common topics and that has developed process maps for cell and gene therapy processes “to establish a common

reference frame and educational tool to streamline process improvement and product commercialisation within the field.”

In particular, he explained how as it stands, there is no common standard for industrial production of cell and gene therapy that is publicly available. BioPhorum aims to bring together leaders working in the field and replace isolation with collaboration. Advantages of harnessing a united force via process mapping include a smoother transition to market and reduced patient treatment costs.

So far, leading companies such as AstraZeneca, Biogen, bluebird bio, Janssen, Lonza, Merck, REGENXBIO, Roche Genentech, Sangamo Therapeutics, and Bayer have collaborated with BioPhorum. The syndicate has created a gene therapy procedure classifying different AAV processes to establish three distinct developmental processes (see figure. 2). BioPhorum found that with a broad representation, industry can progress further and achieve consensus on a more detailed level.

Future Outlooks:

The rapid expansion and technological advances in the cell and gene therapy arena mean achieving a harmonised and thorough standard of practice requires a dynamic effort. Critical factors in ensuring this include procuring a broad industry representation and harnessing automation to eliminate manual procedures.

Looking ahead to the next 10 years, better operational steps and quality control will be some of the expected trends seen in the rapid standardisation of cell and gene therapy. Whilst implementing consensus in development and manufacturing will be a rigorous and demanding task, introducing process mapping can bring cell and gene therapy into the future of medicinal production.

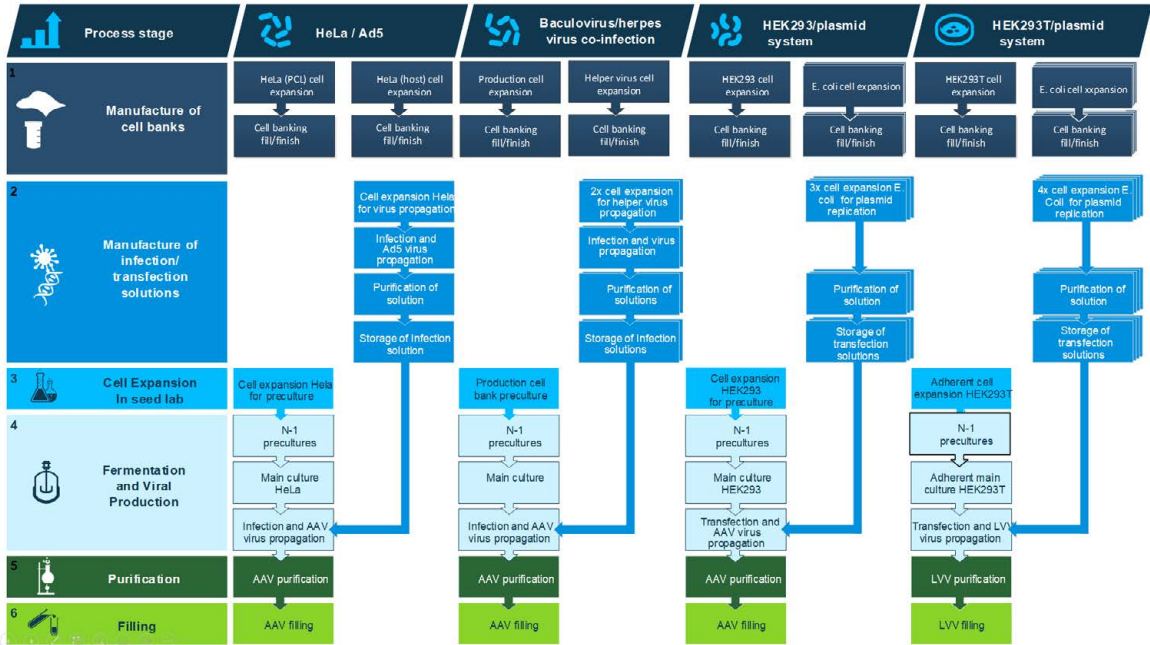


Figure. 2
BioPhorum's Process Map for AAVs

BUILDING THE FUTURE: AAV POTENCY ASSAYS AND CELL THERAPY ANALYTICS

This commentary article looks at the future of AAV engineering and cell therapy analytics, with exclusive insights from Sanofi and Genentech.

Edited by Tia Byer

As part of Oxford Global's Biotherapeutics US Online held on 17th-18th November 2021, Ho-Young (Grace) Lee, Cell Therapy Analytical Development Group Leader at Genentech, and a senior representative from Sanofi, participated in a workshop analysing the future of cell engineering and cell therapy analytics. The workshop was moderated by Lee and attracted an audience of senior representatives from leading pharmaceutical and biotech companies and top research institutions. This commentary explores some of the exclusive insights divulged in the workshop and finds out how best to usher in the next generation of cell-based therapeutics.

Engineering Cell Lines for AAV Potency Assays:

Sanofi kick-started the workshop with a presentation on 'Building For Success: Engineering Cell Lines for AAV Potency Assays'. This explored the use of reporter gene systems to simplify complex potency assays and improve throughput, precision, and costs.

The presentation also delved into the possibility of AAVR overexpressing cells to platform to numerous serotypes and any transgene to improve permissivity and assay signal. The talk concluded with a case study looking at Sanofi's modular cell engineering system, its application to any new gene therapy products in the pipeline, and its implementation in phase-appropriate strategies.

Methodology: The cell-based potency assay is a critical piece of the analytical paradigm for a drug in a clinical trial. It measures both the efficacy and functionality

of the drug candidate and is used for characterising manufacturing changes and for evaluating structure-function relationships of the product. Other potency bioassay requirements include the assay's ability to calculate comparability between batches, measure stability, and enable characterisation data for regulatory submissions. According to a senior representative of Sanofi, "there are a lot of key pieces of the puzzle that we aim to bring together to build the ideal cell-based potency assay".

They continued by explaining "the importance of demonstrating the mechanism of action or MRA of a product and with it the reaction by which that drug is working". Achieving a quantitative readout in a simple assay format facilitates the transferability of method to a quality control lab during the late and commercial phase. Sanofi's acceptance criteria for their cell-based potency assays include requirements such as linearity, precision, accuracy, and indication of stability. The representative went on to claim that "having a strict criterion for these different parameters, means the lab at Sanofi can successfully detect a change in potency".

Findings: Following this, Sanofi divulged some exciting results from the assay developed using the Celigo cytometer whilst researching AAVs expressing GFP (green fluorescent protein). While investigating different time points of cells transfected with AA5, GFP, and a novel serotype called AAVX, the dose-response potency curve demonstrated higher signals 72 hours after infection. The AAVR cells were also shown to have a different signal to the control parental cells. Upon calculating the EC50, which was considerably higher for the control parent, it was concluded that less material

is required for the AAVRs to attain the desired dynamic range and signal. Reflecting on this, the presenter from Sanofi noted how "this is important, especially for gene therapy programmes, because we often work with limited material compared to mAb manufacturing runs". Therefore, an assay that uses less material is preferable.

Insights: The compelling performance of the AAVR compared with the control candidate demonstrated an improved precision on the various parameters of the 4PL fit calculated. In addition to this, the AAVR had an improved F-test for linearity and R2 fit, performing well in the percentage recovery of potency mimics. Strategies for developing the non-clinical GFP assay include cell seeding density and plate uniformity. Sanofi concluded by stating the criticality of starting cell line design strategy as early as the pre-clinical method development stage to ensure successful engineering during phase 1 and eventual implementation during phase 3.

Cell Therapy Analytics:

In the second half of the workshop, Lee introduced the topic of analytics and control strategies with a presentation titled, 'Cell Therapy Analytics: Challenges and Opportunities'. Lee spoke of the unique hurdles facing the industry today and the various technologies and approaches available to usher in the next generation of cell therapy pharmaceuticals. Lee concluded the talk with identification and scrutiny of phase appropriate control strategies and method development.

Consideration and Approaches for Diverse Cell Therapies:

The manufacturing paradigm for CAR T autologous cells is an essential consideration for cell therapy analytics. 1 batch of CAR T autologous cells goes to 1 patient, whilst a single batch of allogeneic CAR T cells go to approximately 100 patients. "The release testing burden is high compared to biologics which can cover 1000 or even 10,000s of patients from 1 batch", Lee explains. She compared the challenge of CAR T cell manufacturing to being "more like scaling out as opposed to scaling up".

There can also be considerable challenges in sourcing raw materials such as cytokines and gene editing reagents. As perishable products, both cold chain supply and custody chain of identity make the paradigm for diverse cell manufacturing complex. Consequently, this explains the high cost of goods, especially for autologous and allogeneic candidates. Other challenges include the various hurdles to commercialisation. In particular, there are multiple cases of pushback by the FDA and EMA, such as refusal to file and complete letter response and even clinical hold from numerous companies.

Control Strategies: From a control strategy perspective, understanding and defining the process parameters of the critical quality attribute (CQA) and setting the meaningful specifications are complicated. For cellular therapies, the starting material is critical to the quality of the final drug product. Autologous therapy starts with the patient's own material, which thus means there are variabilities. In

programmes using gene and genome editing, the critical material quality considerably impacts the overall quality and hence the success of a drug candidate.

To counter this, it is imperative to implement an end-to-end control strategy. "You have to understand the implication of starting material qualities and how this chain of identity and chain of custody will determine the direction of the therapeutic", Lee commented. For optimal results, control strategies should be implemented into the experiments' data management.

Comparability Considerations: As Lee puts it, "the process is the product", and the two should be designed to exist in a symbiotic relationship. Implementing early CQAs will help define comparability strategy and process development. This is because limited product understanding means comparability study would become challenging with process changes. Furthermore, during process improvement, the dosing could be complicated. Some characteristics might not fully be understood, which risks having the new process become defined and mistaken as a new product. Lee identified this as "an implication in the filing strategy".

Insights: Therefore, it is imperative that cell therapy product control be implemented early to ensure successful commercialisation. Analytical development enables process understanding and comparability, and stability studies. Although defining CQAs is challenging, it is a rapidly evolving and iterative process. According to Lee, cell therapy manufacturing and analytical control systems are a paradigm shift that needs a different strategy to understand the product and process from early development. As a solution, data and correlative analysis are required to demystify the CQAs and link them to clinical outcomes.

Future Outlook: The workshop concluded with a panel discussion addressing the challenges of analytics and manufacturing. The two presenters were joined by Bernado Cordovez, Chief Scientific Officer at Halo Labs and addressed the various technologies and techniques available to overcome the difficulties of limited sample amounts and improve reference standard control. As the market for AAV cell-based therapies grows, so will the need for better-defined quality standards and strategisation. The road to developing and manufacturing the next generation of cell therapeutics is an arduous but vital one. Such endeavours hold significant promise for the industry, and here at Oxford Global, we are excited to see what the next couple of years bring.

TACKLING TUMOURS IN-VITRO WITH 3D CELL CULTURES

Raymond R. Mattingly explains the applications of 3D cell culturing at Oxford Global's 3D Cell Culture Symposium

Edited by Tom Cohen

Tumour modelling is absolutely vital to oncology. Researchers need to accurately understand how cancers interact with their microenvironment in order to optimise preclinical development of curative drugs. This is why creating more accurate models has such an impact on the progress of cancer therapies.

Chief among the ways in which modelling can be improved is to culture cells in three dimensions rather than two dimensions. 3D cell modelling better recapitulates the in vivo tumour microenvironment. This is due to the similar morphology and slower growth that the 3D environment can provide.

According to Raymond R. Mattingly, Professor & Chair of Pharmacology & Toxicology at The Brody School of Medicine, East Carolina, 3D cancer avatars have the potential to get very close to the in vivo context. When Mattingly was working with Wayne State University of Medicine, his team experimented with the capability of Tissue Architecture and Microenvironment (TAME) models.

Mattingly and his team of researchers used TAME models to investigate the particular indications of premalignant breast cancer transitioning to carcinoma and type 1 neurofibromatosis (NF1).

Mattingly's Lab and Endpoints for Live Cell Assays

To best simulate how tumours grow and spread in the body, it is important that cell models consider various aspects of the tumour microenvironment. The driving cancer cells are not the only tumour cells that need to be modelled. Tumours can present as complex networks of fibroblasts, inflammatory cells, and blood vessels, among others. "For breast cancer in particular, we need to think about myoepithelial cells and fat cells," said Mattingly.

The assays that Mattingly and his team analysed set out to measure the volume, proliferation, deviation from sphericity, and invasive outgrowths of tumour sites in vitro. This required functional imaging of proteolytic pathways in the tumour microenvironment.

Most of the data that the team collected used confocal microscopy and Z-stack techniques in order to capture images of living cells in real time. Mattingly explained that this can create significant problems with imaging, storage, and analysis, as well as requiring specialist software.

Study one: Breast Cancer

Mattingly first described how they used 3D cell culturing to study breast cancer. In the early stages of cancer metastasis, cancer cells infiltrate into vascular networks, specifically via the lymphatic networks.

Figure 1 (right) shows human umbilical vein endothelial cells (HUVECs) in blue, which form tubes in the 3D cultured matrix. Cancer cells shown in red can be seen to invade the HUVEC tubes and proteolysis is taking place in the sites shown in green.

Although HUVECs are widely used for this kind of modelling, Mattingly commented that the fact that they model blood vessels rather than lymphatic vessels can be a limitation for the cancer indications that they are studying. Cancer cells, especially in breast cancer, are more likely to use lymphatics rather than blood vessels as an initial point of entry.

Next, Mattingly showed us a comparison between a model for microvascular blood endothelial cells (BECs) and microvascular lymphatic endothelial cells (LECs).

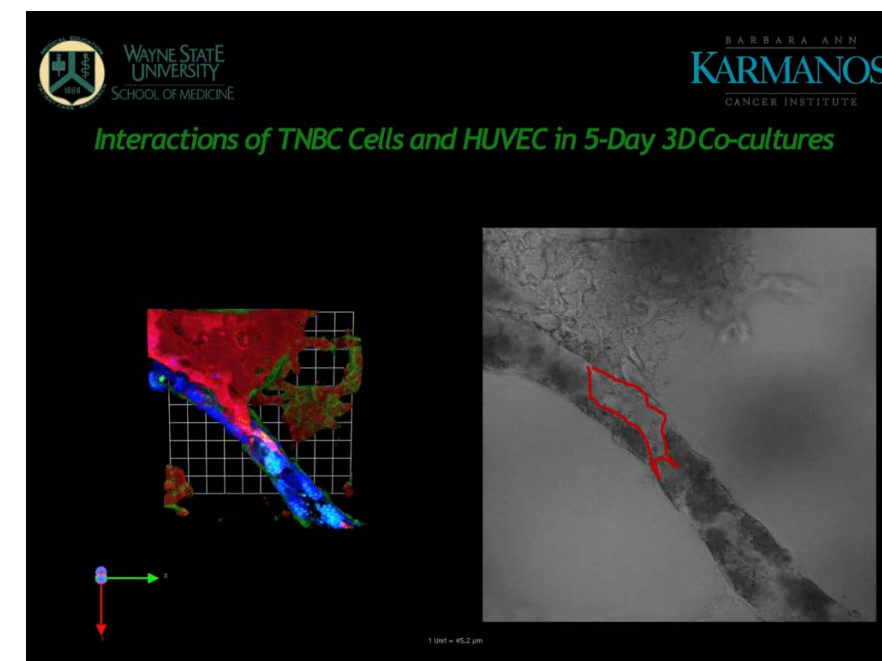
"The fact that they model blood vessels rather than lymphatic vessels can be a limitation for the cancer indications that they are studying"

Looking at the BECs (in Figure 2), the cancer cells show some propensity for interaction and there is a lot of proteolysis, "this is associated with the microvascular endothelial cells seemingly even more than with the cancer cells," said Mattingly.

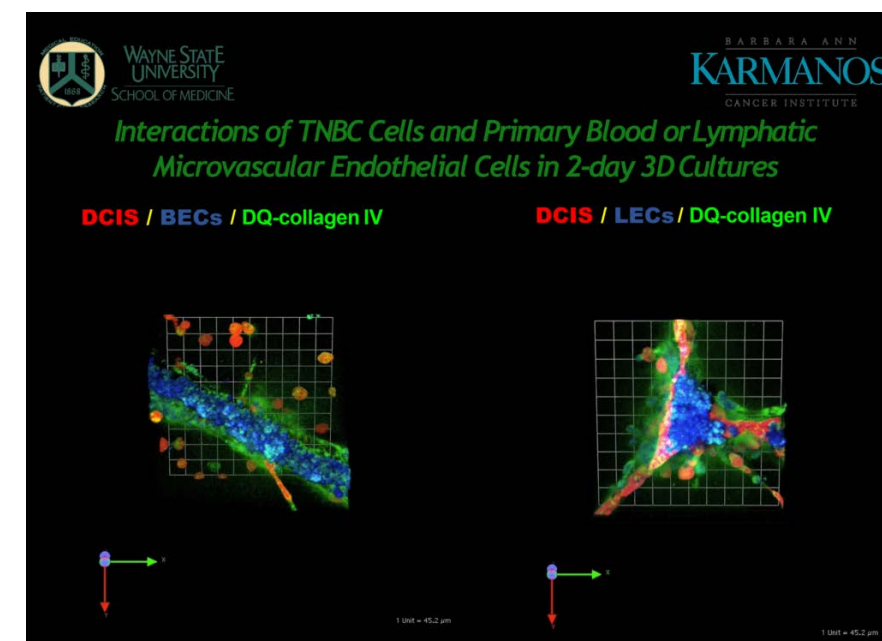
The LECs on the right-hand side of Figure 2 form in what Mattingly calls "prismatic structures," which are characteristic of how they would behave in vivo. Mattingly pointed out how the cancer cells have migrated to the LEC structures and there is a large amount of proteolysis.

This led Mattingly's team to coculture LECs and breast cancer cells (specifically MDA-MB-231) together in 3D. The analysis showed that the cancer cells were significantly stimulated by the lymphatic cells and that the lymphatic cells were "massively" stimulated by the cancer cells.

This work, conducted by Mattingly and his team, demonstrates a very significant point. That modelling lymphatic networks in 3D cultures can provide a detailed picture of cancer cells' initial point of infiltration in the body.



▲ Figure 1 – A slide from Mattingly's presentation showing human umbilical vein endothelial cells (HUVECs) in blue, which form tubes in the 3D cultured matrix



▼ Figure 2 – A slide from Mattingly's presentation, showing 3D cultures of BECs and LECs.

This research could be a great leap toward developing new therapeutic endpoints in the future. Drug developers now have a much clearer way of understanding how breast cancer propagates via the body's pathways, certainly an energising prospect.

Study two: Type 1 Neurofibromatosis

Type 1 Neurofibromatosis (NF1) is one of the more common rare disorders—as defined by the EU and US authorities. The condition is punctuated by the growth of large premalignant tumours along the nerves called neurofibromas. Mattingly clarified that “neurofibromas are complex tumours, presenting many different cell types in the tumour microenvironment.”

“Surgery is usually not possible as a curative measure.”

Although neurofibromas are initially benign, they can cause serious problems due to their large size. They can displace organs or other important structures in the body. Furthermore, they also have a high risk of becoming malignant. Due to their complexity, their mass, and their tendency to wrap around vital structures, surgery is usually not possible as a curative measure.

“NF1 is caused by a loss of neurofibromin which is a negative regulator of the Ras proto-oncogene,” Mattingly told us. “When neurofibromin is missing, Ras becomes too active. This induces too much signalling through pathways such as MAPK and PI3 Kinase drives tumour formation, proliferation, and survival.”

This led some researchers to theorise the use of MAPK MEK inhibitors as a therapy for NF1 patients. Although they had

promise, Mattingly clarified that disappointingly, there was little selective toxicity in the tumour cells versus normal Schwann cells, which indicated this might not be an effective treatment.

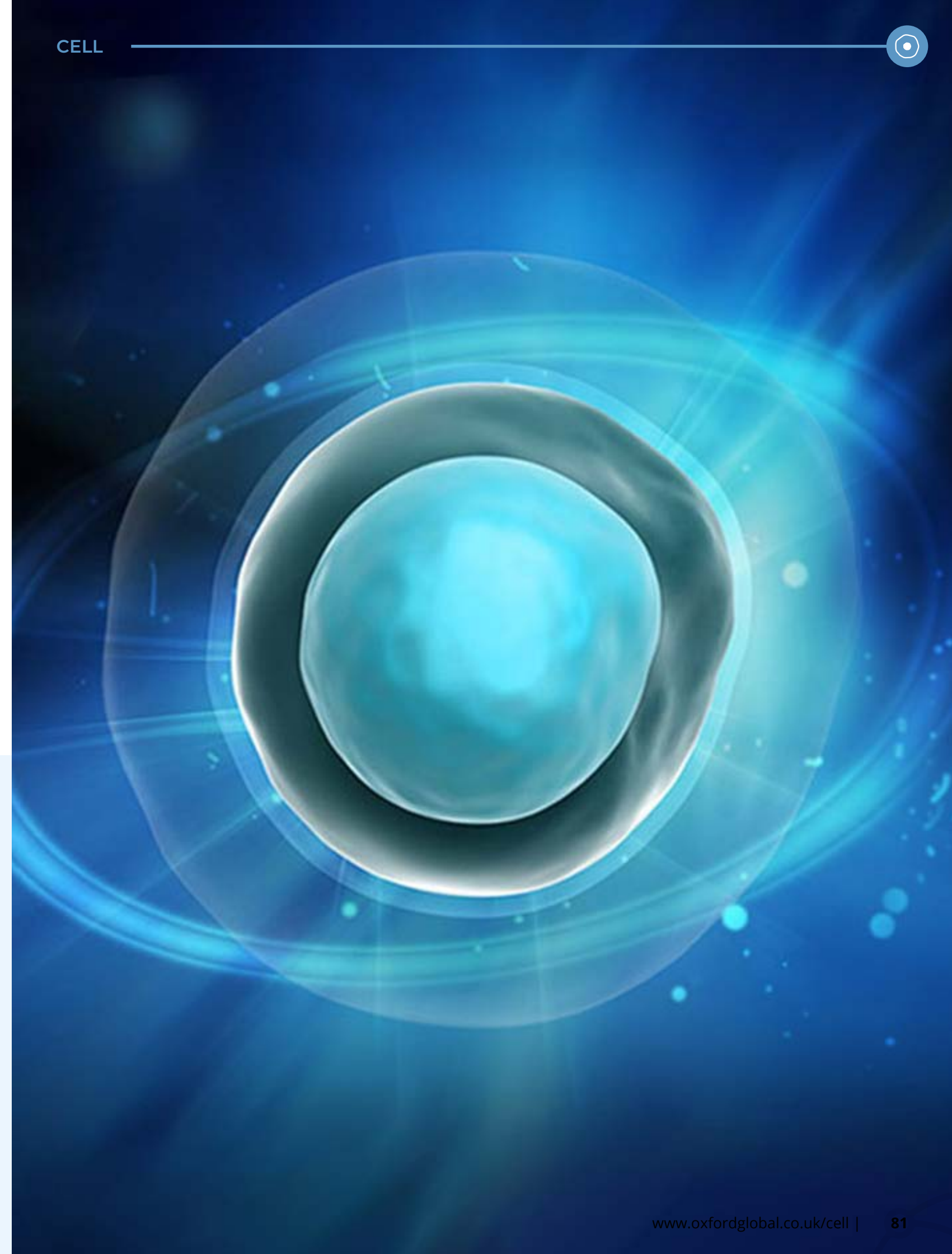
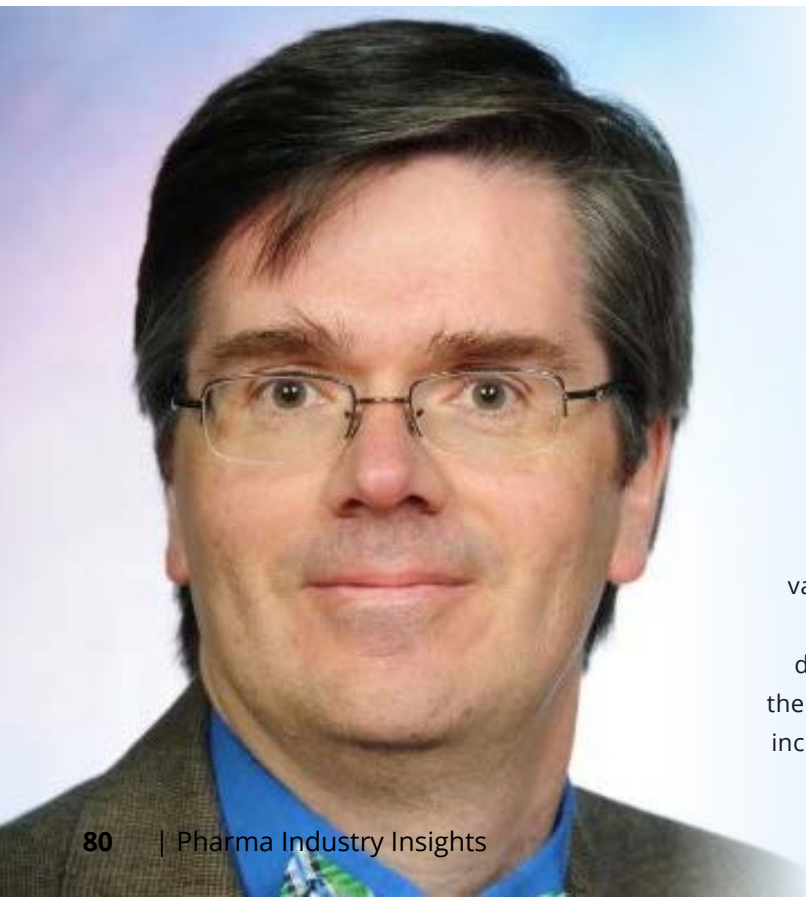
However, Mattingly and his team decided to revisit this idea, this time using 3D cell cultures to model the inhibitors' effect. They analysed the influence of the cell inhibitor selumetinib on the neurofibroma microenvironment and compared their analysis using both 2D and 3D cultures. This comparison hoped to reveal whether the 3D environment would better elucidate the drug's effect. “Truly pathomimetic 3D cultures should be suitable enough to identify the driving pathways that are sensitive to targeted inhibition,” stated Mattingly.

They found that there was a suitable amount of selectivity in their 3D models to serve as evidence for some neurofibromas' sensitivity to the MEK inhibitor. Other teams studying the effect of MEK inhibition in inoperable neurofibromas concurred with this conclusion. Their results found that around half of the paediatric patients saw significant tumour shrinkage with selumetinib.

The drug has now received approval for the treatment of NF1 and is the first drug on the market of its kind. Unfortunately, selumetinib does not treat all cases, so the team are still studying plexiform neurofibromas. Mattingly explained that his team are now “interested in the potential of high-content analysis to think about proteolysis as a problem.”

The advent of 3D cell modelling brings abundant opportunities for drug discovery and development, as demonstrated by Mattingly's research. Bringing an extra dimension to models could indeed expose new horizons of dimensions to therapeutic modalities. Having climbed one mountain, the drug discoverer is now akin to the wanderer above the sea of fog.

Dr. Ray Mattingly is Professor of Pharmacology and Oncology and Chair of the Pharmacology Department at Wayne State University. His research is centered on the identification of effective, new therapeutic approaches to cancers that are driven through activation of Ras signal transduction pathways in the absence of direct oncogenic mutation of Ras. This mechanistic strand underlies both type 1 neurofibromatosis (NF1) and many cases of breast cancer. With the support of a Komen for the Cure award, he established three-dimensional (3D) cell culture models of multiple sub-types of human breast cancer in order to provide tractable and relevant systems in which to identify targeted therapeutics. In subsequent NCI-funded work, he has optimised and validated findings in animal models and patient samples with the goal of translating the work into the clinic. In the context of NF1, he has developed co-culture 3D models of plexiform neurofibromas through the Neurofibromatosis Therapeutic Acceleration Program (NTAP), which includes collaboration with National Center for Advancing Translational Sciences (NCATS) for screening of compound libraries.



SHOWCASING EXPERTS' INSIGHTS FROM EMERGING CELL THERAPY COMPANIES

Emerging experts in the field of cell therapy: Emily Warner, Moin Saleem, and John Maher, answer questions about their science and industry

At Oxford Global's 2021 Cell UK conference, we heard from some up-and-coming names in the world of cell therapy. Genscript's panel discussion set out to showcase emerging companies in the field. The panellists in attendance were Dr Emily Warner, Principal Scientist at Ikarovec; Prof. Moin Saleem, Professor of Paediatric Renal Medicine at Bristol Medical School; and Dr John Maher, Chief Scientific Officer at Leucid Bio. The event was an excellent chance for the panellists to share their thoughts on science and industry.

AAV Manufacture: When to Outsource?

The first question we heard from the audience regarded the manufacture of AAV viral vectors. There has been a recent emergence of companies that can supply platforms that can capably manufacture AAVs to proprietary standards. Due to this, the question then emerges when research labs should begin to trust the outsourcing of this manufacture to third parties over producing them in-house?

Dr Emily Warner explained how her company, Ikarovec, entirely outsourced their AAV manufacture mainly because they are still a small lab. Warner explained that while this has worked for the time being, there are improvements that they

would like to see when it comes to moving into the GMP space. "This is one of the things we've been speaking to Catapult about", said Warner, explaining that Ikarovec has been collaborating with external manufacturers to try and make their AAVs more effective. In particular, modifying AAV vectors' packaging may afford this leap in efficacy.

"Good quality, research-grade material" is the simple factor that companies look for when it comes to AAV manufacture. Warner notes that sometimes quality standardisation can be a limiting factor for third parties in this regard. Better standardisation of quality assurance for AAVs; making sure they're research-grade means that GMP style vectors are used early in development. This removes the obstacle of going back to the drawing board when the product gets to the regulatory stage. "In our experience, it's difficult right now, but it's something we're looking forward to improving; it's a problem that we're trying to solve currently."

Transitioning from Academia to Industry

The next question concerned the panel's experiences with spinning off of university labs and setting up a start-up company. The panel were asked what the driver for this pivot was. We heard from Dr John Maher and Prof. Moin Saleem, who expounded their experiences with spinning-off from academic labs.

Having worked on CAR-T cell therapy for over 20 years, Maher explained that he had initially been somewhat slow to appreciate the commercial potential of the technology when it first started increasing in interest eight years ago. However, he ultimately realised the "huge opportunity" that the therapy offered so much so that it became Leucid Bio's core product. "I honestly feel at the moment that it is really difficult, if not impossible, to do meaningful CAR-T work exclusively as an academic because the funding is simply not there to do this," Maher explained, commenting that it is access to resources which is the primary driver for spin-off companies.

"For the first ten years of my working in CAR-T, it was considered to be an 'ivory tower' activity that rather strange academics, 'boffins' in other words, got up to entertain themselves in the lab. ... And I have to admit, I found that an extremely frustrating experience. ... At King's, we set up a commercialisation Institute led by a man called Mike Garrison. Mike was really keen to try to commercialise these kinds of technologies which universities develop all the time, appreciating that otherwise, there is a significant risk that these technologies may be wasted."

Saleem largely concurred with Maher on this topic, "It's tough to get the amount of funding that you need, particularly for things like manufacturing, analytics, and the steps that you need to get through to clinical trial; then the cost of clinical trials are huge." Saleem emphasises that the objective is to get these therapies to the stage where they can be used by patients as soon as possible. The best way to do that, Saleem thinks, is to have the security and resources of a company to get the product through clinical trials.

"I'm learning it's a commercial world out there, and people are out there also to make their profits. In a way, it's not such a bad thing because it gives us such huge opportunities. After all, the enthusiasm is massive to really get this to work."

Possibilities for CAR-T Cell Therapy in Solid Tumour Cancers

Further regarding Maher's work on CAR-T, one audience member asked how the lessons learned from the method's use on blood cancers may translate to the treatment of solid tumours. Maher responded that they have learned that what works in haematological cancers doesn't work in solid tumours. But this should not detract from the success that CAR-T has had in blood cancers:

"When have we ever had a new cancer medicine, which you apply in the first-in-human setting, where you've got a terminally ill patient population, and you're seeing up to 90% complete response rates?"

However, Maher explained the challenges they are currently facing in tackling solid tumours, stating that "one of the things we need to think about is which molecules we can safely

target." Maher points out that the molecule CD19 is targeted in B-cell-derived blood cancers. While CD19 is expressed on healthy B-cells in addition, immunoglobulin replacement therapy can substitute for the need for these B-cells. As a result, CD-19 can be safely targeted in patients. However, this is not the case when it comes to solid tumours: "we don't have a CD19 analogue", says Maher, "so, what are you actually going to target safely?"

Furthermore, Maher reminds us that T-cell trafficking to solid tumours is also an issue:

"With a blood cancer, you infuse them into the bloodstream. As a result, they have direct access to the malignant cells. In addition, these T cells tend to traffic into bone marrow and lymph nodes, where blood cancer cells also reside. With solid tumours, it's a completely different kettle of fish. How do these T cells actually know where to go? And once they get into a solid tumour, then they have to infiltrate within that environment."

Medical Device Regulations for Local Delivery

We also got the opportunity to hear Saleem's thoughts on regulation, particularly regarding medical devices for local delivery. Saleem mentioned that standard methods of delivering to the kidney did not require any novel devices in the clinic. However, Saleem's targeted development area was to optimise delivery through the renal artery "right through to the glomerulus." Then Saleem noted that the next concern would be to keep the product at the delivery site for as long as possible to get transduction and tweak variables like the pressure and rate of infusion.

"So those are mechanical things that will probably need a proprietary device if we work out the right conditions. ... I think we're at the early stage where we're thinking through those issues and testing them. We've got some excellent ex vivo models of kidneys. And then once we get to the point where we know the mechanical conditions we want to apply to humans in a trial, I think we'd have to go through some regulatory hoops that you'd tell us about to get those things certified."

— Part VII —

DISCOVERY

Advancing and innovating discovery pipelines and research collaborations.

TARGET BASED DISCOVERY

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COMPUTATIONAL DRUG DESIGN & AI

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TARGETED PROTEIN DEGRADATION

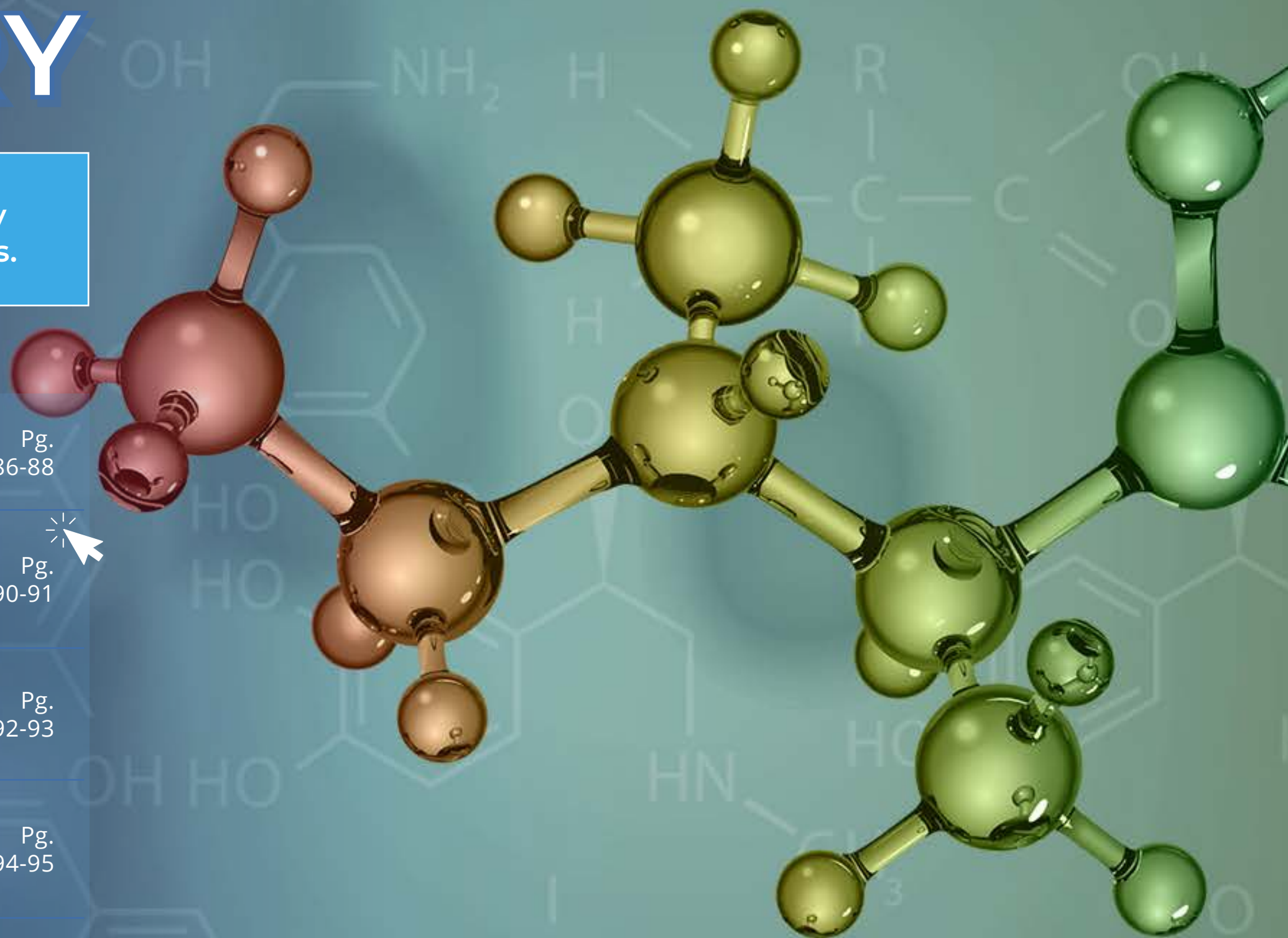
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TARGETED PROTEIN DEGRADATION

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HOW DEL SCREENING IS USED IN LIVING CELLS

Living cells create a more relevant physiological environment for DEL screening, says Dr Iolanda Micco, Associate Director of Chemistry & Alliances at Vipergen

Edited by Tom Cohen

DNA-Encoded Chemical Library (DEL) finding has been a landmark technology in the discovery of drugs, but scientists are eager to improve the efficiency and efficacy of the process. Higher and more reliable hit rates aim to improve DEL technologies, which would fast-track the drug discovery process even further than DEL screening has already taken it. Presenting at Oxford Global's 2021 online Discovery week, Dr Iolanda Micco suggests that implementing DEL screening into living cells, rather than in plastic reaction tubes, represents one such valuable enhancement.

Why Use Living Cells?

The advantage of using living cells in DEL screening is that they provide an environment that has physiological relevance to the target proteins studied, says Dr Micco. The cytosol of a living cell is composed of thousands of macro-molecules that just can't be replicated *in-vitro*. So, using living cells to provide this relevant environment to the target protein would theoretically lead to lower attrition rates and higher success rates.

Another reason DEL scientists are attracted to working with living cells is to exploit them as a vehicle for target protein expression. As these proteins can be expressed directly in the cell, there is no need to purify or even handle the targets. Therefore, workflow is faster, easier, and more efficient

allowing the screening of challenging targets.

The Challenges with Using Living Cells

But how do you get the DEL into a tiny living cell in the first place? Dr Micco's answer is straightforward: inject it. African clawed frog (*Xenopus laevis*) oocytes are prime candidates for DEL injection due to their large size (about a millimetre in diameter), about 100,000 times bigger than somatic cells. The enormity of these cells makes them perfect for the microinjection of DELs. Furthermore, *X. laevis* oocytes are great candidates, as they are inexpensive, widely available, and known to be capable of expressing a wide variety of heterologous proteins.

A second challenge is that DEL members may bind to the endogenous cell proteins instead of the protein of interest, recording hits on irrelevant proteins. Dr Micco explained that the team's solution is to label the proteins of interest with DNA. This DNA allows scientists to differentiate the protein of interest from the superfluous endogenous proteins after achieving hits.

Experiment, Data, and Outcomes

The experiment used cell binder trap enrichment, which Vipergen is currently pioneering at its labs. In each experiment, the fusion mRNA of the protein of interest and 'Prey' was

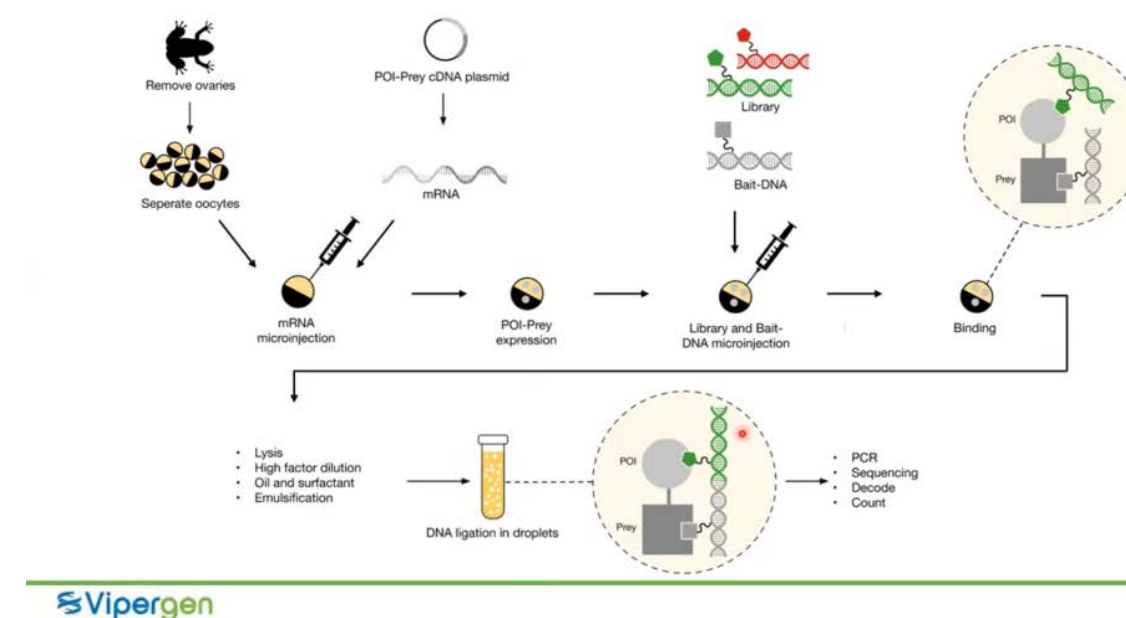


Figure 1 – A slide from Dr Micco's presentation: Diagram illustrating In cell Binder Trap Enrichment (cBTE)

injected into the oocytes initiating them to express the fusion protein. When the protein is expressed, the 'Prey' protein is expressed fused to it, differentiating it from the endogenous proteins in the cell. The scientists then injected DELs into the oocytes along with a DNA-encoded small molecule known to be receptive to the Prey protein: the 'Bait'. The Prey DNA and the DNA barcode for the hit molecule then ligate into one bonded DNA strand (Fig. 1). Therefore, scientists will know whether a hit achieved is receptive to the protein of interest if its DNA barcode contains the Bait DNA when sequenced.

For the data to be collected, the scientists just need to amplify, sequence, and ultimately decode the DNA markers attached to the protein and Prey.

The team at Vipergen selected three proteins of interest to study DEL screening in living cells, these being p38α, ACS2, and DOCK5. Each protein was experimented upon using three

progressive concentrations of the Bait-DNA.

The organisation of this data in this way allows scientists to identify clusters of key compounds for further study and assay.

The most hit target protein, p38α, yielded 154 hits distributed across nine chemical clusters; 21 were synthesised and assayed. They found 19 out of the 21 hits were shown to be inhibitors in an enzymatic assay.

Multiplexing, Orientation, and Competition

Dr Micco also talked about the technique's potential for multiplexing, whereby two Prey mRNA are co-injected into the cell along with two Bait DNA. "Multiplexing is a very powerful approach. Not only does it save time and cost, but it also gives all the information on predicted specificity. So, for those

Heat map – VIPS392

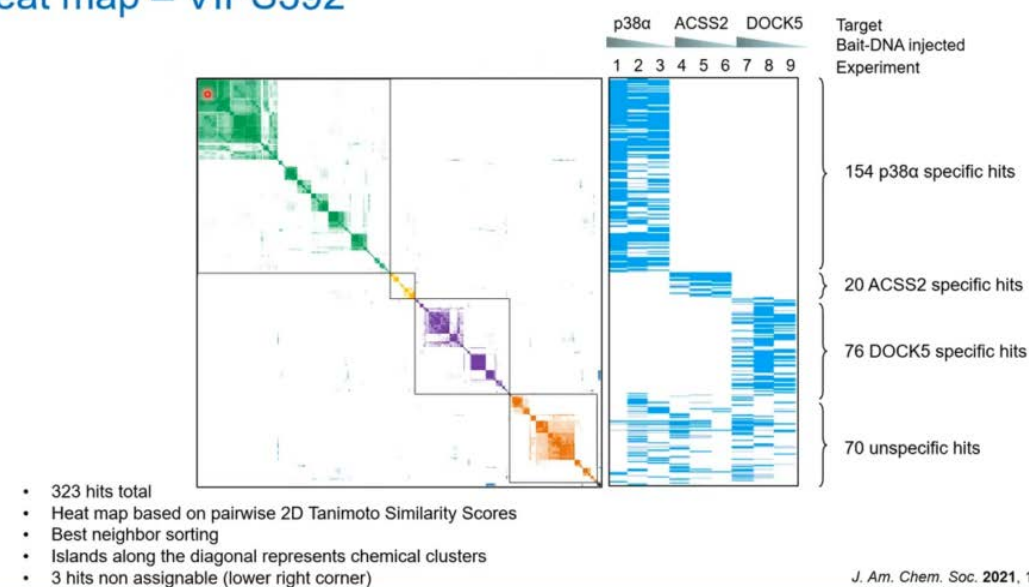


Figure 2 – A slide from Dr Micco's Presentation: compiled heatmap and dataset across all experiments.

applications where specificity is important, it is a very powerful tool.” Dr Micco explained.

They repeated the experiment in various ways to ensure the integrity of the data. The results were similar to the original findings in the following modes: both mono- and duplex-; using different Prey; using both N- and C- terminal fusion. Furthermore, their findings observed no crosstalk between Prey. “There is flexibility, freedom, and confidence with regards to the orientation of the proteins,” says Dr Micco.

Living Cells: a More Efficacious Environment

The techniques that Vipergen are using for DEL in living cells are up-and-coming. Using cBTE, they can screen for various potential targets: Human or pathogenic soluble proteins tolerating either N- or C- terminal fusions. However, there are some challenges for this technology. Integral membrane proteins can't be screened in this way, and the team expects problems for screening proteins that bind strongly to DNA or could be toxic for the cell.

However, Vipergen can screen DELs under more relevant physiological conditions with this technology. DEL screening is, therefore, easier and more efficacious than before, producing lower attrition rates. There is no need to purify the active target protein, which also means an expanded target space and a faster screening process. The experiments performed by Vipergen have shown that the technology is robust, creating reproducible results, and it shows that multiplexing is a viable use of the method.

Iolanda Micco Associate Director of Chemistry and Alliances at Vipergen (Denmark)

Iolanda Micco joined Vipergen (Denmark) in May 2017, where she is currently holding the position of Associate Director of Chemistry and Alliances. Iolanda has obtained her master's degree in Medicinal chemistry at the University of Pisa (Italy) and progressed her studies with her PhD in the same field at the University of Siena (Italy). Iolanda has been working for 15 years in international environments in various and demanding activities, including management and strategic roles. She started her career as medicinal chemist at Siena Biotech where she built her knowledge in the field working on projects from screen to hit to candidate selection in the neurodegenerative area. She taught Chemistry and medicinal chemistry in one of the most prestigious colleges of Singapore for 2 years before moving to Denmark.



At Pivot Park Screening Centre, we have longstanding and proven experience in ultra-High Throughput Screening (uHTS), enabling us to support and accelerate your lead discovery project.

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DISCUSSION GROUP



ALPHAFOLD AND DRUG DESIGN: HAS AI SOLVED BIOLOGY'S 'GRAND CHALLENGE?'

Govinda Bhisetti, Principal Investigator and Head of Computational Chemistry at Biogen, leads a discussion group on what the revolutionary machine learning technology means for drug design.

Edited by Tom Cohen

The world of medicine has been stumped by a 'grand challenge' as influential as it is notorious. Protein folding has for decades been considered enigmatic. The precise way a protein is folded determines much of its functionality within the body, which means mapping a protein's shape is vital to understanding the drugs that target it.

Scientists have long been able to determine the chemical composition of many proteins. Still, their shape is much trickier since there are an astronomical number of structural configurations in which any particular protein can fold.

Enter AlphaFold

The protein folding problem had become such a holy grail for researchers that a biennial competition, Critical Assessment of Protein Structure Prediction (CASP), was launched to promote computational methods of solving the enigma. In 2020, Alphabet's DeepMind entered the second iteration of their tool 'AlphaFold' into CASP14 and achieved such great success that Andriy Kryshtafovych, a project Scientist at UC Davis, declared the protein folding mystery "largely solved".

AlphaFold 2 uses a machine learning approach to assess how a protein spontaneously organises its structure by analysing its amino acid chain. The algorithm is trained using a data pool of over 365,000 pre-sequenced proteins to create accurate

predictions of stable protein structures when given their monomer sequence.

Govinda Bhisetti is a Principal Investigator and Head of Computational Chemistry at Biogen, who has a long-standing interest in protein structure and its application in drug design. Oxford Global hosted a discussion group led by Bhisetti in which the leading experts discussed what improvements they thought AlphaFold 2 would bring to the field of computational drug design.

The Success of AlphaFold 2

Bhisetti began the discussion by explaining his fascination with the success of AlphaFold. It is only recently that we are beginning to see the actual applications of machine learning in the real world. What was once confined to science fiction is now making its way into reality.

AlphaFold 2 has proved the most accurate protein structure prediction method by far. At CASP14, DeepMind's project was over 2.5 times more accurate than the next best contestant (see figure. 1).

Bhisetti then asked our attendees what they thought were the most important practical applications of the technology. The experts' consensus hailed AlphaFold2 as a valuable technology with a bright future. However, its application is still rather in its infancy, mostly requiring combined approaches to make it worthwhile.

DISCOVERY

What Does AlphaFold Mean for Those in the Industry?

"[AlphaFold] is especially useful to optimise the binding mode of the ligand on the protein surface," said Taiji Oashi, a Senior Scientist at Kyowa KIRIN. Oashi's team is working on antibody structures and has had some success in using AlphaFold for predicting them. However, Oashi added that there are some differences in CDR H3 loop compared to data from X-ray crystallography, so there is still room for improvement for AlphaFold's predictions.

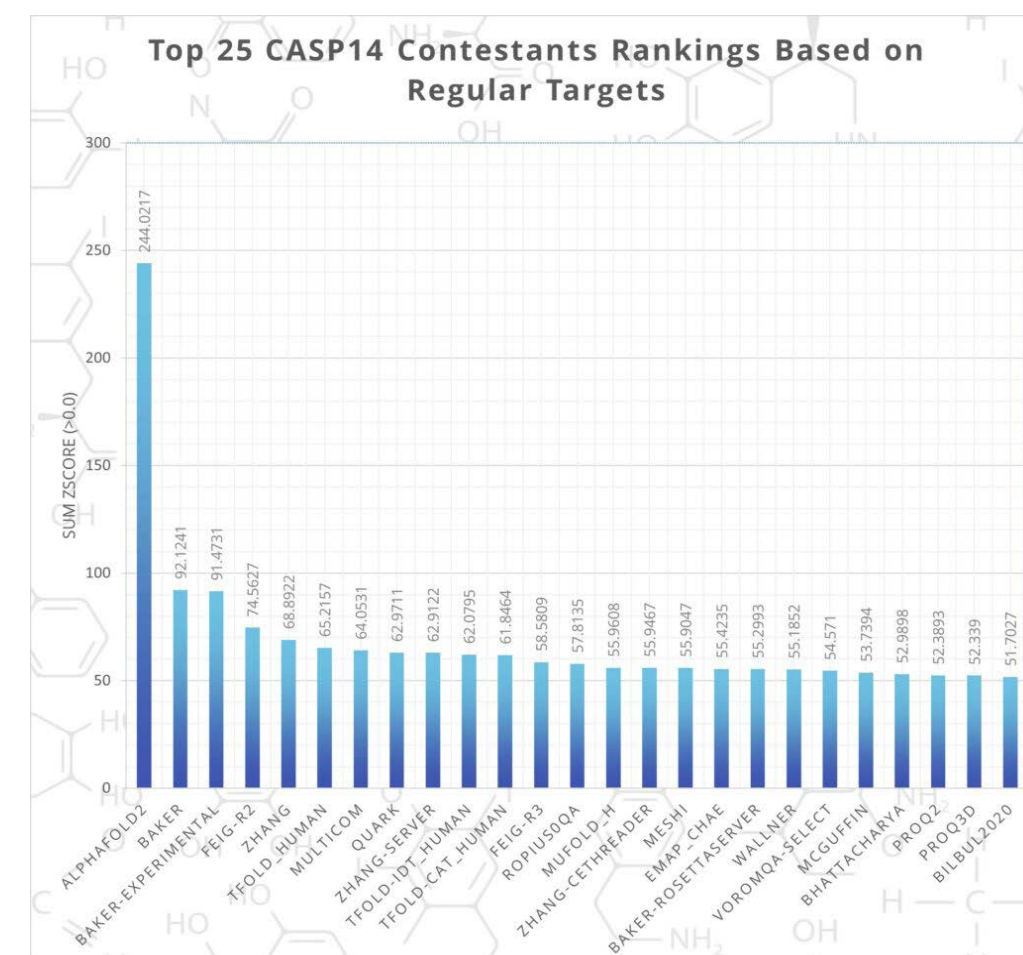
It would be of great use and importance if AlphaFold were to improve toward predicting protein-ligand binding. But still, Oashi is sceptical about whether an AI approach would be the correct method for that kind of prediction. However, they have found a combined approach effective when working with proteins and their structures.

Sam Sinai is a Co-founder and Machine Learning Research Lead at Dyno Therapeutics, which is working on engineering a multimeric 60-monomer protein complex to bind to unknown receptors. Although Sinai's research does not primarily rely on structure models his team uses AlphaFold to investigate regions of proteins in which there is no good crystal structure available.

They use the machine learning tool to see if their modifications to the protein maintain a stable structure. Sinai gave the example of a PLA domain in an AAV they are working on, which is not easily crystallised. His team are using AlphaFold predictions to understand whether the changes they make to its sequence will affect the structure of the protein.

Considering the future of the technology, Sinai commented that "using a machine learning tool like AlphaFold to predict protein-protein binding would be great, but we are far off from being able to reliably address that challenge for a complex multimeric protein complex and potential receptors." He also noted the need to convince collaborators that it would be a good investment of time and resources.

Currently, Sinai's company measures the protein function using high throughput technology after they edit the protein's sequence: "It is still more cost-effective to measure the effects of modifications *in-vitro*." Sinai explained that AlphaFold is fairly incremental in its impact on a multi-faceted problem such as gene delivery.



▲ Figure 1. – CASP14 Rankings, AlphaFold2 Shown as Most Successful. Data from: predictioncenter.org/casp14/

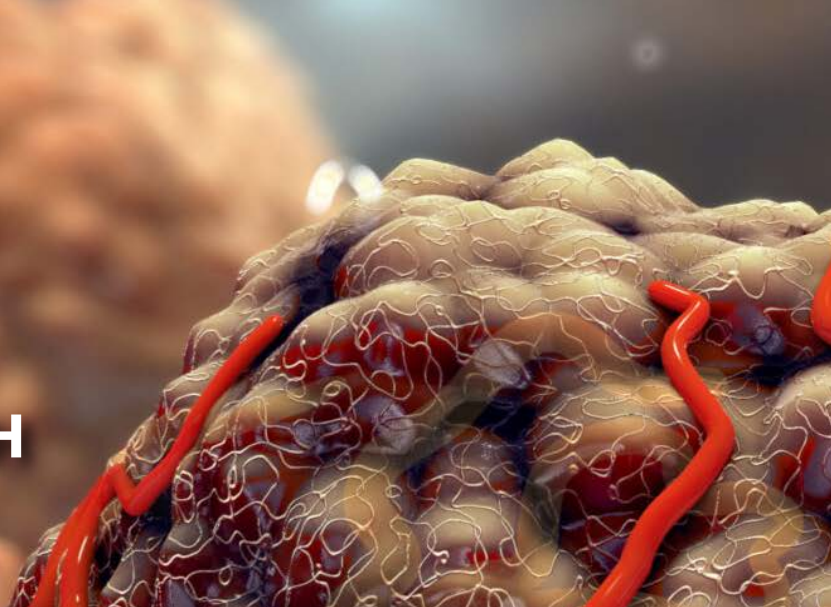
Limitations

Another hurdle is that AlphaFold is trained on already folded structures, which means that it could be too optimistic in folding sequences that are broken. "Sometimes you make a few changes to the sequence, and the protein does not fold properly anymore, but AlphaFold is not telling you that," Sinai added. "Rather, it could approximate the protein back to whatever the closest structure is," he continued. It is unclear if AlphaFold would give you too many false positives to be heavily relied upon for structural predictions at the moment.

The potential of AlphaFold—for the time being—is real, but it hasn't been fully realised yet. Machine learning has the potential to revolutionise how scientists work with the structures of proteins. However, this can make it easy to overlook the fact that the technology is still in its early stages.

Our discussion group members think it will take some time before the computing power and cost-efficiency catches up to make AlphaFold the powerful tool it has the potential to be. However, they are still optimistic about the technology's future.

ORGANOIDS: OPENING NEW DOORS FOR CANCER RESEARCH



Organoids are three-dimensional tissue cultures grown from stem cells. They have been among the most important and exciting developments in medical sciences in the last decade.

Edited by Oliver Picken

Organoids are three-dimensional tissue cultures grown from stem cells. They have been among the most important and exciting developments in medical sciences in the last decade. Their ability to replicate the biology of diseased and healthy organ tissue *in vitro* is helping us understand complicated disease mechanisms and replace faulty cells within the body.

Application and Uses

Organoids allow for the creation of virtually limitless amounts of tissue. Testing of tissues is particularly valuable for rare diseases. Developing treatments for rare diseases is difficult due to small patient populations, an issue that could be dramatically mitigated with organoid use. For example, close to half of all cystic fibrosis patients have the disease due to a rare genetic defect, in many cases only affecting tiny populations and, in some cases, single family lines. The time and money to develop new treatments for each of these genetic defects is sadly not feasible. Fortunately, organoids derived from patients have been used to test if they would respond to existing drugs that would otherwise not be approved due to the expense and risk of administering them to non-receptive patients.

Besides use in rare disease research, organoids have incredible potential for oncology. Tumour organoids open several new doors for cancer research. Compared to other methods, they are better suited to scaling, with the additional advantage of being more translatable to *in vivo* models due to their unique similarities to actual tumours. These applications range from investigating basic disease biology and drug

response screens, to potentially revolutionising the traditional drug development workflow. Using tumour organoids could shift this workflow from a linear to high throughput matrix process, allowing for simultaneous selection of target patient populations and lead candidates.

Patient Derived Organoids

Leo Price, from CrownBio, explains that both healthy tissue and tumour patient-derived organoids can be grown “from the same patient, so basically, we can derive organoids from the healthy tissue, for example from healthy epithelium adjacent to the tumour, and then also have tumour organoids which allows us to run comparative experiments side by side. I think that has a massive benefit compared to other forms of 3D culture, whether it’s spheroids or cell lines, where obviously the healthy tissue is not available”. Several studies have shown that organoid biology more closely reflects patient tumour biology than conventional cell culture. Another advance in *in vitro* to *in vivo* translation is achieved by using the tumour organoids to generate Patient Derived Xenografts (PDX). Here the *in vitro* and *in vivo* tumours are genetically identical.

Testing a new therapeutic in just one tumour model is never sufficient and a typical *in vitro* strategy is used to test in a panel of tumour cell lines. A downside to this approach is that those cell lines are typically generated and propagated through diverse methodologies and with different media. Organoid technology is well standardised and large panels of organoids have been generated using identical procedures. This improves the ability to attribute differential drug responses to specific mutations or sub-classes.

What’s Next for Tumour Organoids?

There are still some challenges ahead for the widespread use of tumour organoids. Last April, at our Organoid Discovery Symposium, we held a panel discussion titled ‘Advancing Organoids in Oncology Drug Development’. One area that

came up repeatedly was the timescale required to grow functional organoids.

Organoids grow much more slowly than traditional cultures, where cancer line lines have been observed doubling in as little as twenty-four hours. This can have a massive impact, particularly in the case of oncology.

Gaston Soria shares his experience, stating that he has “discussed growth rates with several oncologists around the world; they said that would need to get a response within two or three weeks for viability in the clinic.

He continued to explain the challenges with implementing organoid technology “we know that the time frame is quite short for an organoid setup into *ex vivo* drug testing. If we want to move the organoid technology into the *ex vivo* testing of drugs in the clinic, we may need also to explore some hybrid technology. The downside is that may lessen the fidelity of the model but in return give us a bit of speed that we need for cancer patients.”

Leo Price offers an alternative viewpoint, arguing that “These organoids grow slowly, but that is actually a good model of what we see in patients – tumours do not double in cell number every 24 hours like many cell lines. While that makes it a little harder to keep the aggressive timelines that we all are living with, it pays off in the quality of the biology that is allowed to develop.”

Final Thoughts

Organoids have received increasing attention over the past decade and are already showing real-world results. There are high hopes that they will transform our understanding of cancer, unlocking new treatment options and alternative therapies with far fewer side effects than our current methods.

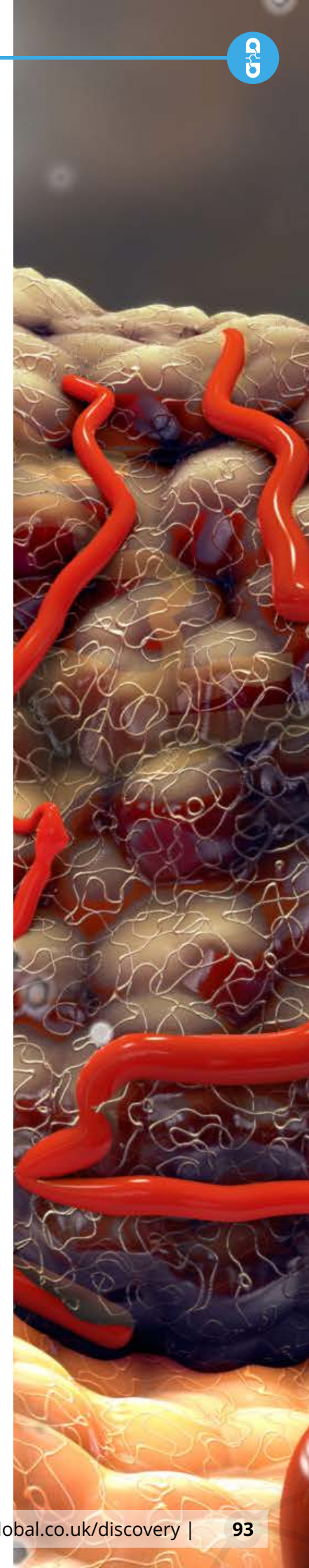
Speaker Biographies

Leo Price – Senior Vice President and head of *in vitro* biology at Crown Bioscience

Leo Price was trained as a cell biologist and has held positions in London, San Diego, Amsterdam and Utrecht in cancer research. From 2005, he ran a research group at Leiden University, where he developed 3D tissue culture-based screening technology. This technology was spun off as Ocello, which offers compound screening and profiling services and since April 2021 is part of Crown Bioscience.

Gastón Soria – Co-Founder & Chief Scientific Officer at OncoPrecision

Gastón has a degree in Genetics and a PhD in Cell Biology from Instituto Leloir and the University of Buenos Aires, including a research internship at the Erasmus Medical Center in the Netherlands. He returned to Argentina in 2012 after conducting his postdoctoral training at the Institut Curie in Paris. Together, these experiences account for 13 years of training in different aspects of cancer research, with focus on DNA replication, DNA repair and epigenetics.



NEXT-GENERATION TARGETED PROTEIN DEGRADATION: BEYOND THE USUAL E3 LIGASES

During Discovery Week 2021, Ian Churcher (Chief Scientific Officer at Amphista Therapeutics Limited) gave a fascinating presentation on recent advancements and novel approaches in protein degradation. This article provides a condensed overview of the key topics covered.

Edited by Oliver Picken

During Discovery Week 2021, Ian Churcher (Chief Scientific Officer at Amphista Therapeutics Limited) gave a fascinating presentation on recent advancements and novel approaches in protein degradation. This article provides a condensed overview of the key topics covered.

History of Protein Degradation

PROTAC (proteolysis targeting chimera)-induced targeted protein degradation (TPD) has become a key area of interest for developing new medicines. As a result, it has and garnered attention from many academic institutions and pharmaceutical companies. PROTACs are small bifunctional molecules which induce ubiquitination of target proteins and employ proteasomes to mediate protein knockdown. It is not an exaggeration to say that PROTACs have opened a new door for novel drug development.

The advancement of the PROTAC field was enabled by the discovery of small-molecule E3 ligase ligands. The most commonly used are CRBN and VHL, followed by MDM2 and IAP, but this is only a tiny fraction of what is possible. The human genome encodes more than 600 varieties of E3 ubiquitin ligase, and there is still a vast area for open to further exploration and development.

Protein degradation using E3 Ligases has had a transformational effect on drug discovery. Catalytic removal of drug target allows exceptionally efficient removal of pathogenic proteins, leading to very high potency effects, both *in vitro* and *in vivo*. There are

a number of molecules using this approach now in the clinic. However, while these current therapies are working well, there is potential for further improvement.

Room for Improvement

Because current drugs are dependent on a very small set of ubiquitin E3 ligases, there are some limitations with the molecules around both the scope that those mechanisms have and the chemistry that they use. The potential for the emergence of tumour resistance is particularly concerning and, while only noted in pre-clinical systems with PROTACs so far, has the potential to lead to reduced duration of clinical efficacy.

We know many targeted oncology treatments will ultimately have problems with resistance and disease progression. But with degraders, there are more opportunities for that resistance to emerge because the degradation mechanism uses a whole range of different cellular proteins, downregulation, deletion, or mutation of any one of which may allow a tumour cell to develop resistance.

The dependence of most PROTACs on a small number of VHL and CRBN-binding warheads with narrow structure-activity relationships also means that the majority of degraders have similar chemical substructures. This, in turn, gives limited chemical options for introduction of desirable chemical properties into PROTACs including oral bioavailability and the ability to enter the CNS. Thus, further research is needed to explore the limits of the degradable area and how to chemically unlock novel ligases.

Cutting Edge Developments: Novel Targeting Methods

Amphista Therapeutics have been exploring exciting new ways to initiate protein degradation. Ian Churcher explains that “we found that there was a number of ways of inducing proximity beyond the usual E3 ligases, in fact, if you look at the ubiquitin-proteasome system, there are a very wide range of different proteins that we could use. First, of course, we have the E3 ligase substrate receptors themselves, and many people are investigating them in a lot of detail. But there’s a lot of other ligase-associated proteins. Indeed, there’s a lot of ubiquitin editing enzymes, such as deubiquitinases. And also, of course, the proteasome itself, where these proteins ultimately go to be destroyed.

We selected a range of these alternative ubiquitin-proteasome systems (UPS) proteins, where small-molecule ligands are known. We then incorporated these ligands into bifunctional molecules which may look a little like PROTACs, but, as they contain our novel warheads, we call them Amphistas. These Amphistas induce the proximity of the target to the cellular degrading machinery leading to potent degradation. We then get all the advantages of a PROTAC approach you would expect in terms of a rapid, selective degradation with long duration of action but, because of the warheads and new degrading mechanisms we’re using, we also see additional advantages across many areas including improved scope of efficacy across a broad range of cells and a good ability to introduce oral bioavailability.”

Conclusion:

Targeted protein degradation has incredible potential to provide numerous novel disease treatments and give new ways to address previously undruggable targets. Current,

first-generation approaches have advanced to the clinic quickly and given a taste of what can be accomplished, and work is underway to unlock further possibilities. The targeted protein degradation field has made rapid progress. Recent developments make a strong case for further investment and research.

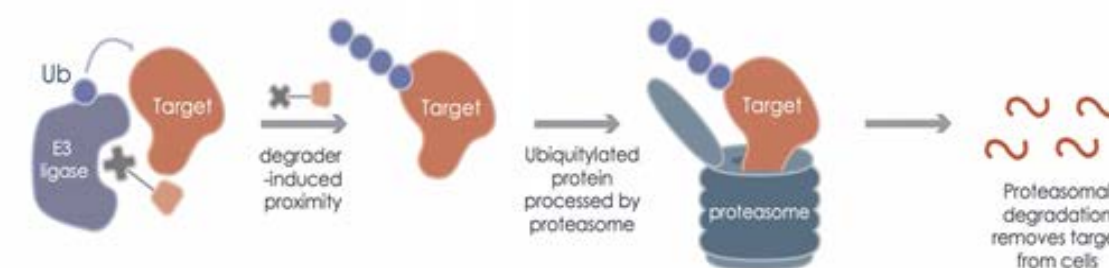
Speaker Biography: Ian Churcher

Chief Scientific Officer at Amphista Therapeutics Limited.

Ian was one of the pioneers of the application of targeted protein degradation to drug discovery as Head of the Protein Degradation Discovery Performance Unit at GSK and through collaboration with Professor Craig Crews (Yale). Ian built a team which demonstrated the viability of the VHL-recruiting PROTAC strategy and advanced a portfolio of projects from early proof of degradation through to clinic-enabling studies.

During a successful career in pharma at Merck & GSK, Ian has contributed to and led a range of discovery projects across many therapy areas from target validation through to lead optimisation and clinical entry. When at GSK Ian also built and led the Fragment-based Drug Design chemistry team as well as other discovery technology areas including chemical biology and compound collection design and acquisition.

More recently, Ian was SVP Drug Discovery at AI biotech BenevolentAI with responsibility for leadership and advancement of a portfolio of drug discovery projects before taking up his current position of CSO at Amphista in 2020. Amphista is using its proprietary next generation protein degrading approaches to advance a portfolio of projects towards the clinic, directly addressing many of the limitations of first generation approaches to further expand the clinical opportunities for the TPD field.



Advantages of TPD in Drug Development

- Catalytic removal of drug target: additional or superior efficacy over inhibitors
- Potent *in vivo* pharmacology - also use vs “underdrugged” targets
- Small molecules – understood development path
- Growing number of PROTAC & glue drugs now in clinic with encouraging initial data

Room for further improvement

- **Reliance on small set of degrading mechanisms:**
- **Scope:** Many tumor cells & targets intrinsically less sensitive to known degrading mechanisms
- **Resistance:** Innate and acquired resistance to PROTACs now a significant concern
- **Druglike properties:** Fixed PROTAC chemistry does not routinely allow eg oral or CNS profiles

THE BIOAVAILABILITY OF ORAL PROTAC MOLECULES FOR ER+ BREAST CANCER

Thomas Hayhow, Associate Principal Scientist at AstraZeneca presents his company's efforts in delivering PROTACs orally.



DISCUSSION GROUP

Edited by Tom Cohen

Estrogen receptor positive (ER+) breast cancer is a type of breast cancer in which its tumour cells have estrogen receptors. In ER+, malignant cells then use the hormone estrogen to grow. Here, E2 ligand estrogen binds to the estrogen receptor and acts as an agonist which activates transcription of downstream genes that are implicated in breast cancer.

The standard method of care for this kind of cancer uses a drug that blocks this mechanism called a fulvestrant. Fulvestrants are selective estrogen receptor degraders (SERDs), agonists that prevent the E2 ligand from binding to the receptor and ultimately degrades the ER.

There are many fulvestrants of this kind that exist as therapies, although they usually involve painful injections and there is no oral substitute. AstraZeneca is one company that have been looking for compounds that could therapeutically use this mechanism with suitable oral bioavailability.

Thomas Hayhow, Associate Principal Scientist at AstraZeneca, presented to Oxford Global's 'Oral PROTAC and PK/PD Profiling' discussion group an account of his team's experience with ER degraders' oral bioavailability. He recounted the development of AstraZeneca's compound AZ'6421, an oral PROTAC with an *in-vitro-in-vivo* disconnect. They have been trying to push towards already existing compounds that antagonise and degrade ERs.

Beyond SERDs and Pursuing PROTACs

The team went out in search of whether PROTACs were viable as an oral drug. They used their earlier SERD (AZD'9496) as a starting point when designing the compound. This was because they knew that it bound to ER nicely. They also used

VHL as the E3 ligase binder, a common choice for PROTACs.

The next task was to investigate what linker they wanted to use to tie the two parts together. Hayhow explained that they had identified a linker that resulted in potent degradation but due to the high hepatocyte level in mouse models, it needed to be improved to be viable for oral delivery.

They named the compound AZ'6421, which showed quick degradation of ER *in-vitro*. To the team's enthusiasm, the evidence showed that the molecule did indeed degrade via a PROTAC-induced mechanism rather than solely SERD degradation. However, there were a few hints of residual SERD-like degradation as well

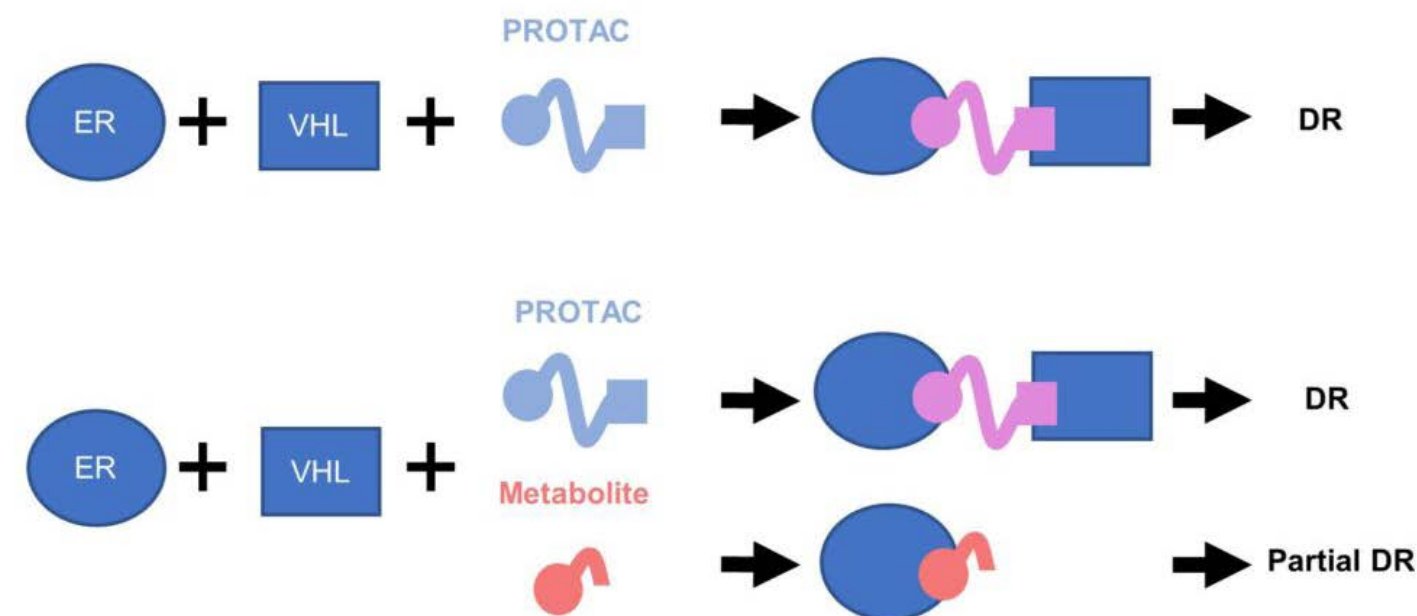
The In-Vivo Problem

However, the AstraZeneca team found unexpected results when testing *in-vivo*. Given the *in-vitro* profiling, they experienced no increase in degradation as dosage increased. As Hayhow said, "*in-vivo* we saw no dose response."

"In-vivo we saw no dose response."

There were a number of issues that the team troubleshooted to work out what was going wrong. They found that the exposure of the drug in the tumour was adequate, the PD effect of the compound was similar, and there were no differential on-target effects such as hypoxia. Therefore, the cause of the problem must be elsewhere.

Hayhow then explained that one theory as to the origin of this problem was that the degrader was experiencing competition from metabolites. Metabolites would curtail the efficiency of their PROTAC as they would take up the ER sites rather than



▲ Figure. 1 – Metabolite reduces ER degradation *in-vivo*.

their compound.

From another troubleshoot, this is indeed what they found. Metabolites were present at much higher free levels than the PROTAC itself and therefore, the metabolites seem to be "completely outcompeting the PROTAC," said Hayhow.

In summary, Hayhow offered the following advice to designers of oral PROTACs: "watch out for metabolites! They can really ruin your PROTAC if you're working *in-vivo*." ER was one of the first PROTAC projects that AstraZeneca had undertaken, perhaps the first ever to go *in-vivo*. Although the compound itself showed some encouraging ER degradation *in-vitro*, it was unfortunately impaired by its metabolite problem *in-vivo*.

Key Parameters for PROTACs to Consider Beyond the Rule of Five

Another attendee raised an interesting question to the group. They asked, when designing PROTACs, what are the key parameters that you need to be aware of in terms of bioavailability? Lipinski's rule of five has been somewhat of a gold-standard for drug design, but does it necessarily translate to the discovery of PROTACs?

"In some ways, I don't think that PROTACs are particularly special molecules other than the fact that

they are a bit bigger than other small molecules"

Hayhow described that there were various publications that have discussed things to keep in mind when designing 'beyond-rule of five' drugs, but says "in some ways, I don't think that PROTACs are particularly special molecules other than the fact that they are a bit bigger than other small molecules."

"So really, trying to stick as close to the rule of five as you can is not a bad place to be," Hayhow continued. Hayhow maintained that keeping the molecular weight and number of rotatable bonds of your molecule as low as possible was good to keep in mind.

Particularly, he stressed that reducing hydrogen bond donors was vital. He gave here the example of VHL, "when embedded, it usually has got two or three hydrogen bond donors which can be enough to shut down bioavailability all-together—putting three of them on an extra-large compound is not putting yourself in good-stead for bioavailability."

— Part VIII —

BIOLOGICS

Accelerating biologics research & development
to optimise their therapeutic potential

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CHARACTERISATION OF THERAPEUTIC OLIGONUCLEOTIDES IN EARLY DRUG DISCOVERY

Oligonucleotides are fast becoming a globally recognised form of therapeutic treatment. But how easy is it to analyse them? Director of AstraZeneca, Tomas Leek takes us through the analytic challenges of the early-stage oligonucleotide characterisation.

Presented by: Tomas Leek, Associate Director at AstraZeneca

Transcribed by: Tia Byer

The Physical and Analytical Chemistry Department at AstraZeneca, provide analytical expertise in the analysis and characterisation of synthetic therapeutic drugs such as oligonucleotides. As part of the Oligonucleotides: Chemistry & Therapeutics Symposium held in April 2021, this article will look at the analytical challenges (LC-MS) in chemically modified ASOs and siRNA and will demonstrate three solution workflows.

Examples of Approved Oligonucleotide Platforms: Antisense Oligonucleotides (ASO) and Small Interfering RNA (siRNA)

Antisense oligonucleotides are typically synthetic single-stranded nucleic strands made up of approximately 18 to 30 nucleotides. ASOs are used for targeted therapy that work by interfering with and suppressing the expression of specific disease-related genes. In turn, they help treat viral infections, cancer growth, and inflammatory diseases.

ASOs hold the capacity for diverse chemical modifications and from an analytical perspective possess an interesting design. For example, in the gapmer design ASOs, the central DNA filled gap is surrounded by chemically modified RNA based flanks. These modifications attempt to protect the internal block from nucleus degradation. Small Interfering RNA (siRNA) represent another promising oligonucleotide platform. The double-stranded siRNA consists of a passenger and guide antisense strand with approximately 20-27 nucleotide length. The analysis of siRNA will not be covered in this presentation.

Analytics Challenges of ASO Impurity Profiling and Chemical Degradation Products:

A vast number of chemical modifications have historically been explored by the academic and industrial therapeutic oligonucleotide community to improve their physicochemical properties and in-vivo PK/PD. Chemically modified ASOs and ASO-conjugates provide a number of challenges to the analytical chemist due to their heterogeneric nature.

Purity analysis and impurity profiling are important check points to support early drug development from an analytical

point and to ensure high quality compounds for in vivo and in vitro assays. The list of commonly detected impurities include for example the synthesis by-products shortmers and longmers, with deleted or additional nucleotides, respectively, next to PS-PO conversions of the phosphorothioate backbone. Depurination of guanine or adenine bases can result from degradation but also from in-source fragmentation. In addition, deamination mainly of cytosine or methyl cytosine can occur, resulting in a mass shift of less than one Dalton. Another level of complexity and further analytical challenge is introduced in ASO-conjugates, due to their linker chemistry and additional targeting moiety attached to the oligonucleotides.

Analytic Workflows: Improving Chemical Characterisation of Naked ASOs and ASO-Conjugates

1.) High Throughput Workflow for Naked ASOs:

In order to support the oligonucleotide synthesis of the ASOs platform, AstraZeneca developed and implemented a high throughput plate analysis workflow for crude synthetic ASOs to confirm product identity and impurity profiling using high-resolution mass spectrometry.

The automated plate analysis workflow includes a short and optimised LC gradient for online desalting and results in co-elution of all ASO-related compounds, followed by UV-MS data acquisition. Deconvoluted MS data is then used to confirm the full-length ASO and to identify impurities from the spectrum fingerprint, facilitated by our newly developed data post-processing tool. This workflow allows a sample throughput of approximately 20 samples per hour.

2.) Ion Pair Separation for ASO-Conjugate Separations:

One strategy to assess the purity of ASOs and ASO-conjugates is to conduct deliberate separation of the sample's content (ASO, targeting moiety and ASO-conjugate) by ion-pairing LC-MS. When tested, significant improvements in chromatography were found, and AstraZeneca could determine the relative content of the full-length ASOs by separating out impurities from the main peak. But by combining information from both UV and MS data purity assessment and impurity analysis of the sample was possible. The main benefit of the ion-pairing approach is that it can be utilised as both a starting point and a pre-screening for further purification.

3.) In-Depth Characterisation of ASO-Conjugates:

An in-depth characterisation workflow with detailed purity and impurity assessment is applied typically for conjugated ASOs prior to in vivo testing. For this analysis, the lab performed method optimisation for each individual conjugate based on the specific physicochemical properties and quantitative information is obtained from integrated extracted ion chromatograms. With this information in hand, an extensive

list of identified impurities can be used for the risk mitigation of the batch. However, this in-depth characterisation requires a greater level of manual and time-consuming assignment and interpretation of the data.

AstraZeneca found that a diverse toolbox is required to best support the ASO and ASO-conjugate platform. For instance, in the early stage, a high-throughput MS without chromatographic separation of naked ASOs is feasible. On the other side, later-stage development relies on in-depth characterisation and impurity profiling of ASOs and ASO-conjugates using ion-pairing chromatography. AstraZeneca concluded that the analysis of ASO-conjugates remains a challenge. Further method development and optimisation are required, particularly regarding automated data analysis tools, differing impurity responses, and the inability to separate all impurities.



Speaker Biography: Tomas Leek Associate Director at AstraZeneca

Leek is an analytical chemist with broad experience in separation science. With a background in chemical engineering, Leek joined AstraZeneca in 1997 as a research scientist. Currently, he is working in a team supporting early preclinical chemistry with analytical science. Over the years, Leek has gained both practical and theoretical experience from MS-hyphenated (UP) LC, GC and SFC separations in GLP/GLS environments.

HARNESSING BISPECIFIC T CELL ENGAGERS AND T CELL PRIMERS IN CANCER TREATMENT

Stimulating T cells using bispecific antibody immunotherapies can lead to transformational clinical benefits for cancer patients. So how exactly is the industry addressing the need for targeted T cell-based immunotherapies?

Edited by Tia Byer

T cell-based bispecific antibody immunotherapies have reshaped treatment paradigms across various cancer types. The global bispecific antibody market is expected to increase to 25 billion USD by 2028, with the number of commercially available products projected to increase by 150% over the next 5 to 6 years. Harnessing the capabilities of immune cells, especially T cells, to enhance anti-tumour activity is a widely adopted strategy in the clinical management of cancer. However, challenges remain, as a wide cross-section of the population do not respond to pre-established cancer treatments, with tumours either refracting or developing resistance. There are also economic and logistical hurdles associated with scaling-up and considerable risk in systematic stimulation, which can lead to toxicity. Nevertheless, stimulating T cells can lead to transformational clinical benefits for cancer patients. Current trends in development include redirecting, reinvigorating, and activating T cells for optimal results. Key players in the field include Abbvie, Alligator Biosciences, Amgen, Bristol-Myers Squibb, F-star, Genmab, Ichnos, Janssen, Regeneron, and Roche.

Bispecific T Cell Engagers – Background and Industry Overview:

Bispecific antibodies are highly potent molecules that facilitate T cell-mediated killing of tumour cells. Simultaneously binding to tumour antigen and CD3ε chains results in the activation of the T cell receptor. Subsequent T cell engagement includes the activation and killing of tumour cells by releasing cytotoxic granules. T cells start to proliferate and expand at the activation site, releasing cytokine and chemokine, which leads to the recruitment of additional T cells. Christian Klein, Site Head Roche Innovation Centre Zurich, explains how “T cell bispecifics are very potent and can mediate so-called serial killing of tumour cells”. He continued, “notably, they can be independent of specificity and the action and differentiation status of T cells”. A major advantage of T cell bispecific antibodies is that they can overcome the various escape mechanisms of cancer cells.

“The field has now essentially moved on to IgG-based T cell bispecific antibodies, with numerous platforms in clinical trials across the industry,” Klein continued. Upcoming platforms include Amgen’s Fc-BiTE, classical 1+1 IgG based T cell bispecifics like Genentech’s TDB platform applied in Mosunetuzumab and Cevostamab, Genmab’s Duobody platform used for example in

Epcoritamab with Abbvie or in Teclistamab and Talquetamb with Janssen, Regeneron’s common light chain 1+1 IgG applied to Odronextamab, and an IgG-scFv platform applied for instance by Xencor for Plamotamab with Janssen. Roche has developed its own differentiated 2+1 TCB platform applied e.g. for development of glofitamab and cibisatamab making use of bivalent binding to tumour antigens.

Peter Ellmark, Chief Scientific Officer at Alligator Bioscience concurs, explaining how...

“there is a great and rapidly increasing need for ... therapies that can expand the number and quality of tumour infiltrating T cells”.

According to Klein, “the interesting aspect of the different T cell bispecific antibody formats making their way to the clinic, is that they essentially enable you to mimic CAR T cell functioning by using off the shelf reagents”. Peter Ellmark, Chief Scientific Officer at Alligator Bioscience concurs, explaining how, “there is a great and rapidly increasing need for such therapies that can expand the number and quality of tumour infiltrating T cells”.

A Promising Landscape: 3 Industry Case-Studies

From T cell priming, redirecting activated T cells, and endogenous and synthetic immunity, the possibilities are numerous. To find out more, we sat down with 3 leading pharmaceutical companies paving the way with their novel T-cell bispecific antibodies treatment strategies.

1.) Roche: ‘Roche-Genentech Cancer Immunotherapy Strategy’

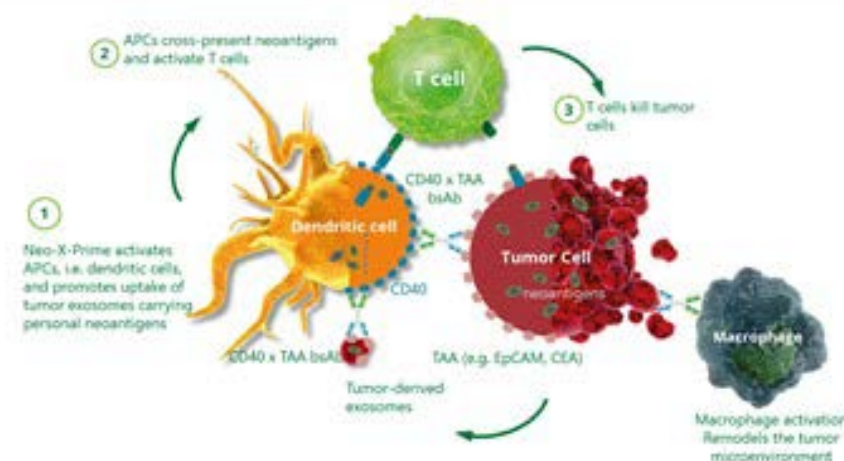
Founded in 1896, Roche is a Swiss multinational healthcare leader that focuses on delivering life-saving pharmaceuticals and cutting-edge diagnostics. Roche-Genentech’s strategy relies on a multi-functional approach to cancer immunotherapy, which makes use of **endogenous immunity approaches**, that rely on pre-existing T cells that have been formed by the body’s immune system to attack the cancer for example by blocking the PD-L1 checkpoint inhibitor or other notable checkpoint inhibitors including TIGIT (tiragolumab), or by simultaneously blocking PD-1 and LAG-3 with a bispecific antibody.

In addition, the synthetic immunity approach is a direction in which Roche and Genentech have various T cell engaging bispecific antibodies in development such as cibisatamab, glofitamab, mosunetuzumab or cevostamab. Roche’s portfolio also harnesses targeted immunomodulators to further boost the activity of T cells, and examples of this include FAP-4-1BBL and CD19-4-1BBL fusion proteins as well as a PD1-targeted IL2v immunocytokine. With multiple engineered antibody projects in the clinical study phase, Roche is set to make big waves in the immunotherapy field.

2.) Alligator Biosciences: ‘Neo-X-Prime’

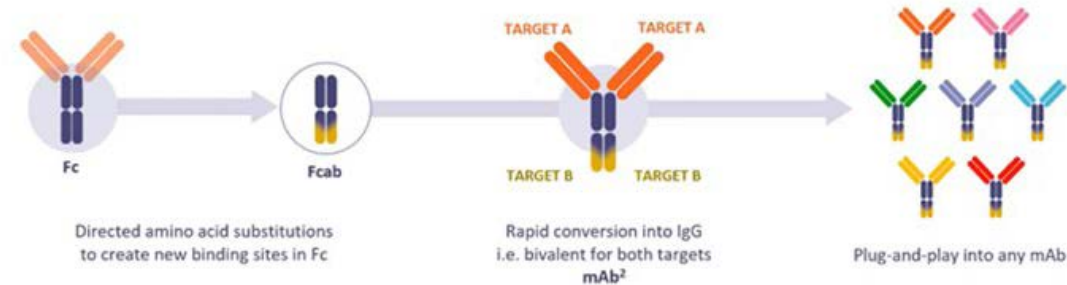
Alligator Biosciences is a Swedish-based clinical-stage biotechnology company entirely focused on immuno-oncology since 2008. Their pipeline includes novel mono- and bispecific antibody technology platforms and have been listed on the Nasdaq Stockholm exchange since 2016. Neo-X-Prime is a neoantigen cross-presentation-inducing bispecific CD40 antibody created by Alligator Biosciences to enhance tumour-specific T cell priming. It is a tetravalent format consisting of two CD40 binding domains and two tumour-antigens. The Fc part of the bispecific is a silenced IgG1 which, according to Ellmark, “is critical when trying to avoid any interaction with Fc

Mode of action of Neo-X-Prime





F-star's Proprietary Technology: Bispecific IgG with Dual Bivalency



gamma receptors". The CD 40 agonist in this antibody depends on cross-linking to induce a strong immune activation, meaning that it will only activate CD 40 when it binds to tumour antigens simultaneously.

Alligator's first-generation Neo-X compound is a technology platform designed and developed to enhance the priming, quantity, and quality of tumour specific T cells. Ellmark explains how "Neo-X provides an opportunity to drive the expansion of T cells which allows for treatment of indications that either has primary or secondary resistance to PD-1 and other checkpoint inhibitors". Additionally, the CD40 antibodies act on myeloid cells and macrophages and can potentially remodel the tumour microenvironment. By mediating cancer activity, Neo-X activates dendritic cells and promotes the uptake of tumour exosomes carrying personal neoantigens. APCs then cross-present neoantigens and activate T cells which leads to the killing of tumour cells. CD40 was identified as the optimal DC target for inducing strong T cell internalisation and priming to tumour antigens with improved functional activity discernible when linked to the antigen.

3.) F-star Therapeutics: 'FS222'

F-star is a clinical stage biopharmaceutical company founded in 2006 with an advanced pipeline of multiple immuno-oncology programs dedicated to creating a paradigm shift in cancer therapy. Its proprietary technology introduces mutations into the Fc domain of an antibody to then generate an Fc antigen-binding domain or 'Fcab'. By subcloning the Fcab into a standard monoclonal antibody construct, a bispecific antibody, termed a mAb2 antibody, is established, which maintains the bivalent nature of the Fab arm and introduces the dual Fcab binding domains.

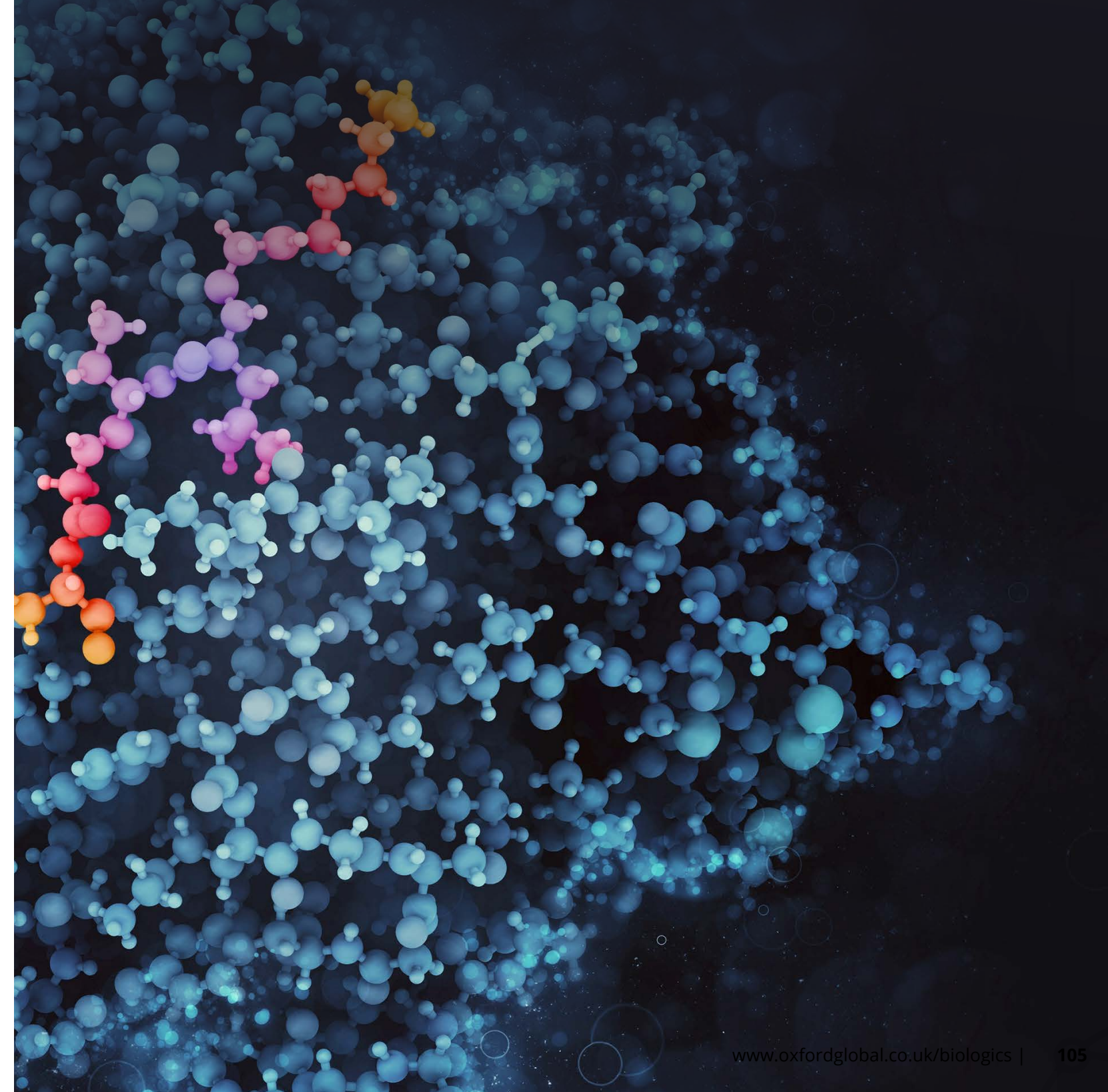
In the coming years, we can expect to witness new product launches globally and favourable policies implemented to aid in the fight against cancer.

FS222 is a clinical-stage programme and tetravalent bispecific antibody designed for optimal T cell activation with minimal toxicity. Ryan Fiehler, Associate Director of Discovery at F-star, stated that "the purpose of FS222 is to redirect activated T cells to the tumour". FS222 simultaneously "releases the brake" on immune control of cancer by blocking the PD-1/PD-L1 pathway and "hits the gas" on immune activation by stimulating the CD137 pathway. The bispecific antibody also induces potent PD-L1-dependent T cell activation, and according to Fiehler, "shows strong anti-tumour activity in multiple models".

What is most promising about FS222 is the potential to avoid dose-limiting hepatotoxicity at therapeutic doses. In designing the technology platform, F-star established a mechanism of action that harnesses, as Fiehler explained, "the three C's: crosslinking, clustering, and conditionality". Resulting T-cell activation, proliferation, and eventual redirection occur to ensure tumour killing disease control. So far, F-star has found that FS222 bound CD137 and PD-L1 simultaneously and tetravalently to elicit potent in vitro T cell activation only in the presence of PD-L1. Studies using different valency combinations showed that tetravalent binding provided superior activity in terms of efficacy and potency in in-vitro assays. Therefore, FS222 presents a unique opportunity versus high affinity CD137 binding bispecific molecules by opening a broad therapeutic window.

Future Outlooks:

With a crowded pipeline, the bispecific antibody market will continue to grow at a double-digit rate. The rise in the number of cancer and chronic diseases, along with various government initiatives and cross-industry collaborations will see the field go (and grow) from strength to strength. In the coming years, we can expect to witness new product launches globally and favourable policies implemented to aid in the fight against cancer. At Oxford Global, we have our eyes firmly peeled and look forward to the latest developments as they unfold.



A NEW CANCER-KILLING BISPECIFIC ANTIBODY WORKING TOWARD THE CLINIC IN 2022

The joint development project by Alligator Bioscience and Aptevo Therapeutics, ALG.APV-527, shows potential in targeting solid tumours.

Edited by Tom Cohen

Joining us on day one of our Biologics UK 2021 conference, Principal Scientist Dr Anette Sundstedt of Alligator Bioscience gave a presentation to Oxford Global. In the presentation, Dr Sundstedt talked about Alligator Bioscience's joint development project with Aptevo Therapeutics for cancer immunotherapy. The new drug, ALG.APV-527 is a 4-1BB and tumor-binding bispecific antibody. 4-1BB has the ability to stimulate the immune cells (anti-tumor specific T cells) involved in tumor control, making 4-1BB a particularly compelling target for cancer immunotherapy.

Potential for Enhanced Potency and Safety

The new drug targets the antigen 5T4, which is expressed in several solid tumour malignancies but has minimal expression in normal tissue. This gives ALG.APV-527 the potential to treat solid tumour indications such as non-small cell lung cancer, head and neck cancer, mesothelioma and others. As an immune cell engager, ALG.APV-527 also targets the costimulatory receptor 4-1BB to activate tumour specific T cells and NK cells in the tumour microenvironment. Importantly, the opportunity to reactivate exhausted tumour specific T cells provides an opportunity to address a key need in immunology. ALG.APV-527 enhances the function of these

immune cells only when in the presence of 5T4 expressing tumour cells, which Dr Sundstedt says "is meant to limit systemic immune activation and liver toxicity which has been observed with other 4-1BB agonistic antibodies."

Previous antibodies that target 4-1BB receptors have struggled with the balance between effectiveness and toleration in the human body. Urelumab, another 4-1BB targeting cancer immunotherapy, was discontinued in phase II trials due to its liver toxicity in doses above 0.3 mg/kg and poor efficacy below that dose. Its contemporary, utomilumab, had the opposite problem; while well tolerated, its biological activity was lacking. ALG.APV-527 was designed to solve these issues and the preclinical data package strongly suggests that this has been achieved. "So far ALG.APV-527 has demonstrated potent anti-tumour effects as well as a good safety profile in pre-clinical settings," said Dr Sundstedt.

In vivo Pre-Clinical Evaluations

Dr Sundstedt's presentation also outlined the findings of in vivo pre-clinical experiments trialling the new drug. The experiments were conducted in tumour models where it was clearly demonstrated that ALG.APV-527 successfully targeted 5T4 expressing tumours.

Experiments also tested the memory response of the immune system after tumour rejection induced by ALG.APV-527

treatment. The memory response was found to induce a broad anti-tumour response as it was found to eradicate both 5T4 expressing and non-expressing tumours, which is in line with the mode of action of the drug and indicates that tumour specific T cells has been (re)-activated by the treatment.

ALG.APV-527 was also tested in comparison to urelumab to assess the toxicity of the new drug. Their findings showed that high doses of urelumab resulted in systemic T cell activation, ulcerative dermatitis, and liver inflammation, while high doses of ALG.APV-527 did not present these effects.

Plans for 2022 Development

Alligator and Aptevo, in their press release on November 12, 2021, announced that ALG.APV-527 is advancing toward the submission of an IND to the U.S. Food and Drug Administration and the initiation of phase I clinical trials. The study will plan to enrol patients with solid tumour malignancies that are likely to express 5T4. The design of the trial will allow to assess safety, pharmacokinetics and preliminary antitumour activity of escalating doses of ALG.APV-527. Additional details of the trial have not yet been finalized.

About ALG.APV-527

ALG.APV-527 is a bispecific antibody with an interesting compound profile and has demonstrated effective cancer-cell killing in pre-clinical models with tumours expressing 5T4. Although still in pre-clinical stages, Alligator Bioscience and Aptevo Therapeutics are planning to advance ALG.APV-527 into clinical trials later this year. The in vivo studies so far show promise and suggest a differentiated profile compared to previous 4-1BB monoclonal antibodies. The phase I clinical trial of ALG.APV-527 will not only assess safety but also determine the dosing regimen as well as exploring activity in several tumour indications likely to express 5T4.

Speaker Biography: Anette Sundstedt

Anette Sundstedt has almost 20 years of experience in research and development in the pharmaceutical/biotech industry with a major focus in immunology and immunotherapy. Previous roles as CSO of Idogen AB and senior research positions at Active Biotech AB and the University of Bristol. Anette has extensive experience of scientific leadership both as project leader and as a project team member with the responsibility for leading biological and pharmacological research in pre-clinical and clinical immunology projects. She holds a M.Sc. in cellular and molecular biology and a Ph.D. in tumor immunology from Lund University.



ADVANCED ANTIBODY ENGINEERING: AI, ML, AND COMPUTATIONAL TOOLS



Oxford Global's Discussion Groups provide a focused networking opportunity for a handful of like-minded experts on a monthly basis. Our April Discussion Group addressing 'Accelerating Antibody Engineering With AI/ML & Advanced Computational Tools and Technologies' brought together an exclusive panel of key opinion leaders to address the market's growth and future outlook.

Edited by Tia Byer

Our April group came together for an hour of specialist discussion about the current state and future directions of antibody engineering with advanced computational technologies. This Discussion Group was a select group of key industry leaders from various pharmaceutical and biotech companies. Notable attendees were senior representatives from GlaxoSmithKline, Pieris Pharmaceuticals, Lundbeck, and Zoetis, to name a few.

Philip M. Kim, Professor at The Donnelly Centre for Cellular and Biomolecular Research at the University of Toronto, led the discussion, whilst Slobodanka Dina Manceva, Senior Scientist at Teva Pharm and W. Blaine Stine, Senior Director, AbbVie, were there to support. Key discussion topics included best practices for implementing Machine Learning (ML) and Artificial Intelligence (AI) and computational tools to harness the development of next-generation antibody therapeutics.

Design of Epitope-Specific Antibodies with Machine Learning Methods:

Kim kicked off the session with a brief overview of the current industry strategies for computational antibody design. Entitled 'Design of Epitope-Specific Antibodies with Machine Learning Methods,' the presentation alluded to optimised therapeutic

model design using ML. Kim opened with the following sentiment: "we live in an exciting time where data availability, computing resources, and novel artificial intelligence methods enable fully de novo design for biologics."

Protein design as a field has come a long way since the 90s. The field relies heavily on three advances of the past decade: data, technology advances in hardware, and artificial intelligence. "We live in an era now where we depend increasingly on physics-inspired models being superseded by data-driven models based on AI and neural networks that capture integral relationships," Kim continued.

Advances in data availability can be channelled into the design of antibodies. Kim identified three integral components of the machinery employed by his lab to determine the parallel structure and sequence models for the identification of peptide binding sites (see figure. 1). Components include transfer learning, graph neural networks, and Attention.

"We live in an exciting time where data availability, computing resources, and novel artificial intelligence methods enable fully de novo design for biologics"

BIOLOGICS

According to Kim, "transfer learning can be used to compensation for peptide-protein data scarcity." Transfer learning can be applied in two ways: pre-training the model on protein-fragment protein complexes and embedding protein sequences with contextualised language models. Speaking on the advantages of graph neural networks, Kim claimed that "graphs are a natural way to represent molecular structure." They are both rotation and translation invariant and do not require discretisation.

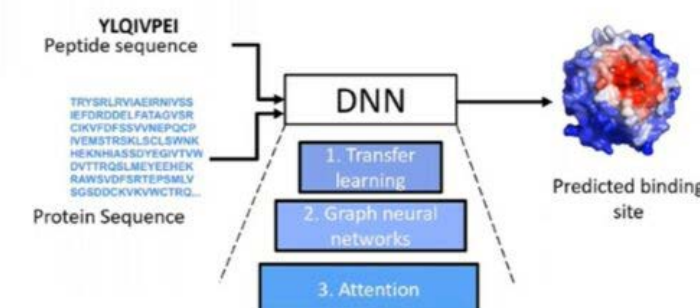
Finally, Attention modules are adapted from the graph input to identify long-range dependencies in sequence inputs. The Kim lab's developed model is primarily composed of attention modules. Its three utilities include learning sequence context in the peptide, learning sequence and structural context in the protein, and learning the relationships between peptide and protein residues. As Kim puts it, "Attention is one of the most elegant ways to capture structural features."

Application Findings: Model Architecture:

The three-component method is then used to initiate Reciprocal Attention, a novel AI feature that captures peptide-

Modern deep learning methods for structural biology: Transfer learning, graph approaches and attention

Parallel structure and sequence models for the identification of peptide/Ab binding sites

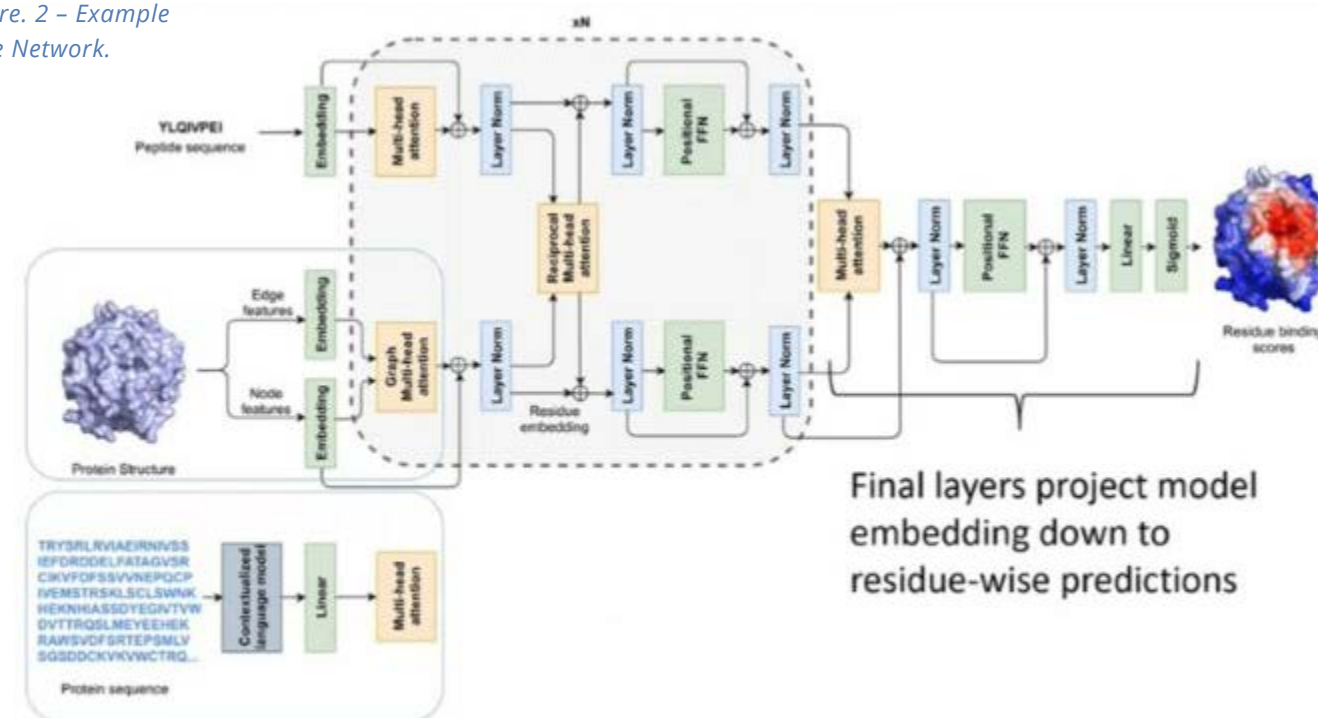


▲ Figure. 1 – Deep Learning Methods for Identification of Peptide Binding Sites. Source: Abdin, Wen and Kim. Communications Biology (under review, also on biorxiv)

protein and protein-protein interactions (figure. 2). Reciprocal Attention allows information to flow in dual directions, from the peptide to the protein and back from the protein to the peptide.

Kim conceptualised the model workflow as consisting of "self-attention layers which allow the model to learn from sequence and structural context." The Reciprocal Attention module then updates protein and peptide embedding while enforcing symmetry. The final layers project model then embeds down to residue-wise predications.

▼ Figure. 2 – Example Peptide Network.





Applications for AI in Antibody Development:

After Kim's presentation concluded, Stine opened the floor to discuss the biggest opportunities for computational AI and ML approaches for discovering new therapeutic biologics. He asked, "what are the most practical applications right now for problem-solving for these emerging methods today? And what can we expect to see on the horizon?" Kim identified the most effective application as being library design for language models. Audience members agreed, with some attesting to its maturation and evident commodification.

Pipeline applications were identified as de novo antibody design in their potential to open a new target space within the field. De novo design refers to the computational procedures used to yield the antibody binding sequence from a target epitope. Kim remarked that one of the most compelling aspects of de novo design is its relative ease of application.

One audience member queried whether "de novo design is still possible in instances of less well-defined loop structures?" The panel agreed that whilst conceptually it is possible, statistical evidence remains insufficient. Possible workarounds include targeting a set of main structural clusters and relying upon a single selection.

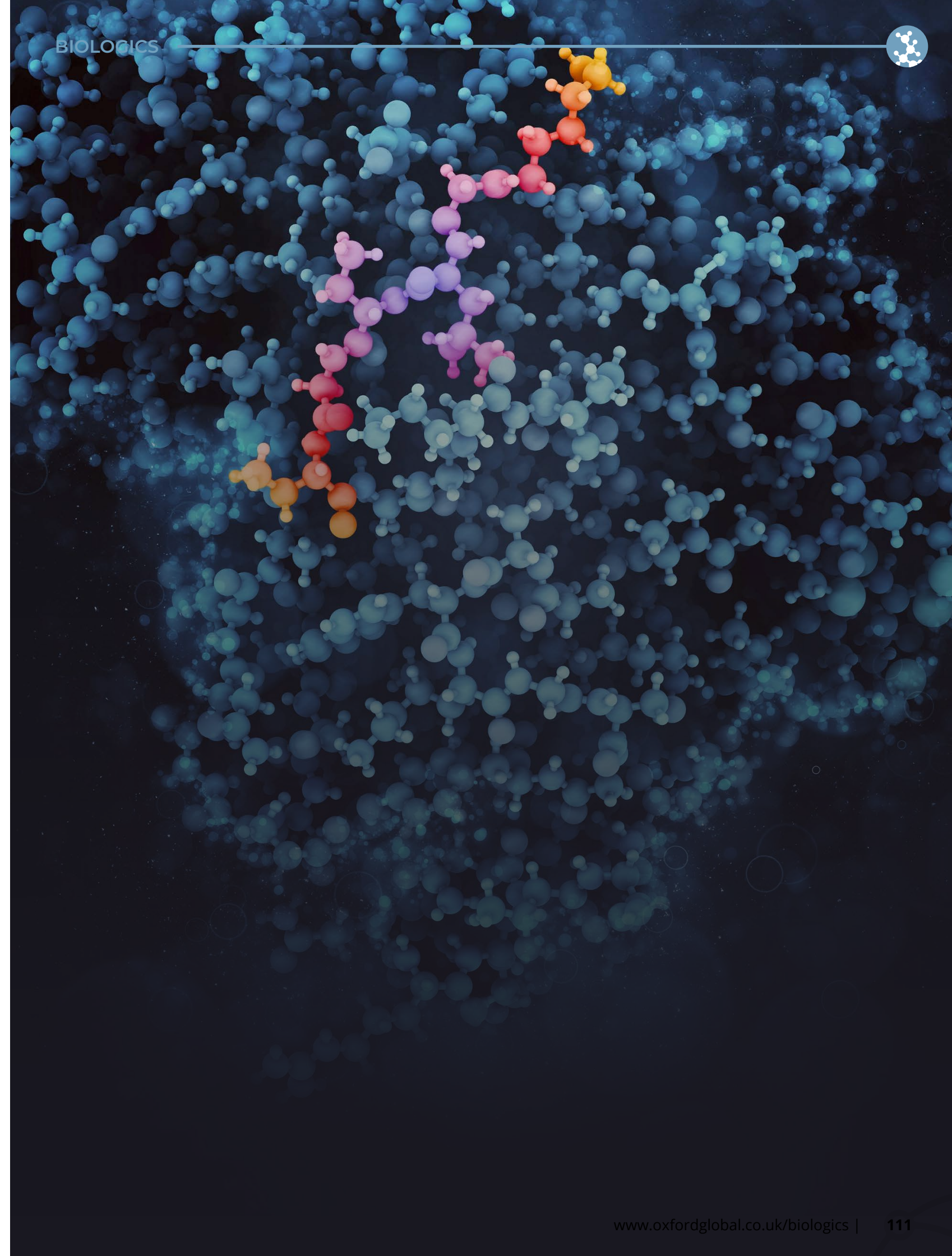
Data Management for Computational Biologics Design:

A further audience member inquired about available applications to manage data output. The said party explained their predicament as follows: "when we apply a similar next-generation sequencing approach, we usually end up with a lot of data, but there is a limit to what we can actually go and test in our lab". Whilst Manceva indicated the value of large data availability, she acknowledged that showing proof of concept early on creates a streamlined process which can help to reduce data volume created during extensive testing.

Kim remarked that one of the most compelling aspects of de novo design is its relative ease of application.

Stine further suggested the potential value of "pushing computational methods to acquire smaller sets of antibodies and by extension guarantee production and characterisation". Additionally, using small-scale methods could facilitate rapid iteration and data generation. This, in turn, provides a method of learning, allowing the model to automatically retrain and redesign accordingly.

The discussion concluded with some final thoughts on the future of AI and ML design for antibody engineering. With the promise of alpha fold technology and an ever-expanding array of biotech start-ups entering the arena, the field of antibody-based therapeutics is certainly catching momentum. At Oxford Global, we couldn't have been more pleased with the turnout for our April biologics Discussion Group. The conversation was engaging, the debate stimulating, and the industry insights invaluable.



PEPTIDES CONGRESS: MARKET TRENDS AND INDUSTRY CHALLENGES

What are the key market trends and challenges facing the Peptides therapeutic industry today? We sat down with an advisory group of leading pharmaceutical and academic experts to discuss our latest Peptides Congress and the interesting insights it disclosed.

Edited by Tia Byer

Between the 26th and 28th of April last year, Oxford Global held its annual Peptides Congress as part of its Biologics Europe Series Online Event. The event was co-located with the Proteins & Antibodies Congress, and Oligonucleotides Symposium. Running from the 26th to the 27th the Peptides Congress provided an in-depth look at the critical developments in peptide synthesis, chemistry, analytics, and novel disease treatments. The virtual event showcased a range of pharmaceutical and biotech industry leaders, as well as expert academic speakers. Advisory board members consisted of key figureheads from Nimble Therapeutics, Boehringer Ingelheim, Sanofi, and Almac Group, and principal academics from the University College Dublin, University of Toronto, and Ulster University. In the post-event report, we have gathered some highlights from the events, including a summary of the key topic area discussed, and the major trends and challenges facing the peptide industry today.

The Event – Key Topic Areas:

Key topic areas covered in Day 1 of the Peptides Congress focussed on peptide chemistry, analytical development, formulation, and delivery. Featured presentations discussed advances in peptide purifications as well as accelerated peptide synthesis. Gyros Protein Technologies' presentation on how peptide-based epitopes and therapeutics are synthesised for COVID-19 provided a fascinating insight

into peptides chemistry. The talk, 'Considerations For Optimising High-Throughput Synthesis Of SARS-CoV-2 Peptides For Epitope Analysis', racked in an impressive 94 views, making it the most popular session of Day 1 and the entire Biologics Eu event. Bachem's spotlight presentation on their latest innovations in engineering microspheres and nanoparticles for peptide delivery also attracted a considerable audience. The 'Spotlight On New Technologies' session received 80 + views and touched on Bachem's molecule Hiving technologies, as well as their latest venture into process developments of oligonucleotides and NCEs.

Day 2 provided an in-depth look at numerous peptide therapeutic case studies and novel disease treatments. Featured presentations addressed the complexities of personalised cancer vaccine manufacturing as well as the development of De Novo peptide design. However, the two most popular presentations covered immune cell targeting and bioactive peptides. Bicycle Therapeutics, talk 'A Fully Synthetic Tumor Targeted Immune Cell Agonist (TICATM) Engages CD137 To Induce Immunologic Elimination Of Nectin-4 Expressing Tumors' was led by its Vice President of Chemistry and received 73 views. It discussed the possibility of using immune cell targets and synthetic chemistry to assemble tumour targeted immune cell agonists. The second most-watched presentation of the day, with 61 views, was the University of Queensland's 'Discovery Of Bioactive Peptides And Peptide-Based Drug Design'. In this Professor David Craik examined the use of crop plants as expression systems for the production of



pharmaceutically active cyclotides.

Market Trends: Formulation Frontiers, Trending Technologies, and Sustainability in Manufacturing

The peptide therapeutics industry is currently estimated to reach over \$ 46 billion by the end of 2025. Despite its increasing number of commercial successes over the past few years, our board members believe the full potential of the industry has yet to be unleashed. During the post-event advisory meeting, board members discussed and highlighted some of the expected key market trends currently facing peptide therapeutics.

Orally Available Peptide:

Formulating orally available peptides has been one of the big scientific breakthroughs of the past couple of years. Along with protein therapeutics, many peptides require parenteral administration to avoid degradation by stomach enzymes. Parenteral administration refers to non-oral administration techniques such as injections. Approximately 95% of all peptides are delivered parenterally, meaning there are currently only 4 drug products that are being administered orally. As injections can be a big deterrent for patients, oral delivery has become especially desirable and extensive efforts have been made to develop more 'consumer-friendly' delivery strategies. On such exciting development includes the advances made in semaglutide delivery. Semaglutide is a peptide-drug used to treat diabetes and obesity. Previously it was only available as an injection however in 2020 its oral use was approved for market.

Technological Developments:

Other advances in delivery include new technologies such as pumps. ALZET's Osmotic model administer peptide therapeutics by pumping them around the body and can, with continuous delivery, maintain an effective level of short half-life proteins. Pump technologies possess several advantages over conventional delivery methods. With injections, compounds can be eliminated rapidly by the body, which affects the variation level of recombinant proteins in plasma and tissue. Pump administration, on the other hand, produces valuable tissue-specific effectiveness and minimises toxicity. Whilst the ALZET model has been around since the 1970s and pertains only to animal studies, its human application is up and coming. Device makers are also working on the development of microneedles for painless injections and medicinal patches.

Making Manufacturing Greener:

Looking ahead we can expect to see the implementation of green initiatives for peptide manufacturing. Peptide synthesis is a complex and arduous process associated with a significant risk of failure. Failure during the Phase 1 or 2 batches can cost up to \$5.5 million and non-surprisingly produces immense amounts of waste. Besides, current state-of-the-art peptide synthesis employs high doses of reagents and solvents,

both of which are extremely hazardous to the environment and ecosystems. Using flow chemistry during discovery and development could provide a solution to this. Flow chemistry lends itself to potentially greener peptide modifications such as reducing the risk of excess during solid-phase reduction and reducing excess amino acids. This in turn makes for a more cost-effective approach to peptide therapeutics.

Challenges: Discovery, Developability, and Manufacturing

Regulations: One of the key challenges scientists face whilst creating peptide therapeutics in clinical trials is regulation. The two chief regulatory bodies are the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA). Currently, there appears to be a lag between the two committees and the regulations they produce. This is especially the case with regulations concerning synthetic peptides; the EMA having already set clear directives and the FDA not. Uncertainty coupled with the need to control individual impurities within peptides makes for a laborious and variable pre-clinical stage testing. The FDA is also yet to publish certain directives on isomeric impurities.

Increasing Bioavailability: Bioavailability refers to the extent a drug is made available to its targeted biological destination. This is measured by both the rate and fraction of the dose that reaches its intended target. Bioavailability also determines the active effect of the drug. Oral peptides such as semaglutide and octreotide have a bioavailability reading of 1 %. Low bioavailability is a problem for oral drugs as they by nature must work extra hard to overcome and resist the gastrointestinal environment. The stomach acts as a physical and biochemical barrier for the absorption of peptides which results in low bioavailability and increased risk of inefficacy. To circumvent this, bioavailability values need to increase to around 5% or 10%. And whilst certain devices claim to achieve this, the issue is whether there will be a huge toxicity price to pay.

Upscaling and Cost: As the field of peptide therapeutics rapidly expands, the need to upscale will become paramount. In addition to this, the level of peptide complexity being produced is increasing. Industry growth presents several logistical problems, however. Reducing the cost of manufacturing will prove difficult due to the competitive nature of the commercial process. A possible novel solution could be to produce cleaner peptides, which then reduces the time required time and effort required for purification. For this, manufacturers will have to introduce alternative solvents and alternative technologies, which in themselves will take time to streamline.

These market trends and the important insights provided by the advisory board will inform the structure and content of the next annual Peptides Congress held as part of the Biologics Europe event.

Speaker Biographies

**Lukasz Frankiewicz,
Senior Product Specialist
(EMEA) at Gyros Protein
Technologie**

Frankiewicz has close to 20 years experience working in Peptide Chemistry and has obtained his PhD from the University of Warsaw. He has worked as a Post Doc in Belgium and with universities and private companies in Dublin, Ireland. Frankiewicz has also been involved in Project Management of the synthesis of peptides, peptide libraries and the chemical synthesis of proteins with a company in France.



**Seamus White, Business
Development Manager at
Bachem**

Seamus White is a Business Development Manager for Peptides and Oligonucleotide and has over 20 years working in the Pharma and CMO industry. He specialises in HiPo Oncology API, Peptide and ADC warhead and has experience leading SHE and Management Systems Auditor initiatives. White holds a BSc in Chemistry and Pharmacology, and a MCIPs from the Chartered Institute Procurement Supply.



**Claudia Murar, Business
Development Manager
at Bachem**

Claudia Murar, PhD, first joined Bachem in 2018 and has since been made Business Development Manager of new chemical entities, peptides and oligonucleotides. Previously Murar worked as a Pioneer Fellow at ETH Zurich University where she conducted research on developing breakthroughs in the fields of oncology and autoimmune disease. She received her PhD at ETH Zurich and also spent time studying in France, Japan, the UK and the US.



**Kevin McDonnell, Vice
President at Bicycle
Therapeutics**

As Vice President of Chemistry for Bicycle Therapeutics, Dr. McDonnell is responsible for advancing bicyclic peptide therapeutics in the oncology discovery programs, with a focus on immuno-oncology. He began his career developing FcRn targeted peptides and Fc fusion protein therapeutics at Syntonix Pharmaceuticals and Biogen. obtained his PhD with Prof. Barbara Imperiali at MIT. McDonnell has previously worked for BIND Therapeutics where he led the discovery chemistry efforts.



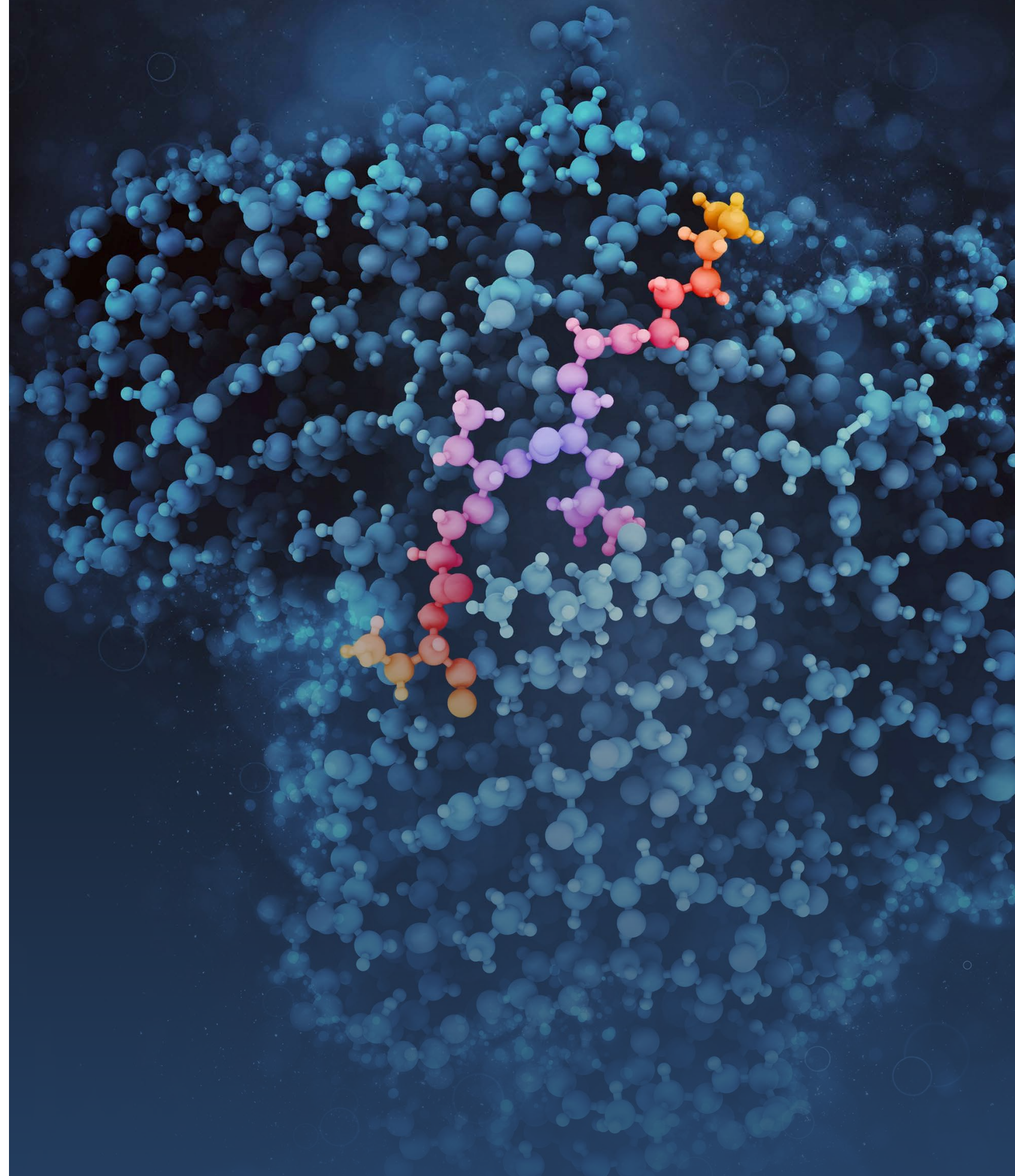
**Zina Zokouri, Business
Development Manager
at Bachem**

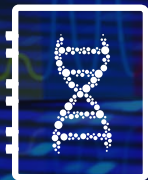
Zina Zokouri joined Bachem in 2020 and works as a Business Development Manager. Previously Zokouri has worked for technology firms such as Valantic and Evolva, where she researched metabolic pathway engineering for the biosynthesis of plant-derived compounds in yeast and development of a transcription-factor-based biosensors for the detection of those compounds.



**David Craik, Professor
of Chemistry at The
University of Queensland**

David Craik is a Professor of Chemistry and Australian Research Council Laureate Fellow at the Institute for Molecular Bioscience, The University of Queensland, Australia. His research focuses on applications of cyclic peptides, toxins and NMR in drug design. He is a Fellow of the Australian Academy of Science and received the Ralph F. Hirschmann Award from the American Chemical Society. He is author of 620 papers and has trained 70 PhD students.





OMICS VENDOR DIRECTORY

The below list of Vendors all work in fields within Omics, and provide cutting-edge solutions in the following fields and more:

- Bioinformatics/Data Science
- Clinical Diagnostics
- Clinical Genomics
- DPCR
- Drug discovery
- Gene Expression
- Genome Editing Technologies
- Next Generation Sequencing
- Single Cell Analysis
- Single Cell Bioinformatics/Computation Analysis
- Single Cell Clinical Development
- Single Cell Epigenetics
- Single Cell Genomics
- Single Cell Imaging
- Single Cell Metabolomics
- Single Cell Methods
- Single Cell Proteomics
- Single Cell Transcriptomics
- Spatial Biology
- Translation

10x Genomics	https://www.10xgenomics.com/
ACTIVE MOTIF	https://www.activemotif.com/
Agilent Technologies	https://www.agilent.com/
Akoya Biosciences	https://www.akoyabio.com/
Beckman Coulter	www.beckman.com
BEX CO	https://www.bexnet.co.jp/english/
Biogazelle	https://services.biogazelle.com/
bioMérieux	https://www.biomerieux-industry.com/pharma-healthcare
Bio-Rad	https://www.bio-rad.com/
Bio-Techne	https://www.bio-techne.com/
Bruker Corporation	https://www.bruker.com/en.html



Canopy Biosciences	https://canopybiosciences.com/
CeGaT Gmbh	https://www.cegat.de/
Cellenion (Scienion)	https://www.cellenion.com/
Charles River	https://www.criver.com/
Codex DNA	https://codexdna.com/
Covaris	https://www.covaris.com/
CYTENA	https://www.cytena.com/
Cytiva	https://www.cytivalifesciences.com/en/us/
Cytosurge	https://www.cytosurge.com/
Diagenode	https://www.diagenode.com/en
DNA Script	https://www.dnascript.com/
Dovetail Genomics	https://dovetailgenomics.com/
Emulseo	https://www.emulseo.com/
Eppendorf	https://www.eppendorf.com/UK-en/
Epredia	https://epredia.com/
Flow Robotics	https://flow-robotics.com/
Fluent Biosciences	https://www.fluentbio.com/
Fluidigm	https://www.fluidigm.com/
FORMULATRIX	https://formulatrix.com/
GeneFirst Limited	https://www.genefirst.com/
GENEWIZ	https://www.genewiz.com/en-GB?sc_device=Mobile
Honeycomb Biotechnologies	https://honeycomb.bio/
Illumina	https://www.illumina.com/
Inorevia	https://www.inorevia.com/
Integrated DNA Technologies	https://www.idtdna.com/pages/products/next-generation-sequencing#methods



Ionpath	https://www.ionpath.com/
KromaTiD	https://kromatid.com/
Lexogen	https://www.lexogen.com/
Lumencor	https://lumencor.com/
Lunaphore Technologies	https://lunaphore.com/
Menarini Silicon Biosystems	http://www.siliconbiosystems.com/
Merck	https://www.merckgroup.com/en
MGI	https://en.mgi-tech.com/
Miltenyi Biotec	https://www.miltenyibiotec.com/GB-en/
Mission Bio	https://missionbio.com/
Nanostring Technologies	https://www.nanostring.com/
NEO New Oncology	https://www.siemens-healthineers.com/laboratory-diagnostics
NimaGen	https://www.nimagen.com/
Novogene	https://en.novogene.com/
OPTOLANE Technologies	https://optolane.com/?ckattempt=1
PacBio	https://www.pacb.com/
Parse Biosciences	https://www.parsebiosciences.com/
Partek	https://www.partek.com/
PerkinElmer	https://www.perkinelmer.com/uk/
Pillar Biosciences	https://pillar-biosciences.com/
Purigen Biosystems	https://www.purigenbio.com/
QIAGEN	https://www.qiagen.com/us/
Quantabio	https://www.quantabio.com/

RareCyte	https://rarecyte.com/
Rebus Biosystems	https://rebusbio.com/
Resolve Biosciences	https://resolve-biosciences.com/
Roche Diagnostics	https://diagnostics.roche.com/
Samplix	https://samplix.com/
Scipio bioscience	https://scipio.bio/
SensID	https://www.sens-id.com/
seqWell	https://seqwell.com/
Single Cell Discoveries	https://www.scdiscoveries.com/
Source Bioscience	https://www.sourcebioscience.com/
SPT Labtech	https://www.sptlabtech.com/
Stilla Technologies	https://www.stillatechnologies.com/
Synthego	https://www.synthego.com/
Tag.bio	https://tag.bio/
Takara Bio	https://www.takarabio.com/
Tecan	https://www.tecan.com/
TwinStrand Biosciences	https://twinstrandbio.com/
Twist Bioscience	https://www.twistbioscience.com/
Ultivue	https://biomarker.ultivue.com/
VectorBuilder	https://en.vectorbuilder.com/
Veranome Biosystems	https://veranomebio.com/
Vizgen	https://vizgen.com/
Yokogawa	https://www.yokogawa.com/uk/



BIOMARKERS VENDOR DIRECTORY

The below list of Vendors all work in fields within Biomarkers, and provide cutting-edge solutions in the following fields and more:

• Assay Development

• Bioinformatics

• Biomarker Discovery & Development

• Biomarker Technologies

• Biomarkers Analysis

• Clinical Development

• Clinical Trial Design & Operations

• Clinical/Translational

• CTC

• Cytometry

• Diagnostic Biomarkers

• Diagnostic Development

• Digital Biomarkers

• Genomic Biomarkers

• Liquid Biopsies

• Multiplexing

• Non-Invasive Biomarkers

• Patient Selection & Stratification

• Personalised Medicine

• Sample Management & Biobanking

• Translational Research

10x Genomics <https://www.10xgenomics.com/>

ABCAM <https://www.abcam.com/>

Affinity Biomarker Labs <https://affinitybiomarkerlabs.com/>

Akoya Biosciences <https://www.akoyabio.com/>

Alderley Park Limited <https://www.alderleypark.co.uk/>

Almac <https://www.almacgroup.com/>

ANGLE <https://angleplc.com/>

APIS Assay Technologies <https://www.apisassay.com/>

Arc Regulatory <https://www.arc-regulatory.co.uk/>

Arima Genomics www.arimagenomics.com

Audubon Bioscience <https://audubonbio.com/>

Avacta <https://avacta.com/>

BIOMARKERS

Beckman Coulter

www.beckman.com

Belgian Volition

<https://volition.com/>

BioAgilytix

<https://www.bioagilytix.com/uk/>

Biocartis US, Inc

<https://www.biocartis.com/en>

Biogazelle

<https://services.biogazelle.com/>

Biognosys

<https://biognosys.com/>

BioLizard

<https://lizard.bio/>

Bio-Techne

<https://www.bio-techne.com/>

Bioview

<https://bioview.com/>

Canopy Biosciences

<https://canopybiosciences.com/>

Caprion Biosciences

<https://cellcarta.com/>

Causaly

www.causaly.com

Celerion

<https://www.celerion.com/>

Cerba Research

<https://www.cerbaresearch.com/>

Charles River

<https://www.criver.com/>

Concept Life Science

<https://www.malvernpanalytical.com/en/about-us/our-brands/concept-life-sciences>

Crown Bioscience

<https://www.crownbio.com/>

Definiens

DermTech

<https://dermtech.com/>

Digital Science

<https://www.digital-science.com/>

EMD Millipore Corporation

<https://www.merckmillipore.com/GB/en>

engine the biomarker company

<http://engine-gmbh.de/>

Epistem

<https://www.epistem.co.uk/>

Eurofins Discoverx Corporation

<https://www.discoverx.com/home>

Fios Genomics

<https://www.fiosgenomics.com/>

Fluidic Analytics

<https://www.fluidic.com/>



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Fluidigm	https://www.fluidigm.com/
Gyros Protein Technologies	https://www.gyrosproteintechnologies.com/
Hematogenix	https://www.hematogenix.com/
HistologiX	www.histologix.com
HTG Molecular Diagnostics	https://www.htgmolecular.com/
Human Metabolome Technologies	https://humanmetabolome.com/
Immunologix Laboratories	https://www.immunologixlabs.com/
Indica Labs	https://indicalab.com/
Indivumed	https://www.indivumed.com/
Interactive Software	https://www.interactivesoftware.co.uk/
Intertek	https://www.intertek.com/
Invicro	https://invicro.com/
Keen Eye	https://www.keeneye.ai/
Labcorp	https://drugdevelopment.labcorp.com/
LGC	https://www.lgcgroup.com/
Luminex	https://www.luminexcorp.com/eu/
Lunaphore Technologies	https://lunaphore.com/
Merck	https://www.merckgroup.com/en
Meso Scale Discovery	https://www.mesoscale.com/
Metabolon	https://www.metabolon.com/
Miltenyi Biotec	https://www.miltenyibiotec.com/GB-en/
MiRXes	https://mirxes.com
MLM Medical Labs	https://www.mlm-labs.com/
Nanostring Technologies	https://www.nanostring.com/

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NeoGenomics	https://neogenomics.com/
Novai	https://www.novai.co.uk/
Olink	https://www.olink.com/
Oncimmune	https://www.oncimmune.com/
Owlstone Medical	https://www.owlstonemedical.com/
Pelago Bioscience	https://www.pelagobio.com/
Personalis	https://www.personalis.com/
PPD	https://www.ppd.com/
PRA Health	https://www.iconplc.com/
Precision For Medicine	https://www.precisionformedicine.com/
Propath Ltd	https://propath.co.uk/
Proteome Sciences	https://www.proteomics.com/
Quanterix	https://www.quanterix.com/
Radiomics	https://radiomics.bio/
Randox	https://www.randox.com/
Roche Diagnostics	https://diagnostics.roche.com/
Rules-Based Medicine (RBM, a Q2 Solutions Company)	https://rbm.q2labsolutions.com/
Sapient Bioanayltics	https://sapient.bio/
Scailyte	https://scailyte.com/
Sengenics	https://sengenics.com/
SensID	https://www.sens-id.com/
SomaLogic Inc	https://somalogic.com/life-sciences/
The Science Behind	https://www.thesciencebehind.com/
Tissue Solutions	https://www.tissue-solutions.com/
Ultivue	https://biomarker.ultivue.com/
Unilabs	https://unilabs.com/
Universal Biosensors	https://www.universalbiosensors.com/
Ziath	https://ziath.com/



IMMUNO VENDOR DIRECTORY

The below list of Vendors all work in fields within Immuno, and provide cutting-edge solutions in the following fields and more:

- Advanced Models
- Antibody-based Therapies
- Assay Development
- Autoimmunity
- Biomarkers & Translational Research
- Cancer Vaccines
- Cell Therapy
- Clinical Development
- Drug Discovery & Development
- Genomic Tools
- Immuno-Oncology
- Oncolytic Viruses
- Precision Medicine
- Preclinical Development
- Screening Tools
- Spatial Biology Tools
- Spatial Omics Tools

10x Genomics	https://www.10xgenomics.com/
ABCLONAL	https://abclonal.co.uk/
ABL Inc.	https://ablinc.com
Agilent Technologies	https://www.agilent.com/
Axion Biosystems	https://www.axionbiosystems.com/
Batavia Biosciences	https://www.bataviabiosciences.com/
Beckman Coulter	www.beckman.com
Benchling	https://www.benchling.com/
Biocytogen	https://biocytogen.com/
Bio-Rad	https://www.bio-rad.com/
Bio-Techne	https://www.bio-techne.com/

IMMUNO



Cell Signalling Technology	https://www.cellsignal.co.uk/
CELLINK	https://www.cellink.com/
Cerba Research	https://www.cerbaresearch.com/
Charles River	https://www.criver.com/
Concept Life Science	https://www.malvernpanalytical.com/en/about-us/our-brands/concept-life-sciences
Crown Bioscience	https://www.crownbio.com/
Cytiva	https://www.cytivalifesciences.com/en/us/
Cytognos	https://www.cytognos.com/
Dovetail Genomics	https://dovetailgenomics.com/
Enplas Europe	https://www.enplas.co.jp/english/
Eurofins Discoverx Corporation	https://www.discoverx.com/home
Eurofins Discovery	https://www.eurofinsdiscoveryservices.com/
Fluidigm	https://www.fluidigm.com/
GemPharmatech	https://gempharmatech.us/en/
Genedata	https://www.genedata.com/
genOway	https://www.genoway.com/
GenScript	https://www.genscript.com/
HistologiX	www.histologix.com
Immunitrack	https://www.immunitrack.com/
Indivumed	https://www.indivumed.com/
INOVOTION SAS	https://www.inovotion.com/
Ionpath	https://www.ionpath.com/
JPT Peptide Technologies	https://www.jpt.com/



Labcorp	https://drugdevelopment.labcorp.com/
Lunaphore Technologies	https://lunaphore.com/
Merck	https://www.merckgroup.com/en
Miltenyi Biotec	https://www.miltenyibiotec.com/GB-en/
MLM Medical Labs	https://www.mlm-labs.com/
Nanostring Technologies	https://www.nanostring.com/
Navinci	https://navinci.se/
NeoGenomics	https://neogenomics.com/
Nexelis	https://nexelis.com/
Nordic Bioscience	https://www.nordicbioscience.com/
PBL Assay Science	https://www.pblassaysci.com/
PeproTech	https://www.thermosfisher.com/peprotech
PerkinElmer	https://www.perkinelmer.com/uk/
Personalis	https://www.personalis.com/
PharmaLegacy	http://www.pharmalegacy.com/
Pharmatest	https://pharmatest.com/
Promega	https://www.promega.co.uk/
ProQinase GmbH	https://www.reactionbiology.com/
Repositive	https://repositive.io/
Scailyte	https://scailyte.com/
Sino Biological Europe GmbH	https://www.sinobiological.com/



Solentim	https://www.solentim.com/
Synexa Life Sciences	https://www.synexagroup.com/
Sysmex	https://www.sysmex.co.uk/
Takara Bio	https://www.takarabio.com/
Tissue Solutions	https://www.tissue-solutions.com/
Visiopharm	https://visiopharm.com/
WuXi AppTec	https://www.wuxiapptec.com/



PHARMATEC VENDOR DIRECTORY

The below list of Vendors all work in fields within PharmaTec, and provide cutting-edge solutions in the following fields and more:

- AI & Machine Learning
- Analytical Solutions
- Automation Solutions
- Bioinformatics
- Data Science
- Digital Transformation / Technologies
- Digitalisation
- Discovery Informatics / IT
- Enterprise IT
- Lab Tech
- Logistics, Supply Chain
- Manufacturing / Biomanufacturing
- Mobile Robots
- Pharma Data
- Process Chemistry
- Production Scale Up
- QA/QC
- Smart Labs

Appian	https://appian.com/
BENCHLING	https://www.benchling.com/
BINOCS	https://discover-binocs.com/
Biorelate	https://www.biorelate.com/
Catalent	https://www.catalent.com/
ChemAxon	https://chemaxon.com/
Collaborative Drug Discovery	https://www.collaborativedrug.com/
Eppendorf	https://www.eppendorf.com/UK-en/
Gearu	https://gearulabs.com/
Genomenon	https://www.genomenon.com/

PHARMATEC

GenScript	https://www.genscript.com/
LabTwin	https://www.labtwin.com/
Linguamatics	https://www.linguamatics.com/
Merck	https://www.merckgroup.com/en
Omron	https://www.omron-healthcare.co.uk/
ONTOFORCE	https://www.ontoforce.com/
Owkin	https://owkin.com/
PureStorage	https://www.purestorage.com/uk/
Schrödinger	https://www.schrodinger.com/
SciBite	https://www.scibite.com/
Stäubli	https://www.staubli.com/en-gb/
Synthace	https://www.synthace.com/
The Hyve	https://www.thehyve.nl/
United Robotics Group	https://www.unitedrobotics.group/
Waters	https://www.waters.com/nextgen/gb/en.html

FORMULATION & DELIVERY VENDOR DIRECTORY

The below list of Vendors all work in fields within Formulation & Delivery, and provide cutting-edge solutions in the following fields and more:

- Alternative Drying Technologies
 - Bioprocessing
 - CGT
 - Characterization
 - CMC
 - Continuous Manufacturing
- Delivery Drug
 - Device Development
 - Dosage
 - Drug Product Development
 - Formulation
 - Inhalation
- Lyophilization
 - Nanotechnology
 - Predictive Modelling
 - RNA Platforms

ABER Instruments	https://aberinstruments.com/#gref
ADARE PHARM SOLUTIONS	https://adarepharmasolutions.com/
Advanced Instruments	https://www.aicompanies.com/
Aptar CSP Technologies	https://www.csptechnologies.com/
Ashland	https://www.ashland.com/
Asymchem	https://www.asymchem.com/
BASF	https://pharma.basf.com/solutions/biopharma
BioDuro-Sundia	https://bioduro-sundia.com/
Cambrex	https://www.cambrex.com/
CAS	https://www.cas.org/
Catalent	https://www.catalent.com/

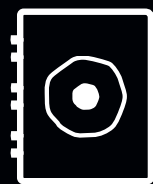
FORMULATION

Celanese	https://healthcare.celanese.com/
Contract Pharmaceuticals Limited	https://cplltd.com/
CoreRx	https://www.corerxpharma.com/
Datwyler	https://datwyler.com/
Emergo	https://www.emergobyul.com/
Emultech	https://www.emultech.nl/
Evotec	https://www.evotec.com/en
Experic	https://expericservices.com/
Foster Delivery Science	https://www.fosterdeliveryscience.com/
FreeThink Technologies Inc	https://freethinktech.com/
Gerresheimer	www.gerresheimer.com
Halo Labs	https://www.halolabs.com/
Harro Höfliger	https://www.hoefliger.com/
Inabata America Corporation	https://us.inabata.com/
Intec Pharma	http://www.intecpharma.sk/
Intertek	https://www.intertek.com/
leon-nanodrugs	https://www.leon-nanodrugs.com/
Leukocare	https://leukocare.com/
Lonza	www.pharma.lonza.com
Matralix	https://www.matralix.com/
MedPharm	https://www.medpharm.com/us/
Merxin	https://www.merxin.com/
MG2 S.r.l.	https://www.mg2.it/

Millipore SAS	https://www.merckmillipore.com/GB/en
Miltenyi Biotec	https://www.miltenyibiotec.com/GB-en/
Minnetronix Medical	https://minnetronixmedical.com
Munit	www.munit.com
Nanexa	https://nanexa.com/
Nanologica	https://nanologica.com/
Nanopharm	https://nanopharm.co.uk/
NETZSCH Premier Technologies	https://grinding.netzsch.com/
New England Biolabs	https://www.neb.uk.com/
NextPharma	https://nextpharma.com/
NOF CORPORATION	https://www.nofamerica.com
Occlugel SAS	http://www.occlugel.com/
Pfanstiehl	https://pfanstiehl.com/
Pfizer CentreOne	https://www.pfizercentreone.com/
Pharmaceutics International	https://www.pharm-int.com/
PION	https://www.pion-inc.com
ProDigest	https://prodigest.eu/
Quay Pharma	https://www.quaypharma.com/
Quotient Sciences	https://www.quotientsciences.com/
Recipharm	https://www.recipharm.com/



RedShiftBio	https://www.redshiftbio.com/
RNAcore	https://www.houstonmethodist.org/research/our-research/cores/rnacore/
Schrödinger	https://www.schrodinger.com/
SCIEX	https://sciex.com/
Sever Pharma Solutions	https://www.severpharmasolutions.com/
SiSaf	https://www.sisaf.com/
Technobis	www.crystallizationsystems.com
Telstar	https://www.telstar.com/
tesa Labtec	https://www.tesa-labtec.com/en
Thermo Fisher Scientific	https://www.thermofisher.com/uk/en/home.html
Trelleborg Group	https://www.trelleborg.com/en
Unchained Labs	https://www.unchainedlabs.com/
Upperton Pharma Solutions	https://www.upperton.com/
Wyatt Technology	https://www.wyatt.com/
Xeris Biopharma	https://www.xerispharma.com/
Zerion Pharma	https://www.zerion.eu/



CELL VENDOR DIRECTORY

The below list of Vendors all work in fields within Cell, and provide cutting-edge solutions in the following fields and more:

- 2D & 3D Models
- Analytics & Characterisation
- Bioprinting
- Bioprocessing Solutions
- Cell Culture & Media
- Cell Lines
- Commercialisation
- Cryopreservation
- Gene Editing
- Genomic Tools
- Manufacturing
- Microphysiological System Technology
- Pre-Clinical & Clinical Development
- Quality Control
- Raw Materials
- Supply Chain
- Tissue Engineering
- Viral & Non-Viral Vectors

Abcam	https://www.abcam.com/
ABER INSTRUMENTS	https://aberinstruments.com/#gref
Advanced Instruments	https://www.aicompanies.com/
Asimov	https://www.asimov.com/
Axion Biosystems	https://www.axionbiosystems.com/
Benchling	https://www.benchling.com/
Berkeley Lights	https://www.berkeleylights.com/
Biocair	https://www.biocair.com/
Bioneer A/S	https://bioneer.dk/
Bio-Techne	https://www.bio-techne.com/

CELL

Caltag Medsystems / CELLnTEC	https://www.caltagmedsystems.co.uk/
Catalent	https://www.catalent.com/
C-CIT Sensors	https://www.c-cit.ch/
Cellenion (Scienion)	https://www.cellenion.com/
CELLINK	https://www.cellink.com/
CellTool GmbH	
Cellular Highways Ltd	https://www.cellularhighways.com/
CelVivo	https://celvivo.com/
Cergentis	https://www.cergentis.com/
ChemoMetec	https://chemometec.com/
CPC Colder Products Company	https://www.cpcworldwide.com/
CYTENA	https://www.cytена.com/
Cytomos Ltd	https://www.cytomos.com/
Eppendorf	https://www.eppendorf.com/UK-en/
FOX BIOSYSTEMS	https://foxbiosystems.com/
FUJIFILM, Irvine Scientific	https://www.irvinesci.com/
GENEWIZ	https://www.genewiz.com/en-GB?sc_device=Mobile
GenScript	https://www.genscript.com/
Halo Labs	https://www.halolabs.com/
Horizon Discovery	https://horizondiscovery.com/
IDBS	https://www.idbs.com/
Intertek	https://www.intertek.com/
iotaSciences	https://www.iotasciences.com/

Kerry	https://www.kerry.com/
KromaTiD	https://kromatid.com/
Life Science Group	https://lifesciencegroup.co.uk/
Lipotype GmbH	https://www.lipotype.com/
Malvern Panalytical	https://www.malvernpanalytical.com/en/
Manchester BIOGEL	https://manchesterbiogel.com/
Miltenyi Biotec	https://www.miltenyibiotec.com/GB-en/
NecstGen	https://necstgen.com/
Nova Biomedical	https://www.novabiomedical.com/
Novo Nordisk Pharmatech A/S	https://novonordiskpharmatech.com/
Ovizio Imaging Systems	https://ovizio.co/3MEw6hl
OXGENE	https://oxgene.com/
PeproTech	https://www.thermosfisher.com/peprotech
PROGEN	https://www.progen.com/
PyroScience	https://www.pyroscience.com/en/
Reading Scientific Services Ltd	https://www.rssl.com/
Roche Diagnostics	https://diagnostics.roche.com/
RoslinCT	https://www.roslinct.com/
Sartorius	www.sartorius.com/octet
SCIEX	https://sciex.com/

Solentim	https://www.solentim.com/
Symcel AB	https://symcel.com/
Synthego	https://www.synthego.com/
TestAVec	https://www.testavec.com/
Thermo Fisher Scientific	https://www.thermofisher.com/uk/en/home.html
Tissue Solutions	https://www.tissue-solutions.com/
ValitaCell	https://valitacell.com/
VectorBuilder	https://en.vectorbuilder.com/
Wyatt Technology	https://www.wyatt.com/



DISCOVERY VENDOR DIRECTORY

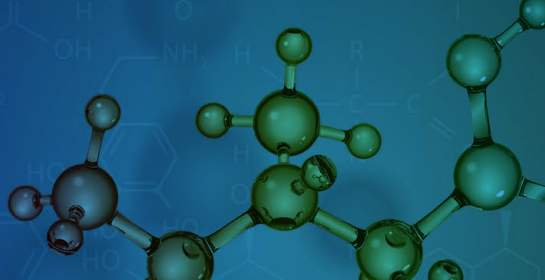
The below list of Vendors all work in fields within Discovery, and provide cutting-edge solutions in the following fields and more:

- AI/Machine Learning
- Automation
- Biologics-based discovery
- Cell Culture
- Chemical Biology
- Computational Chemistry
- Covalent Inhibitors
- DEL
- Disease Modelling
- Medicinal Chemistry
- Molecular Drug Design
- Organ on Chip
- Organoid Development
- RNA based discovery
- Safety/DMPK
- Screening Solutions
- Targeted Protein Degradation

10x Genomics	https://www.10xgenomics.com/
ADMESCOPE	https://www.admescope.com/
Apex Molecular	https://apexmolecular.com/
Arctoris	https://www.arctoris.com/
Aurelia Bioscience	https://www.aureliabio.com/
Axion Biosystems	https://www.axionbiosystems.com/
Axol Bioscience	https://www.axolbio.com/
BioTheryX	https://biotheryx.com/
Birdotech Europe GmbH	https://www.birdotech.com/
bit.bio	https://www.bit.bio/
Cambridge Crystallographic Data Centre	https://www.ccdc.cam.ac.uk/

DISCOVERY

Causaly	www.causaly.com
ChemAxon	https://chemaxon.com/
Chempartner A/S	https://chempartner.com/
Chempass	https://chempass.ai/
Collaborative Drug Discovery	https://www.collaboratedrug.com/
Concept Life Science	https://www.malvernpanalytical.com/en/about-us/our-brands/concept-life-sciences
Creoptix	https://www.creoptix.com/
Crown Bioscience	https://www.crownbio.com/
DefiniGEN	https://www.definigen.com/
Dotmatics	https://www.dotmatics.com/
Elsevier	https://www.elsevier.com/en-gb
Evotec	https://www.evotec.com/en
Fluidic Analytics	https://www.fluidic.com/
Fujitsu	https://www.fujitsu.com/uk/
GalChimia	https://www.galchimia.com/
GemPharmatech	https://gempharmatech.us/en/
Graphcore	https://www.graphcore.ai/
HexagonFab	https://www.hexagonfab.com/
Hubrecht Organoid Technology	https://huborganoids.nl/
Iktos	https://iktos.ai/
Inoviem Scientific	https://www.inoviem.com/
IRBM	https://www.irbm.com/



LAXAI Life Sciences	http://www.laxai.com/
Lhasa Limited	https://www.lhasalimited.org/
Liverpool ChiroChem	https://www.liverpoolchirochem.com/
Luminex	https://www.luminexcorp.com/eu/
Magnitude Biosciences	https://magnitudebiosciences.com/
Mcule	https://www.mcule.com
MedChemExpress	https://www.medchemexpress.com/
MercachemSyncom	https://mercachemsyncom.com/
Metabolon	https://www.metabolon.com/
Miltenyi Biotec	https://www.miltenyibiotec.com/GB-en/
Mimetas	https://www.mimetas.com/en/home/
Molecular Devices	https://www.moleculardevices.com/
Nanome	https://nanome.ai/
Ncardia	https://www.ncardia.com/
Newcells Biotech	https://newcellsbiotech.co.uk/
NUVISAN	https://www.nuvisan.com/home.html
OncoDesign	https://www.oncodesign.com/
Owkin	https://owkin.com/
Pelago Bioscience	https://www.pelagobio.com/
PHABIOC (InnoME)	https://www.phabioc.shop/en/
Pivot Park Screening Centre	https://www.ppscreeningcentre.com/

Promega	https://www.promega.co.uk/
Reaction Biology	https://www.reactionbiology.com/
Recipharm	https://www.recipharm.com/
Sai Life Science	https://www.sailife.com/
Sartorius	www.sartorius.com/octet
Schrödinger	https://www.schrodinger.com/
Selvita	https://selvita.com/
siTOOLS Biotech	https://www.sitoolsbiotech.com/
Sygnature Discovery	https://www.sygnaturediscovery.com/
Syngene International Ltd	https://www.syngeneintl.com/
Tissue Solutions	https://www.tissue-solutions.com/
Torx Software	https://www.torx-software.com/about/
Twist Bioscience	https://www.twistbioscience.com/
WuXi AppTec	https://www.wuxiapptec.com/
ZeClinics	https://www.zeclinics.com/



BIOLOGICS VENDOR DIRECTORY

The below list of Vendors all work in fields within Biologics, and provide cutting-edge solutions in the following fields and more:

- ADC
- AI/ML
- Antibody Design
- Antibody Libraries
- Antibody/Protein Engineering
- Bioanalysis & Characterisation
- Computational Tools
- Expression/Purification
- Oligo Analytical Development/CMC
- Oligo Synthesis
- Oligo Therapeutic Development
- Peptide Chemistry
- Peptide Therapeutic Development
- Process Development
- Production and Manufacturing
- Protein Therapeutic Development
- Screening & Automation
- Stability
- Vaccine Analytics/CMC
- Vaccine Design
- Vaccine Development
- Vaccine Platforms

AbCellera <https://www.abcellera.com/>

ABCLONAL <https://abclonal.co.uk/>

Admescope <https://www.admescope.com/>

Agilent Technologies <https://www.agilent.com/>

Akoya Biosciences <https://www.akoyabio.com/>

AlivaMab Discovery Services, LLC <https://alivamab.com/>

Almac <https://www.almacgroup.com/>

AmbioPharm <https://www.ambiopharm.com/>

American International Chemical <https://www.aicma.com/>

BIOLOGICS

ATUM <https://www.atum.bio/>

Bachem <https://www.bachem.com/>

Belyntic <https://belyntic.com/>

Benchling <https://www.benchling.com/>

Berkeley Lights <https://www.berkeleylights.com/>

Bionamic AB <https://bionamic.io/>

Bio-Rad <https://www.bio-rad.com/>

Biotage GB Ltd <https://www.biotage.com/>

Bio-Techne <https://www.bio-technique.com/>

Bio-Works <https://www.bio-works.com/>

Carterra Bio <https://carterra-bio.com/>

CEM <https://cem.com/uk/>

Chemical Computing Group <https://www.chemcomp.com/>

Cytiva <https://www.cytivalifesciences.com/en/us/>

ENPICOM N.V <https://enpicom.com/>

EnzyTag <https://enzytag.com/>

Eppendorf <https://www.eppendorf.com/UK-en/>

e-Zyvec SAS <https://www.e-zyvec.com/>

Fairjourney Biologics <https://fjb.pt/>

Fluidic Analytics <https://www.fluidic.com/>

FOX BIOSYSTEMS <https://foxbiosystems.com/>

FUJIFILM Diosynth Biotechnologies <https://fujifilmdiosynth.com/>

GemPharmatech <https://gempharmatech.us/en/>



Genovis	https://www.genovis.com/
GenScript	https://www.genscript.com/
GL Biochem	http://www.glbiochem.com/en/index/index.aspx
Glycotope GmbH	https://www.glycotope.com/
Gyros Protein Technologies	https://www.gyrosproteintechnologies.com/
Halo Labs	https://www.halolabs.com/
HexagonFab	https://www.hexagonfab.com/
IBIS Technologies	https://www.ibis-spr.nl/
ImmunoPrecise Antibodies	https://www.immunoprecise.com/
InstaDeep	https://www.instadeep.com/
Iris Biotech GmbH	https://www.iris-biotech.de/
Lonza	www.pharma.lonza.com
MABSilico	https://www.mabsilico.com/
Maxcyte Inc	https://maxcyte.com/
Merck	https://www.merckgroup.com/en
Molecular Devices	https://www.moleculardevices.com/
Pepscan	https://www.pepscan.com/
PolyPeptide Group	https://www.polypeptide.com/
Polypure AS	https://polypure.com/
ProvePharm Life Solutions	https://www.provepharm.com/
Reading Scientific Services Ltd	https://www.rssl.com/



RedShiftBio	https://www.redshiftbio.com/
Refeyn	https://www.refeyn.com/
Rodon Biologics	https://www.rodonbiologics.com/en
Sartorius	www.sartorius.com/octet
SCIEX	https://sciex.com/
SGS	https://www.sgs.com/
Sino Biological Europe GmbH	https://www.sinobiological.com/
Sphere Fluidics	https://spherefluidics.com/
Teledyne ISCO	https://www.teledyneisco.com/en-us/chromatography
Twist Bioscience	https://www.twistbioscience.com/
Unchained Labs	https://www.unchainedlabs.com/
Vivitide	https://vivitide.com/

Thank you for reading



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2022