CATAPULT
Medicines Discovery

Medicines Discovery Catapult

MDC Connects

A Guide to **Complex Medicines**

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MDC Connects: A Guide to Complex Medicines

MDC Connects is a series of weekly webinars which ran in 2021. The sessions set out to **educate**, **inform**, and **advise** the community in drug discovery, focusing on recent trends and practice, encapsulating insight from experts in these areas.

The sessions were aimed at small companies developing their own medicines but were equally open to the wider drug discovery community.

This was the second series of MDC Connects webinars exploring the opportunities and challenges presented by the discovery and development of new drug modalities, namely complex medicines. The webinars were a guide through the steps needed to take an idea from concept to the clinic, and heard from both pioneers in developing novel medicines, as well as the experts who support them.

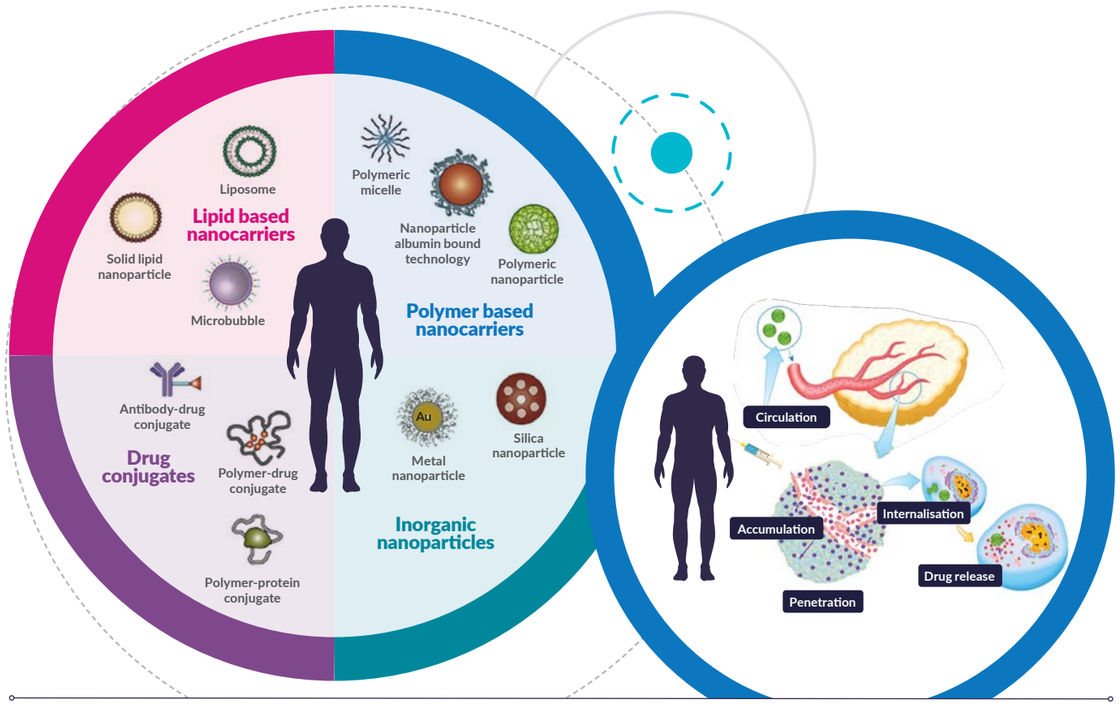
Each webinar was delivered by three experts, totalling 18 speakers from 15 organisations, and this guide includes a summary of each presentation, which together create an important and consolidated resource of modern drug discovery knowledge. It would not have been possible without the community's support, and a special thank you is extended to the partners who took part in MDC Connects. Please take a look at their websites to see if there are ways in which they can help you.

Thank you, and enjoy the MDC Connects: A Guide to Complex Medicines 2021.

Dr Sarah Brockbank
Medicines Discovery Catapult

Complex Medicines:
Why, What, When? Opportunities and Challenges

The medicines industry is going through a period of change. While small molecule therapeutics still make up 90% of approved medicines, patient expectations are driving the industry towards targeted, precision treatments, which require a shift towards stratified, complex medicines, with more challenging discovery and development needs.



Complex medicines include a variety of advanced drug chemistries and therapeutic targeting vehicles, comprising complex APIs (e.g., antibody drug conjugates, oligonucleotideconjugates), advanced delivery mechanisms (e.g., intratumoral injection, targeted therapies) and complex dosage form or formulations (e.g., nanomedicines).

To embark on new complex medicines discovery programmes, scientists require distinct drug discovery expertise, specialist technical skills, access to equipment and infrastructure and drug delivery know-how, as well as regulatory and commercialisation support. Such specialised technical and commercial assistance for start-ups and spin-outs is growing in the UK.

In the first webinar, Dr Duygu Yilmaz introduced this exciting, new, and rapidly growing field of complex medicines, while Dr David Cook described how to assess the commercial opportunity and Prof. Peter Simpson outlined the current state of research in the UK.

Complex Medicines:
Target Landscape

Dr Duygu Yilmaz, Senior Scientist
Medicines Discovery Catapult

Drug Discovery Trends

Progress in molecular biology and biotechnology has led to a shift from small molecules to non-small molecule-based drugs, including proteins polymers, nucleic acids and mixtures.

The timeline below highlights the delay between the discovery of a technology and its introduction to the market.

For instance, while RNA interface mechanisms were discovered in 1998, it wasn't until 2018 that the first siRNA was approved. Many more new drug modalities and complex medicines are expected in the coming years following the human genome project, and the introduction of next generation sequencing and gene editing technology.

Why Beyond Small Molecules?
Progress in Molecular Biology and Biotechnology

1990 Antibody engineering technology
1998 Discovery of RNAJ mechanism
2003 Completion of Human Genome Project
2005 Next generation sequencing
2010 First synthetic cell
2011 Discovery of TALENs
2013 CRISPR-Cas9 for genome editing in eukaryotic cells

1980 — PCR Technique — **1990** — **2000** — **2010** — The new era of FDA approved therapies — **2020**

1982 First recombinant drug, human insulin, approved
1998 First antisense oligonucleotide approved
2000 First antibody drug conjugate approved
2016 First clinical trials of CRISPR-Cas 9
2017 First CAR T therapy for cancer is approved
2018 The first siRNA approved
2019 3 more ADCs approved
2020 First mRNA vaccine approved

What do Molecular Technology Advances Mean?

Advances in molecular technology have led to a greater understanding of human disease, and the pathways and mechanisms involved. They have also meant that previously inaccessible drug targets are now being reached using antibody drug conjugates, drug peptide conjugates and gene therapy. These new modalities require drug delivery technologies such as polymeric nanoparticles, inorganic nanoparticles, or lipid-based nanoparticles, which can pass biological barriers to deliver the novel moieties. They also allow poorly soluble or unstable therapeutic moieties, or highly toxic compounds, to be used with minimal side effects.

Nanomedicines in Clinical Practice

Nanomedicines are a popular delivery mechanism for oncology targets as they help to minimise the systemic toxic side effects of the active pharmaceutical ingredients. A good example is Vyxeos, where the active substances – daunorubicin and cytarabine – are contained in liposomes for protection. This allows the drugs to be transported into the cancer cells. Within anaemia, the use of nanoparticles addresses the toxicity issues associated with free iron in the circulation, while the COVID-19 vaccine uses mRNA technology.

Biological Characterisation of Complex Medicines

Despite all these opportunities for complex medicines with more than one chemical entity, determining their pharmaceutical properties is more challenging compared to small molecules.

There are five main principles in the characterisation of a complex medicine:

1. Circulation
2. Accumulation
3. Penetration of the target
4. Internalisation
5. Release of the active pharmaceutical ingredient

Each process needs to be tracked from the systemic level to the subcellular level in order to characterise the complexes. Positron emission tomography (PET), near infrared imaging, and mass spectrometry imaging can assess circulation, accumulation and penetration of complex medicines, whilst immunofluorescence and mass spectrometry can assess internalisation and drug release. The biodistribution, organ and tumour accumulation of the complex medicines can be studied using immune-imaging, whilst microscopy allows internalisation, cell trafficking and efficacy to be monitored.

It is clear that advances in the genomics era have driven the diversification of drug targets, and molecular biological techniques and drug delivery technologies have enabled science to go beyond small molecule approaches, despite the challenges that complex medicines bring.

Dr Duygu Yilmaz, Senior Scientist, Medicines Discovery Catapult

During her PhD, Duygu worked on functionalised liposomal drug delivery systems, which can release the drug payload in a highly sensitive manner, in response to stimulus in the environment. Duygu has experience in various other drug delivery systems, including polymeric nanoparticles and cell-penetrating peptides. During Duygu's time at MDC, she was developing microscopy-based methods to study intracellular entry and trafficking of nanoparticles.

Complex Medicines:
Exploring the Commercial Opportunity

Dr David Cook, Chief Scientific Officer
Blueberry Therapeutics

What is a Complex Medicine?

Essentially, a complex medicine does not fit the 'traditional' drug category. There are several reasons why it could be complex:complex active ingredients, complex formulations, and a complex route of delivery, or any combination of these.

Developing a Complex Medicine

As a rule, simple is often best, so it is essential to first consider the rationale for developing a complex medicine, its advantages and the commercial opportunity it offers.

Complex medicines may require specialist equipment and expertise to produce, which can have manufacturing implications, as well as producing challenging regulatory pathways and safety assessments. Clinical testing can be difficult, and factors such as blinding with complex forms, formulation with novel excipients which require additional vehicle arms, mode of delivery, and placebo/comparators all need to be considered for clinical testing. The time and cost of R&D is therefore likely to be higher.

If it is so Hard, Why Bother?

Complex medicines should be considered as sometimes they are the solution to the problem we are facing. The COVID-19 pandemic is a fitting example, with the Pfizer/Moderna vaccine utilising a complex formulation form that allows the intracellular uptake of molecules that would never normally cross the lipid bilayer.

Complex medicines can lead to new modalities, multi-active medicines and improved solubility, while also offering targeted or enhanced distribution and sustained or on-demand delivery. Why is this important? Because it ultimately benefits the patient, clinicians, payers, and society.

Complex medicines can present new commercial opportunities; they can open new drug targets and classes, enable drug repositioning, produce new dosing regimens, and provide additional IP protection that can extend the lifetime of a product. Complex medicines can also offer improved safety and greater benefits such as risk profiles with targeted delivery, improved efficacy and better patient compliance.

Questions that Need Answering

Is complex medicine a logical solution to the problem you are facing? Would a simpler solution be just as effective, and at a lower cost? Do you understand the problem you are trying to solve?

The fundamental questions are, why would this be used, and is my medicine better than what is already available?

It is critical to start with the problem of an unmet medical need and find the solution, a complex medicine could often be the logical answer to that need.

Dr David Cook, Chief Scientific Officer, Blueberry Therapeutics

David Cook has a background in biochemistry, immunology and molecular biology, with a PhD from Imperial College, London, and three years academic postdoctoral experience. He has worked in the pharmaceutical industry for over 20 years, including 17 years in a wide variety of R&D roles at AstraZeneca (AZ) both in the UK and the US. Whilst at AZ, David worked on numerous drug projects, from target selection to supporting market products. He was also heavily involved in an in-depth analysis of AZ's drug portfolio and understanding why projects fail and was the lead author on AZ's "5Rs Paper" which described the learnings from this exercise.

David joined Blueberry Therapeutics as Chief Scientific Officer in 2014 and is responsible for leading the research activities of the business, which are focused on developing new treatments for common dermatological diseases.

Complex Medicines:

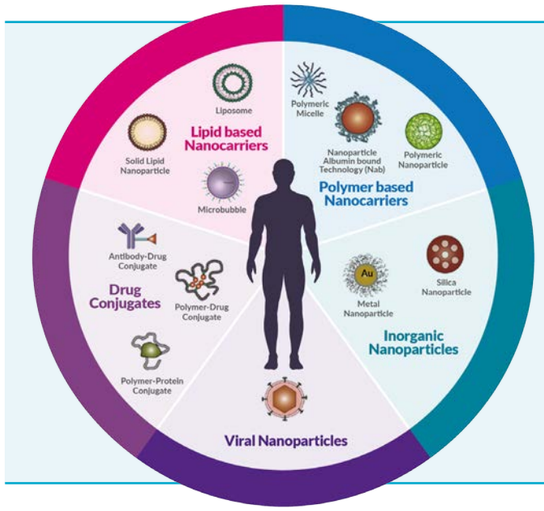
Where are we now, and where could we get to?
Understanding the UK R&D Opportunity in Complex Medicines

Prof. Peter Simpson, Chief Scientific Officer
Medicines Discovery Catapult

Putting the UK at the Forefront of New Technology

MDC supports complex medicine innovators and to date has been involved in 17 complex medicines projects, from large scale companies through to early-stage academic collaborators.

An influential example of complex medicine innovation is the mRNA COVID-19 vaccines from Moderna and Pfizer/BioNTech, which will probably drive the rapid growth of nanoparticle technology over the next few years. The potential for antibody drug conjugates and oligonucleotides has also been recognised by UK pharmaceutical and biotech companies investing in this technology.



- The Antibody-Drug Conjugates market was valued at £1bn in 2016 and is estimated to reach £7.5bn by 2025
- Oligonucleotide therapeutics market is expected to reach $6bn by 2027
- Huadong licensed Immunogen's antibody-drug conjugate in $305m deal
- Genetech is paying $30m upfront and up to $1.7bn in milestones to Bicycle Therapeutics (UK) on immuno-oncology bicyclic conjugates therapies
- Takeda Pharmaceutical licensed COUR Pharmaceutical Development's mid-stage nanomedicine candidate CNP-101 for up to $420m in milestones

Improving the UK's Success

To better understand and support the growing complex medicine landscape, MDC, in partnership with the Centre for Process Innovation (CPI), has engaged with stakeholders across the UK and published a report highlighting successes, challenges and future recommendations.

Technical challenges include access to imaging facilities for pre-clinical investigations; the need for new bioanalytical methods and validation assays; support for collaborative science and technology partnerships; and sharing of expertise. Innovators in complex medicines need government backing and investment, national coordination of infrastructure and a support network. There needs to be more thought given to the next generation of researchers too, and many graduates entering the drug discovery field require training to understand complex medicines and help grow the industry.

The key to success is guidance. How do you take a novel technology, a novel molecule, a novel modality, from initial pre-clinical research through to a clinically ready drug candidate? Practical support is needed from technology characterisation to validation and industrialisation; from the early pre-clinical research journey, through regulatory approval and clinical trials, into the clinic.

Improved delivery technologies and scalable manufacturing is needed. Nanoparticle delivery is challenging and, whilst advances have been made, the technologies are still not optimal. Through a better understanding of product composition, physiochemical properties, stability, delivery and performance, these technologies could become more readily accessible for industrialisation.

What are the Solutions?

A national laboratory infrastructure would provide a single-entry point for companies to receive support from pre-clinical assessment, process development, scalability and manufacturing. It would offer expertise and education on drug development, clinical trials design and regulatory issues, and help provide confidence to investors. Partnerships and collaborations would bring different experiences and skills together to help push forward product development.

Regional Focus

A regional focus for complex medicines innovation has been proposed, which would centre around transforming productivity in complex medicines with a multi-faceted approach. This would support innovation, business, growth and skills development, with local access to complex characterisation facilities, business support and accelerators, and then building from regional up to national collaboration.

Actions
What Is The Next Step?

- Publish and publicise the report findings
- Use this seminar series to begin to connect the community better
- Pilot alliances and build investable propositions
- Create a more effective and coherent voice

Bioanalysis of drug candidates and delivery mechanisms

Cell uptake	Biodistribution
Target engagement	Pre-clinical imaging
Pathway signalling	PK/PD
Biomarket discovery	Tissue pathology
Target product profile	

Translations

- Clinical biomarkers
- Plasma and tissue omics

Prof. Peter Simpson, Chief Scientific Officer, Medicines Discovery Catapult

During Peter's time at MDC, his expert biology teams collaborated with universities and SMEs around the UK, generating innovative technology and assay methodologies to accelerate progress in therapeutic discovery. Peter has previously been Director of N8 Research Partnership, establishing multi-university research collaborations across the North of England and leading strategic engagement on innovation policy.

In 2020, at the start of the Covid pandemic, Peter led the establishment of the Alderley Park Lighthouse Lab, which employed more than 600 staff and has delivered ~9 million Covid tests over the past year.

Peter also has twenty years' experience leading innovative drug discovery departments and programmes within major pharmaceutical companies including Merck and AstraZeneca, and sits on multiple national and international advisory boards.

DISCOVERY | DEVELOPMENT | COMMERCIALISATION

RESEARCH — DISCOVERY — PRE-CLINICAL — CLINICAL — ON PATENT — OFF PATENT

Medicine manufacturing innovation and development

- Oral solid dose
- Nanomedicines
- Viral vectors
- Therapeutic proteins
- Microbiome
- Storage and monitoring
- Packaging and distribution
- Patient adherence

Commercial drug manufacturing

The full report is available on the **Medicines Discovery Catapult website**

Complex Medicines:

Selection and Characterisation of the Lead

The selection and characterisation of the lead molecule for a complex medicine have many similarities with conventional drug discovery. Although questions remain regarding biological activity, target engagement and druggability, the methods of characterising these parameters can require very different and more complicated methods of analysis.

This session explored assays, technologies, and capabilities that can be employed to select and characterise the lead molecule. Tilly Bingham outlined the development of assay cascades for complex medicines and the analytical techniques required to fully characterise the test item and ensure data can be reproducibly generated. Dr James Szczerkowski described the application of advanced microscopy techniques to determine the binding, internalisation, and intracellular trafficking of the drug cargo, and Dr Rebecca Thompson described how cryo-electron microscopy can be used to characterise membrane-bound structures such as liposome or exosome delivery vehicles.

Complex Medicines:

The Challenges of Developing an Assay Cascade for Complex Medicines

Dr Tilly Bingham, VP Science
Concept Life Sciences, a Malvern Panalytical brand

What Do We Need to Consider?

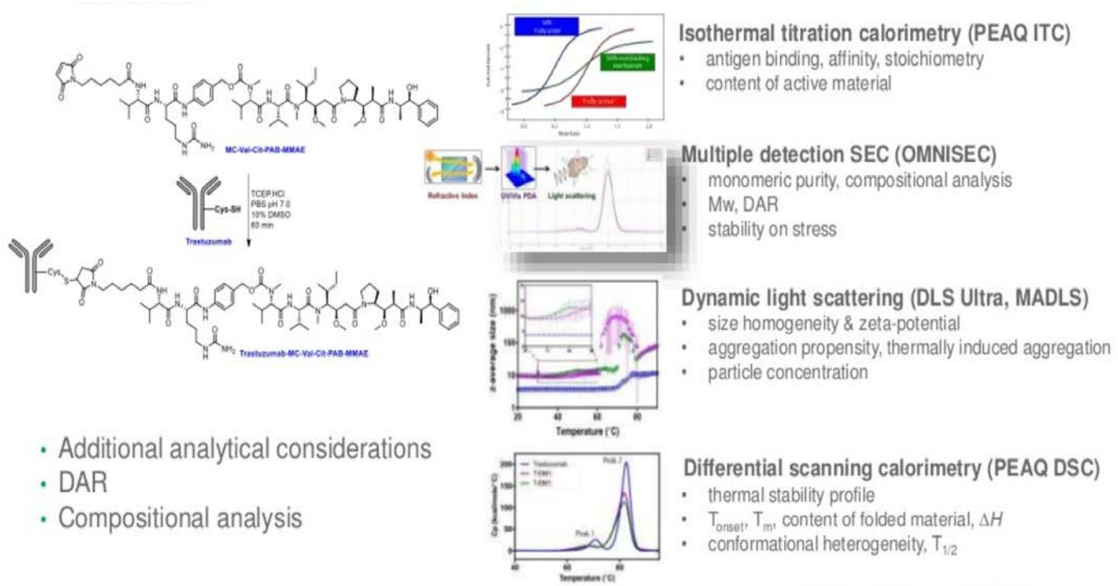
The development of assay cascades for complex medicines follows similar principles to 'traditional' medicines. The cascade needs to incorporate predictive models indicating the medicine's performance in humans, based on the planned route of administration and formulation. It should consider the best *in vitro* and *in vivo* models to confirm the mechanism of action (MoA) and the desired phenotypic effect, as well as the absorption, distribution, metabolism, excretion and toxicology of the complex medicine. The cascade culminates in a suitable PK/PD model in a disease-relevant animal model, which is linked to the disease in humans via a suitable translational biomarker.

For a complex medicine, developing assays to answer these questions can be very challenging. How will the complex medicine be made and analysed to ensure the reproducibility of what is tested in the assay cascade? Is this analysis translatable for use in *in vivo* studies? How is the developability assessed and evaluated? Aggregation propensity and thermal stability are examples of medicine developability characteristics that need to be considered during the optimisation of a complex medicine.

Cascade Assay Case Studies

Antibody Drug Conjugates (ADCs)

This example shows the bioconjugation of a cytotoxic warhead to trastuzumab, an HER2 receptor targeting antibody in breast cancer. The graphic below highlights the importance of selecting suitable analytical techniques for characterising the prototype medicine. In addition to properties such as antigen-binding (affinity and stoichiometry), the monomeric purity and drug antibody ratio (DAR), measured with a technique such as mass spectrometry or SEC-MALS, are defining characteristics of ADCs. When evaluating the developability of the ADC, analytical techniques such as Dynamic Light Scattering (DLS), to assess size homogeneity and aggregation propensity, together with DSC assessment of thermal stability, are also important.



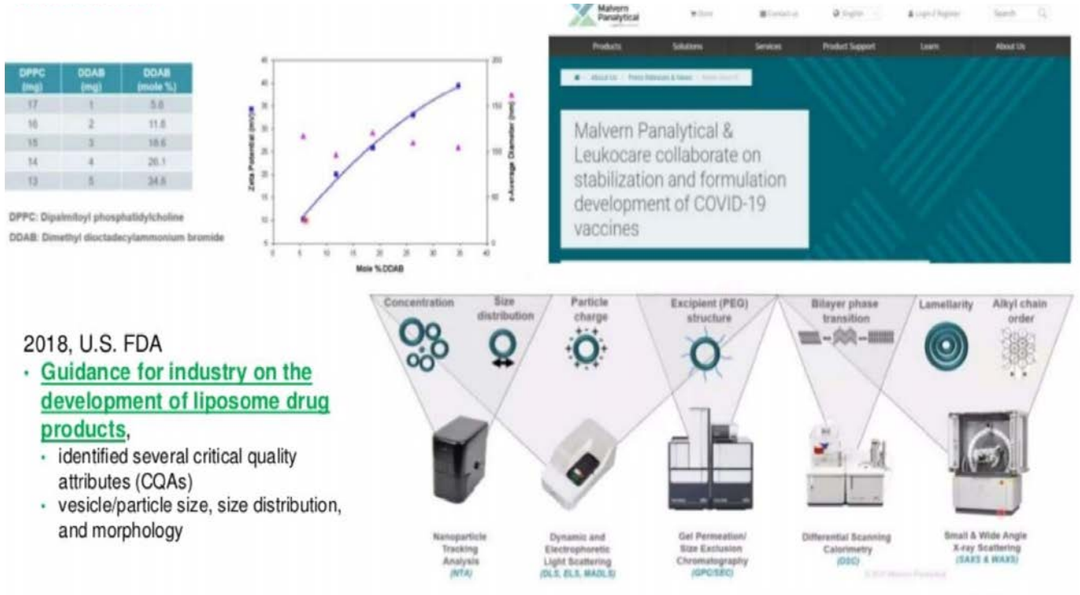
Liposomes, LNPs and RNA Delivery Systems

A topical example can be used to demonstrate the more complex analytical techniques used to characterise potential liposome drug products, recently brought to the fore due to COVID-19.

Of importance are the techniques employed to assess the charge of the lipid composition, which is relevant for cellular uptake, tissue targeting and clearance.

The US Food and Drug Administration (FDA) has issued guidance on the properties and physicochemical attributes which are important in the development of liposomal-complex medicines.

The take-home message when developing assay cascades for complex medicines is that although the questions the assay cascade needs to answer are the same as for 'traditional' medicines, the analytical characterisation of the complex medicine becomes a much more important consideration. It is essential to ensure that what is tested is fully characterised and that the data can be reproducibly generated. There is also a need to be forward-thinking in terms of the developability of the medicine, with an eye on future manufacturing requirements.



Dr Tilly Bingham, VP Science, Concept Life Sciences

Tilly Bingham has over 18 years' experience in the pharmaceutical sector in 'large pharma' (Organon, Schering-Plough, MSD), biotech (Redx Pharma) and currently with contract research organisation Concept Life Sciences. Tilly's early career was spent working in CNS therapeutic areas. More recently, as Head of Research and Operations at Redx Pharma, she was involved in the discovery and development of oncology and fibrosis clinical and pre-clinical candidates, including the porcupine inhibitor RXC004 and BTK inhibitor LOXO-305. Tilly is currently overseeing multiple programmes covering the pharmaceutical, agrochemical and petrochemicals sectors.

Complex Medicines:

Cellular Internalisation and Trafficking of Complex Medicines

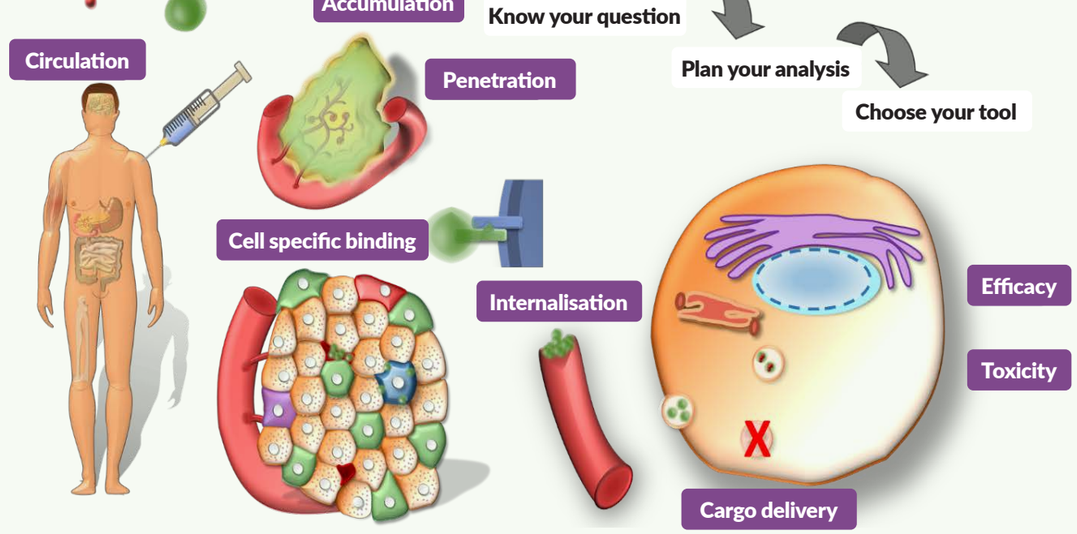
Dr James Szczerkowski, Postdoctoral Scientist in Advanced Microscopy
Medicines Discovery Catapult

Answering Complex Medicine Questions with Microscopy Techniques

Complex medicines open up the possibility of targeting previously undruggable targets with an improved therapeutic index and better safety. However, to achieve this unique challenges need to be overcome.

In simple terms, there is the cargo (the drug) and a delivery technology that needs to be stable and capable of circulating in the body, to the target tissue. The drug needs to accumulate at the target and have enough diffusional potential to penetrate further than the first layer of cells, or to bind to a specific part of the cell if a receptor has been used in the delivery technology. Once the cargo has been internalised, it is important to know how much has been internalised, what is the effect on the cell – specifically whether it has the intended effect or whether it is toxic - and whether this toxicity is because of the drug or the delivery technology.

Microscopy a Powerful Tool to Medicine Interact with a Biological System

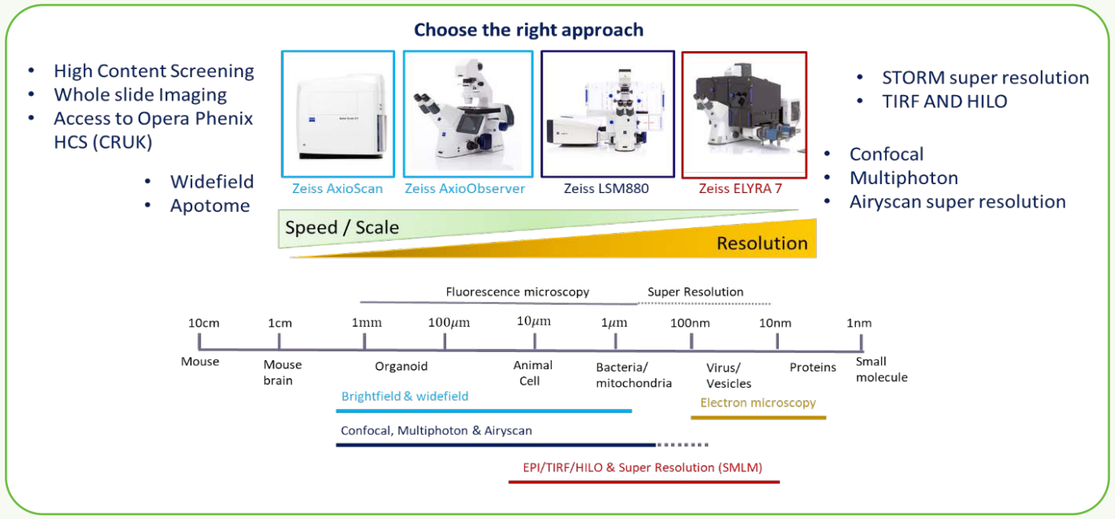


Planning an Analysis

What are the Questions?

It is key to design the project around the questions that need to be answered. The right approach and imaging equipment need to be selected, which will depend on the scale being examined; the smaller the scale, the higher resolution that will be needed.

MDC Microscopy and Complex Medicines



What are the Goals?

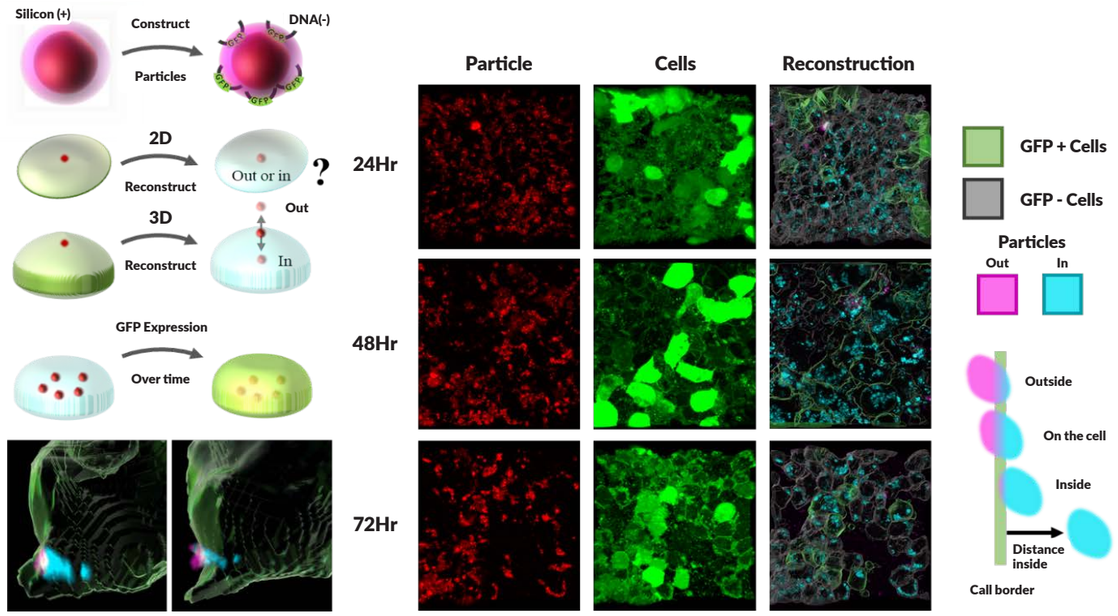
Is the goal to study cell binding, internalisation trafficking or real-time tracking? Will you be looking at tissue or spheroid? Once this has been determined, the analysis method and the equipment can be selected, whether a simple hit-or-miss assay, a co-localisation analysis, 3D reconstruction of the whole cell or real-time tracking with live cells.

Case Study - *in vitro* Analysis of Silica Nanoparticles

The study evaluated the internalisation of silica nanoparticles and the desired effect. Positively charged silica nanoparticles and plasmid DNA encoding green fluorescence protein (GFP) were combined. The negative DNA is electrostatically attracted to the positive silica nanoparticles. The bound particles were incubated with the cells, and the efficiency of uptake, and any subsequent GFP expression, was determined using confocal microscopy.

The cell mask was defined, and the cells and silica nanoparticles reconstructed. Using 3D microscopy, it was possible to determine if the silica nanoparticles were inside or outside the cell by linking the internalised particles to GFP expression, with images taken at different time points. Basic questions include how many particles are internalised; when and how are they internalised; and was the cargo, in this case GFP protein, successfully released and expressed? As the analysis was in 3D, it was possible to zoom into the area of interest, i.e. GFP cells, to visualise where the particles were within this region.

N4 Pharma - Nuvec Nano Particle



Dr James Szczerkowski, Postdoctoral Scientist, Medicines Discovery Catapult

James Szczerkowski is a Postdoctoral Scientist in Advanced Microscopy with Medicines Discovery Catapult, helping support projects through experiments design, image acquisition and analysis.

James acquired a PhD from King's College, London, defining the dynamics of mitochondrial networks during Wnt3a-mediated asymmetric cell division of mouse embryonic stem cells. James utilised a myriad of microscopy techniques and developed custom scripts for image analysis and data extraction. From automated pipelines to high throughput screening, his work focused on 3D cellular reconstruction, subcellular distribution and tracking, and the characterisation and morphology changes of cells in real-time, as they interact with their environment.

CATAPULT Medicines Discovery

Complex Medicines:

CryoEM in Characterisation and Quality Control of Complex Medicines

Dr Rebecca Thompson, Head of Faculty Biological Sciences Research Facilities and Deputy Director
Astbury Biostructure Laboratory, University of Leeds

Cryo-electron microscopy (CryoEM) is a method that images specimens preserved in a very thin film (50–100nm) of vitreous ice. This preserves samples in a 'native-like' state, so the subsequent images of biomolecules can be processed to yield 3D reconstructions of macromolecular complexes with close to atomic resolution. CryoEM allows the routine study of complexes ranging in size from 100 kDa to much larger MDa complexes, although structures even smaller and larger have been solved.

Case Studies

Many complex medicines can be studied using a single particle processing approach, which takes the 2D information and turns it into a 3D structure. Below shows images of an icosahedral virus in grey, the subsequent 2D class averages, and its reconstitution into a 3D structure where *de novo* model building is possible.

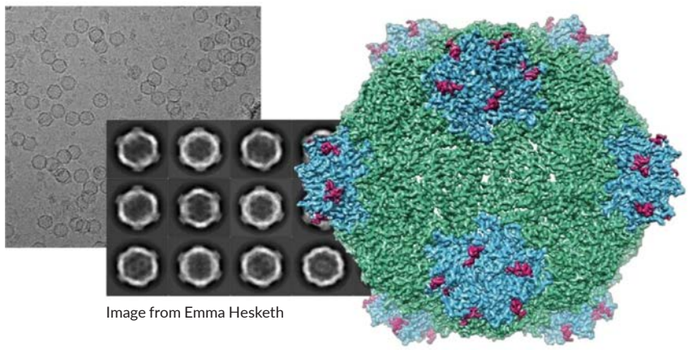


Image from Emma Hesketh

The technique can solve the structure of a wide range of molecules, e.g. target macromolecules with antibody Fab fragments, nanobodies or protein binders bound to a target. This enables scientists to understand and rationalise the inhibitory effects of a molecule mechanisms by which viruses might bind proteins, generate statistics on whether adeno-associated viruses used as gene delivery vectors contain the cargo, or demonstrate heterogeneity of samples.

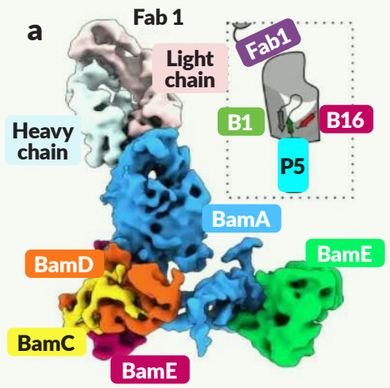
For the characterisation of membrane-bound structures such as liposomes or exosomes, Cryo electron tomography can be applied. This technique allows multiple images to be taken at different angles and tomograms to be produced. These images can show the morphology of the exosome or liposome, and also show how heavily the particle is packaged, which can help the understanding of liposome or exosome delivery to a cell.

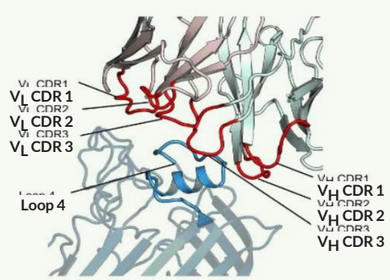
CryoEM can be used to give a rapid idea of morphology, size and distribution of delivery vehicle and cargo in the characterisation of complex medicines.

The next generation of detectors are providing new opportunities for rapid data acquisition, reducing the time and therefore cost from sample to structure.

Fab Fragment Binding

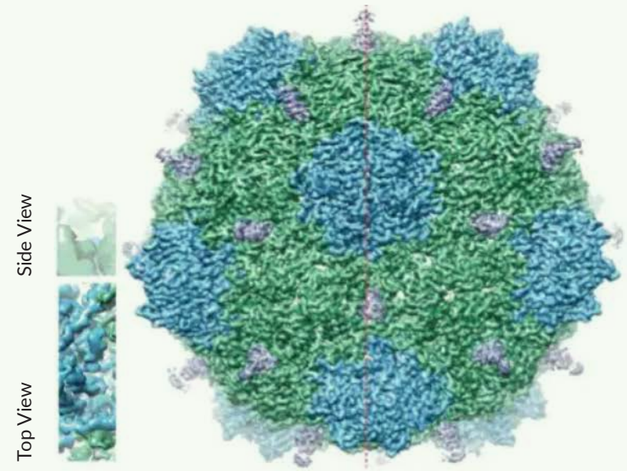
- BAM function is dependent on conformational switching between and 'open' and a 'closed' conformation
- Solving the structure of a BAM:Fab complex indicated that the Fab had locked BAM in the 'open' conformation, rationalizing its inhibitory effect





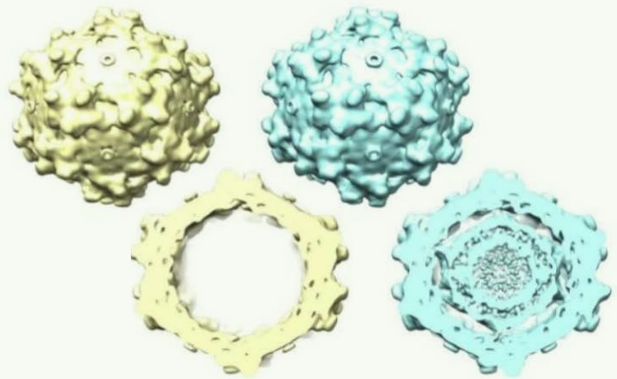
Affirming Binding

- Plant viruses can cause devastating losses to agriculture and are therefore a major threat to food security
- The rapid identification of virally-infected crops allowing containment is essential to limit such threats, but plant viral diseases can be extremely challenging to diagnose
- Structure was solved of affimers (artificial binding proteins) and a model plant virus Cowpea mosaic Virus



Adeno - Associated Virus

- Pre-clinical and clinical successes in AAV-mediated gene replacement, gene silencing and gene editing have helped place AAV as the ideal therapeutic vector
- Empty capsids can range from 20% to over 98% in vector preparations when produced by standard transient transfection production pipelines
- Full:empty ratios need to be characterized



Dr Rebecca Thompson, Head of Faculty Biological Sciences Research Facilities and Deputy Director, Astbury Centre

Rebecca Thompson oversees the management of a range of research facilities, including CryoEM capabilities, with two state-of-the-art Titan Krios microscopes. Rebecca's current research interests span CryoEM and include developing and optimising workflows for high-resolution structure determination of macromolecular complexes by single particle analysis and using cryo-electron tomography to image cells and organelles.

Complex Medicines:

Understanding the Interplay Between Complex Medicines and Biological Systems

This webinar considered how to develop a molecule with the potential to be a medicine. As with small molecule compounds, complex medicines also interact uniquely with environmental and biological systems. These interactions can affect stability, exposure, biodistribution and the potential for toxicities such as immune cell activation, and therefore need to be fully characterised to deliver efficacious medicines with minimal toxicity.

Dr Zahra Rattray described a bioanalytical toolbox for characterising and analysing the protein corona, and how this affects the complex nanomedicine's interaction with the surrounding environment and influences its fate in the body. Jayne Lawrence focussed on model biomembranes and how these can be used to understand and optimise the delivery of therapeutic cargoes to the cell. Finally, Robert Wheller described the challenges of measuring pharmacokinetics (PK), stressing the importance of bioanalysis techniques which can measure the exposure of the entire complex drug, the cargo or the delivery vehicle.

Complex Medicines:

The Early Assessment of Prototype Nanomedicine Nano-bio Interactions - 'Where Nano Meets Bio'

Dr Zahra Rattray, Chancellor's Research Fellow
Strathclyde Institute of Pharmacy and Biomedical Sciences, Laboratory of Complex Bioanalysis
University of Strathclyde

What is a Protein Corona, and Why Measure It?

Complex medicines and nanomedicines are not inert systems, they are reactive particles that interact with their surrounding environment. When introduced to biological media such as blood, the interaction of nanomedicines with their surrounding environment results in the spontaneous surface adsorption of biomolecules, altering their biological and chemical identity. This is referred to as the protein corona. The protein corona has a fundamental impact on a nanomedicine's physicochemical properties and its unique interactions with biological systems. The protein corona can affect immune cell activation, circulation time and biodistribution, the colloidal stability of nanomedicines and the formation of particle depots influencing drug release.

Early characterisation of nanomedicine critical quality attributes, and their impact on interactions with biomolecules, can inform the design of novel, safe and efficacious nanomedicines. Bioanalytical tools exist that can be used to predict or characterise the protein corona at multiple scales, including *in silico* tools (e.g. atomistic or molecular dynamics modelling). Cellular models (*in vitro*), pre-clinical models (*in vivo*), and human studies can be used to measure the biological fate of nanomedicines at different scales, each providing a different level of information on their performance.

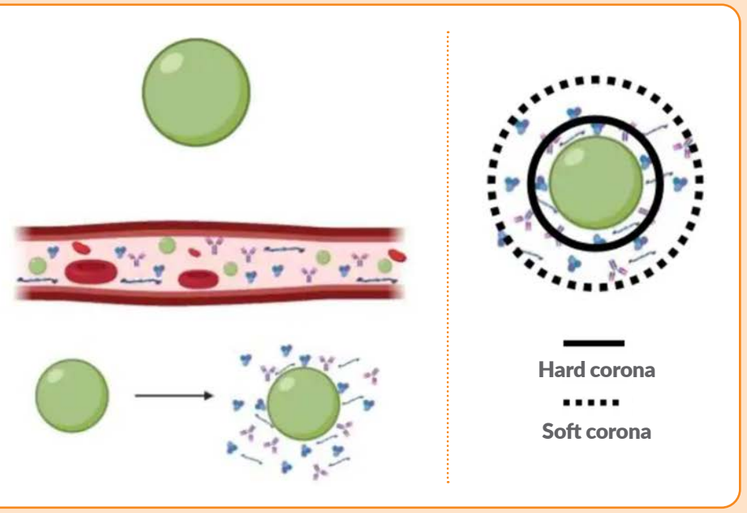
The Biological Fate of Complex Medicines

Particle Properties

- Composition
- Surface charge
- Morphology
- Size
- Cargo
- Concentration

Environmental Parameters

- Incubation media
- Protein content and composition
- Duration
- Temperature
- Flow rate



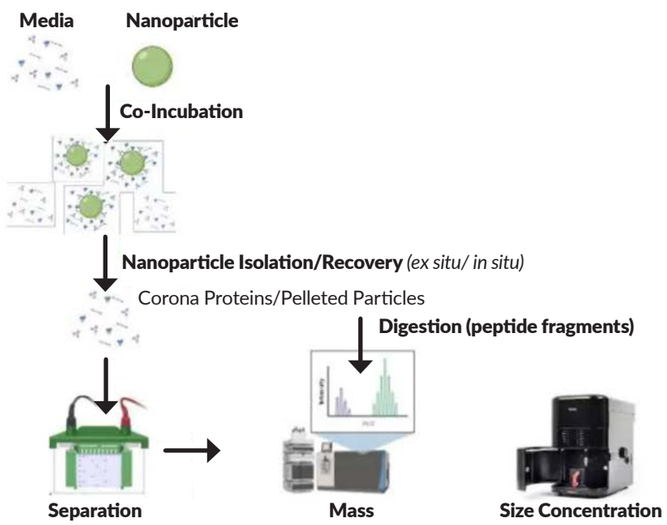
The Protein Corona *in vitro* and *in situ* Bioanalytical Toolbox

A typically reported pipeline for analysing the protein corona, following nanomedicine incubation with protein containing or cell culture media, is shown below. Nanoparticle isolation methods are routinely used to measure the protein corona. However, in recent years *in situ* or minimally disruptive analytical techniques are increasingly being implemented in the analysis of the nanomedicine protein corona.

Recent advances in field flow fractionation (FFF) technologies and capabilities have seen increased implementation of this technology for the high-resolution analysis of nanomedicine interactions with biological media. FFF overcomes the challenges associated with nanomedicine isolation from incubation media, and through its hyphenation with multiple analytical detectors, can provide a multiparametric assessment of the influence of the protein corona on nanomedicine physicochemical parameters.

In addition to advancements in analytical technologies and capabilities, increasing attention is being drawn to the biological relevance of conditions under which the protein corona is being measured. In the era of precision medicine, high-throughput microarray microfluidics based analyses, which mimic biorelevant conditions, and their multiplexation with analytical capabilities and multiscale models, can be used to predict and measure the protein corona. Measurement of the protein corona and its subsequent impact on nanomedicine safety and efficacy is critical for delivering nanomedicines with minimal toxicity.

The Protein Corona Bioanalytical Toolbox - *in vitro*
Reported for metallic, inorganic, polymeric, etc



Structural
- Circular Dichroism/FTIR
- Raman
- NMR

Quantification
- Protein assays
- LC-MS/ICP-MS
- Imaging TEM/cryo-TEM

Identification
- MS
- SDS-PAGE
- ELISA

Affinity/Dynamics
- ITC/QCM

Dr Zahra Rattray, Chancellor's Research Fellow, University of Strathclyde

Zahra Rattray is an interdisciplinary translational pharmaceutical scientist with over ten years' experience of working in the academic, industry and clinical sectors, developing a diverse molecule portfolio. Zahra received her PhD in Drug Delivery from the University of Manchester in 2013 and completed a postdoctoral research position at Manchester, developing new analytical pipelines for profiling antibody drug product stability.

Zahra has significant formulation experience from her time at AstraZeneca Pharmaceuticals as both a pre-clinical and late-stage formulation scientist. Zahra completed a postdoctoral research position at the Yale School of Medicine, in partnership with Patrys Ltd, where she explored the potential of cell-penetrating autoantibodies as DNA damage repair agents for the treatment of glioblastoma, and as targeting ligands for drug and gene delivery systems. Zahra is currently a Chancellor's Research Fellow at the University of Strathclyde, where her team explores the development of bioanalytical measurements for profiling the nanoparticle protein corona and the role of nuclear import in cancer progression.

University of Strathclyde

Complex Medicines:
Interaction of Colloidal Gene Delivery Vehicles with Model Biomembranes

Prof. Jayne Lawrence, Head of the Division, Division of Pharmacy and Optometry
University of Manchester

MANCHESTER 1824
The University of Manchester

The interaction of colloidal gene delivery vehicles with model biomembranes are described here as an exemplar of the interaction of any nanoparticulate system with any cell membrane.

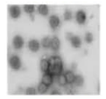
Preparation of Lipoplexes

Gene delivery vehicles such as lipoplexes can interact with both early and late-stage endosomal membranes. The lipoplexes studied here were prepared by mixing vesicles containing cationic lipids, e.g. DODAB, in the presence or absence of the lipid DOPE, with the nucleic acid, e.g. DNA.

Ideally, drug delivery vehicles should be designed to enter the cell by phagocytosis or endocytosis but which can also escape the endosome before it matures into the lysosome - "the dustbin of the cell". The interaction between the lipoplexes and the early- and late-stage endosomes can be studied using a range of different techniques and model endosomal membranes, which mimic both the early and late endosomes to determine if differences exist between them.

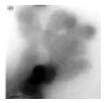
Lipoplexes (LD)

Vesicles mixed with nucleic acid



vesicles

DODAB with/ without a 1:1 molar ratio of DOPE



lipoplexes

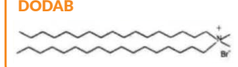
ct DNA

0.1 mg mL^{-1} of ct DNA

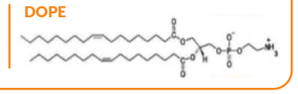
DODAB : ct DNA charge ratio of 2:1

Vesicle Composition

DODAB



DOPE



Lipoplex Properties

Preparation	Apparent hydrodynamic size (nm)	Zeta potential (mV)
DODAB/ DODAB-DOPE Vesicles	**61-84**	**47-53**
DODAB/ DODAB-DOPE Epoplexes	**110-120**	**36-49**

Preparation using deuterated DODAB or D_2O substituting for H_2O made no difference to physico-chemical properties

Example Techniques

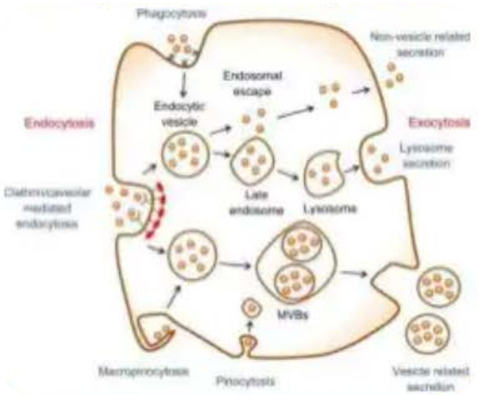
- **Langmuir trough experiments** measure the surface tension in the presence of lipids, and here shows that the surface tension changes measured suggests that DOPE enters the endosomal monolayers
- **Brewster angle microscopy** allows half the surface of the endosomal membrane to be visualised so that its interactions with the lipoplexes can be observed. This shows that the separation of lipids in the endosomal monolayers increases in the presence of the lipoplexes
- **Specular neutron reflectivity** can detect components interacting with the model endosomal membranes and quantifies the number entering the monolayer. Here DODAB is seen to enter the endosomal monolayers and is increased in the presence of DOPE

The interaction of lipoplexes with giant unilamellar vesicles containing fluorescent dye, and visualised *in situ* by confocal microscopy, reveals that DOPE enters the membrane and alters its integrity of the membrane, allowing DNA to be translocated into the vesicle.

The above study shows there is a differential interaction with the lipoplexes in the presence or absence of DOPE, and a difference in the interaction with early and late endosomal monolayers.

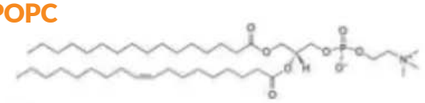
In conclusion, different techniques are used to obtain different information, and using several techniques in combination can provide a good picture of the system being characterised.

Cell Uptake of Nanoparticles



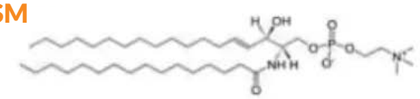
Model Endosomal Membranes

POPC

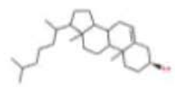


DOPE

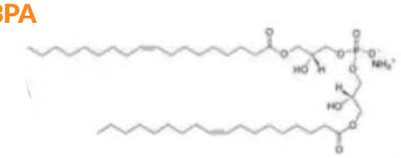
SM



CHOL



LBPA



Composition of the Model Endosomal Membranes

Composition (mo1%)	EEM	LEM
POP C	**40**	**61**
DOPE	**20**	**16**
Spingomyelin (SM)	**6**	**-**
Cholesterol (CHOL)	**34**	**17**
Lysobisphosphatidic acid (LBPA)	**-**	**6**

Prof. Jayne Lawrence, Head of the Division of Pharmacy and Optometry, Manchester University

Over the past 25 years, Jayne has pioneered the use of neutron scattering techniques in pharmaceutical science. Before then, with the exception of neutron crystallography, neutron scattering was not used in the pharmaceutical science community. However, it is now gaining an increasing acceptance amongst both pharmacy academics and pharmaceutical industrialists as an invaluable tool in answering a range of important questions that cannot be answered by any other available techniques. For example, Jayne has successfully used neutron diffraction to elucidate the organisation of lipids in models of the stratum corneum of human skin and as a means to understand the selectivity of the antifungal drug amphotericin B, towards fungal verses mammalian membranes.

Jayne's use of small angle neutron scattering has helped in the formulation of microemulsions with enhanced drug solubilising capacity and offered an explanation of how polymers help to stabilise drug nanoparticles. Most relevant for this webinar is how neutron reflectivity has been invaluable in helping to determine how gene delivery vehicles, and the nucleic acid they contain, interact with cell and endosomal membranes, thereby aiding the design of improved gene delivery vehicles.

The University of Manchester

Complex Medicines:

Importance of PK and Bioanalysis for Drug Development, and Challenges of Bioanalysis for Complex Medicines

Robert Wheller, Associate Scientific Director
LGC

Pharmacokinetics (PK) and Bioanalysis

Measuring PK within medicine development allows the drug or excipient concentration to be linked to efficacious or toxic effects, allowing a safe dosing regimen to be calculated. Well established, robust bioanalytical techniques exist for PK measurement within biological samples, such as LCMS or immunoassay.

Challenges in Complex Medicine PK and Bioanalysis

The increased heterogeneity of complex medicine chemistries and formulations present a challenge for *in vivo* measurement. It is important to understand what the assay measures so that the results have relevance and reflect the state in the biological system. The integrity of the medicine, or formulation through sample collection, storage and extraction, must be considered, and the material used to construct the calibration standards should reflect the drug products.

Case Study - Antibody Drug Conjugate (ADCs)

ADCs, although established in the oncology field, present some interesting bioanalytical challenges. They are a heterogeneous mixture, with numerous payload conjugation sites and biotransformation possibilities leading to many different forms.

Antibody-Drug Conjugate Bioanalysis

ADCs exist as a heterogeneous mixture

- Payload conjugation site
- Biotransformation

Currently no single assay can measure all forms - but subsets can be analysed

Total Antibody

Conjugated Antibody Payload

Free Payload Metabolites

A single assay is not capable of measuring all these forms, so a number of factors are considered when selecting an appropriate assay:

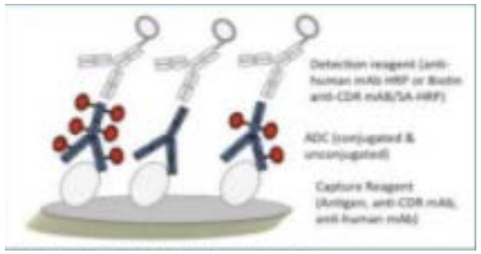
- Different conjugation techniques of the payload
- Linker technology (cleavable/non-cleavable)
- Reagent availability
- Laboratory technology expertise

Possible assay formats include total antibody, free payload, conjugated antibody, conjugated payload, and drug antibody ratios (DAR), as shown below.

ADC Assay Specifics

Total Antibody

- Cannot discriminate if molecule is active
- Typically IA
- Reagents easily sourced
- Consider impact of conjugated payload
  - Asses ACC:naked antibody admixtures



Free Payload

- High potency - need to ensure low exposure
- *Ex vivo* Stability studies
- Typically LC-MS
- Monitor metabolites or with attached linker
- Consider interconversion during extraction

A single assay measurement cannot solve the heterogeneous nature of complex medicines, so careful consideration should be given to what needs to be measured, what can be measured and whether novel approaches are available to help solve these challenges.

ADC Assay Specifics

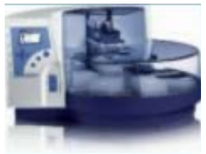
Conjugated Antibody

- Measure by IA
- Reagent availability (Anti-payload antibody)
- Consider sensitivity to the amount of conjugated payload

Conjugated Payload

- Measure by hybrid IA -LC-MS
- Cleavable linker?
- Non-cleavable linker?
  - Protein digestion approach
  - Consider conjugation technique - number of surrogates

- Both will measure active form of drug product
- Measurements will most likely not match
  - LC-MS is sensitive to number of attached payloads
  - Immunoassay likely to not be fully sensitive
- Challenge with controls to characterise conjugate sensitivity



Drug-Antibody Ratio (DAR)

- Measure how ratio shifts across a time profile following dosing
- Measurement can contextualise conjugate assay results
- Qualitative measurement
- Hybrid IA - LC-MS approach
  - Intact analysis using HR-MS
- Approach offers limited sensitivity
  - Only useful after high dose administration

Robert Wheller, Associate Scientific Director, LGC

Robert Wheller has worked in the field of bioanalysis for the last 16 years, starting within the DMPK group at GSK, gaining experience in small molecule, oligonucleotide and protein quantitation using LC-MS/MS and LBA techniques. Robert moved to LGC as a principal scientist, leading a team performing protein LC-MS/MS bioanalysis and, providing technical oversight for these challenging assays. Robert's latest role as Associate Scientific Director focuses on supporting the wider LC-MS/MS bioanalytical group, ensuring the scientific rigour of methods that are developed, directing research projects aligned with the departmental strategy and building external reputation.

Complex Medicines:
A Glance at Novel Drug Delivery Systems

At this midpoint in the webinar series, we heard from the pioneers developing delivery technologies and how they are applying these to create innovative complex medicines. They discussed the advantages offered by the different delivery technologies and the challenges which need to be addressed, with particular emphasis on early consideration of methods for manufacture and scale-up.

Dr Yvonne Perrie introduced the advantages offered by using lipid nanoparticles (LNPs) to deliver RNA, the lipids commonly used and the parameters for manufacture. Dr Louise Coletta discussed therapeutic microbubbles for the targeted delivery of cytotoxic drugs as a way of improving the therapeutic window of oncology treatments, and finally Prof. Helen McCarthy described a unique RALA peptide, which can deliver therapeutic RNA vaccines directly to antigen presenting cells.

Complex Medicines:
Lipid Nanoparticles: So Much More than a Little Fat Blob

Dr Yvonne Perrie, Professor in Drug Delivery
University of Strathclyde

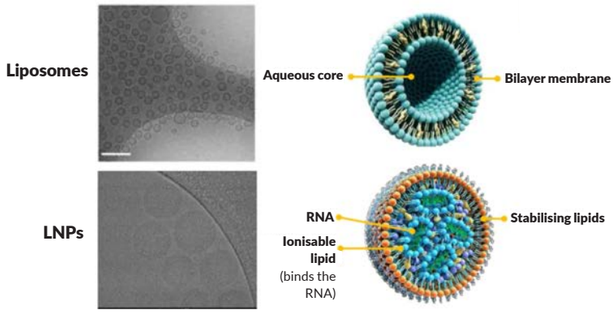
Liposomes and Lipid Nanoparticles

A key component of using RNA in complex medicines is that it only needs to enter the cytoplasm, whereas DNA requires a plasmid which needs to reach the nucleus. However, RNA cellular uptake can be impaired by degradation, as well as its anionic and hydrophilic nature, which limits passage through the cell membrane. As a result, an effective delivery vehicle is required.

Liposomes and lipid nanoparticles (LNPs) offer several advantages as delivery vehicles for both RNA and DNA. Liposomes feature a lipid bilayer with an aqueous core – the drug can be encapsulated in either. LNPs, like lipids, have a cationic lipid which binds the RNA, although they have a non-aqueous pore and are much smaller, at just 50nm.

An effective LNP offers protection from degradation, high potency, delivery and cellular uptake of the mRNA, and appropriate biodistribution to minimise toxic side effects. It should also be manufacturable with the ability to scale up, which should be considered early in the drug development programme.

Liposomes and LNPs

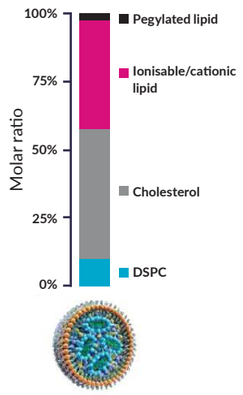


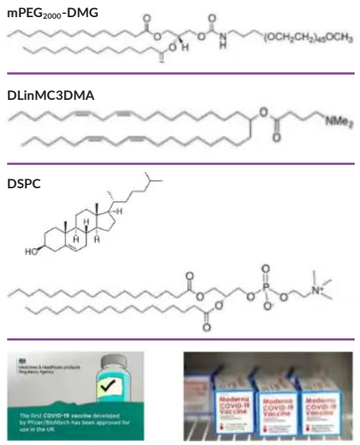
Commonly Used Lipids and LNP Production

Cationic/ionisable lipids such as DOTAP, DLin-KC2-DMA and DLin-MC3-DMA are commonly used. The latter two have an apparent pKa of 6.4–6.7 and can bind anionic RNA when combined with stabiliser lipids. When the pH is raised to 7.4, the outside of the LNP becomes neutral.

The parameters for manufacture and scale-up to GMP are described above. Central to LNP production include mixing the components using the staggered herringbone mixer or toroidal mixer, the ratio of the aqueous phase and ethanol, and the flow rate. Critical quality attributes to consider are size, PDI and zeta potential of the LNP, lipid yield, loading and release profiles, which should lead to good potency, appropriate biodistribution and *in vivo* efficacy.

Combine these with Stabiliser lipids:





Dr Yvonne Perrie, Professor in Drug Delivery, Strathclyde Institute of Pharmacy and Biomedical Sciences, the University of Strathclyde

In 1998, Yvonne gained a PhD from the University of London for her investigation into the role of liposomes for DNA vaccine delivery. Yvonne then worked in London with the drug delivery company Lipoxen Technologies for two years, developing their liposome drug delivery platform technology prior to moving into academia to set up her own research group.

Yvonne joined Aston University in 2000 and was appointed Professor in Drug Delivery in 2007, and was Head of the Aston Pharmacy School from 2009 – 2016. In 2016, Yvonne moved to the University of Strathclyde as Professor in Drug Delivery and is a Vice Dean (Research) for the Faculty of Science. Yvonne's research is multi-disciplinary and focuses on the development of nanomedicines to facilitate the delivery of drugs and vaccines, thus providing practical solutions for current healthcare problems.

Complex Medicines:
'Bursting with Promise' - Precision Drug Delivery with Therapeutic Microbubbles

Dr Louise Coletta, Group Leader
St James's University Hospital

Therapeutic microbubbles for targeted, triggered drug delivery of cytotoxic drugs have been developed to try and improve treatment of colorectal cancer and liver metastases.

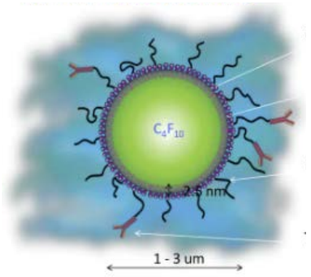
What are Microbubbles?

Microbubbles are gas encapsulated lipid spheres of ~2µm in size. A therapeutic microbubble for targeted, ultrasound-triggered drug delivery is composed of a liposome attached to the phospholipid shell of a microbubble. When injected intravenously, the microbubble uses the circulatory system to locate and bind the tumour blood vessels via the targeting molecule on its surface. Once bound, an ultrasound destruction pulse is delivered, which bursts the microbubbles. This system provides a better therapeutic index, with less offsite toxicity.

To investigate how therapeutic microbubbles release their drug and improve PK/PD responses, microbubbles were manufactured using microfluidic on-chip technology. The addition of the liposomal payload and the targeting antibody produced therapeutic microbubbles. Using three different payloads - luciferin, Irinotecan and its metabolite SN38 - the physical properties of the therapeutic microbubbles, namely their binding capability, drug release and ultrasound characteristics, were assessed.

Optimising Therapeutic Microbubbles

1. The Microbubble



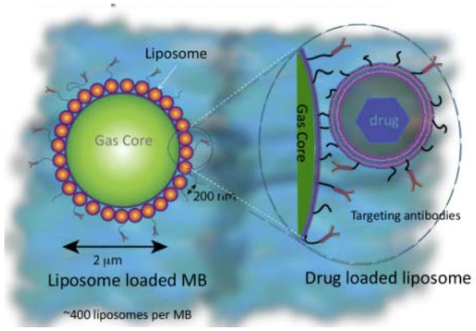
Gas Core
Low solubility

Lipid Shell
Resistance to gas diffusion
biocompatibility

PEG Layer
Improved biocompatibility

Targeting Ligand
Antibody, aptamers, peptides target MBs

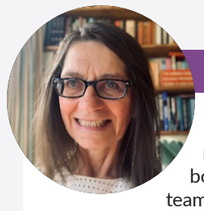
Therapeutic Microbubbles



How Does this Work?

In vivo biodistribution and metabolism experiments with therapeutic microbubbles demonstrated that the cytotoxic drug irinotecan, and its metabolite SN38, were only detected in tissue which had received the therapeutic microbubbles with the ultrasound trigger, and reduced tumour volume was apparent. Encapsulating the more potent SN38 saw greater inhibition of tumour growth.

Therapeutic microbubbles can improve the therapeutic index of cytotoxic drugs by increasing the percentage of the injected dose that reaches the tumour, whilst limiting the bioavailability in normal tissues.



Dr Louise Coletta, Group Leader, Leeds Institute of Medical Research, St James's University Hospital

Louise Coletta's interests lie in the molecular mechanisms of disease and the development of novel cancer therapeutics. Louise's group focuses on pre-clinical evaluation and translation using both *in vitro* and *in vivo* models. In recent years, Louise has worked in a multidisciplinary team with physicists, engineers, clinicians and chemists engaged in the development of therapeutic microbubbles for targeted, triggered drug delivery.

Complex Medicines:
Under the Radar: Alternative Delivery of mRNA Vaccines

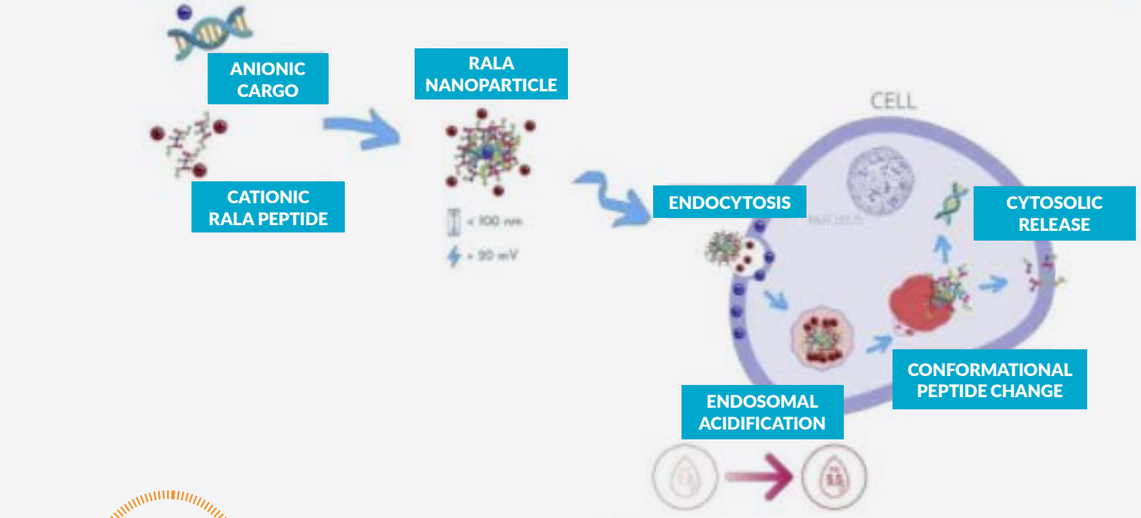
Prof. Helen McCarthy, Chair of Nanomedicine in the School of Pharmacy at Queen's University Belfast and CEO
pHion Therapeutics

What is RALA?

RALA is a thirty amino acid peptide which is positively charged, with an arginine backbone, hydrophobic leucine and cysteine residues that bind the mRNA and form a nanocomplex. RALA can enter antigen presenting cells without changing the receptor status on the cell so that entry is undetected. Particles enter by clathrin-mediated endocytosis, and once inside, the pH drops and the RALA motif changes confirmation and fuses with the endosomal membrane. This allows a highly efficient escape of the cargo in antigen presenting cells, evoking a CD8 T cell response.

Peptide mRNA nanoparticles are specifically designed to enter the antigen presenting cells that reside in the dermal layers of the skin intradermally. Using genetically engineered mice, it has been demonstrated that RALA mRNA nanoparticles do not activate an innate immune response. RALA also stabilises mRNA, so that it can be stored at room temperature.

Technology Enters Cell Without Toxicity



Pre-clinical HPV Vaccine Development

RALA/Cy5-mRNA nanoparticles injected into the ear of mice intradermally were shown to migrate from the site of administration to draining lymph nodes in the neck. A high percentage of the cargo, two hours post injection, was seen in the intradermal CD11c+ antigen presenting cells; this decreased after 96 hours. Further studies using splenocytes stimulated with the E7 peptide confirm a strong CD8 response. This response has translated into therapeutic tumour models.

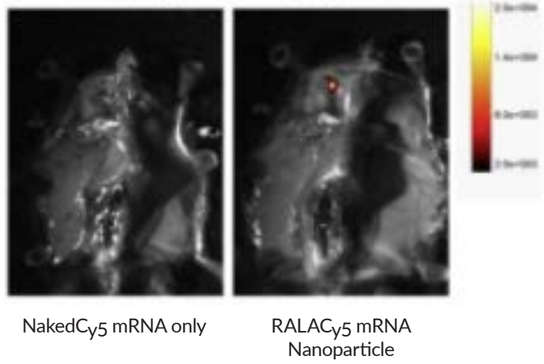
Why HPV?

Whilst vaccines exist to prevent HPV, therapeutic options for those with the virus are limited. 90% of HPV infections are caused by HPV16 and 18, and these lead to a high incidence of cervical cancer. Further work at pHion Therapeutics using HPV 16 and 18 has shown the CD8 response leads to regression of those tumours.

Plans for clinical trials and manufacture are now in place. Using a nano assembler, it has been possible to show that microfluidics can be used to scale-up the manufacture of these RALA/mRNA vaccines ready for the clinic.

RALA/mRNA Therapeutic Vaccines

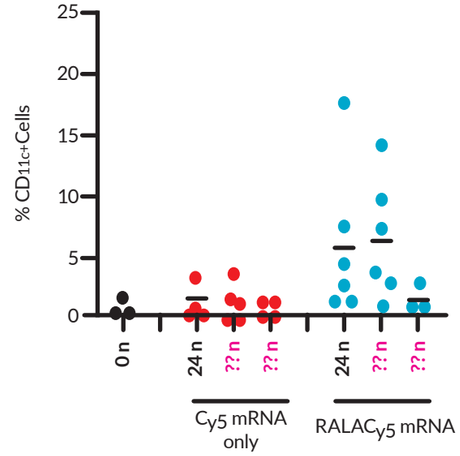
RALA/C_{y5}-mRNA nanoparticles migrate from the site of administration to draining lymph nodes in the neck (imaged at 2h post-administration)



NakedCy5 mRNA only

RALACy5 mRNA Nanoparticle

RALA/C_{y5}-mRNA enter $CD11c^+$Antigen Presenting Cells



Prof. Helen McCarthy, Chair of Nanomedicine in the School of Pharmacy at Queen's University Belfast and CEO, pHion Therapeutics

Helen McCarthy's research team has focused on the development of non-viral delivery systems for nanomedicine applications. These biomimetic peptide systems are designed to overcome the extra and intracellular barriers so that the macromolecular payload can be delivered at the destination site, in order to exert the optimal therapeutic effect. The widespread utility of these delivery systems has led to a spin-out company, pHion Therapeutics, which is currently developing a pipeline of peptide/mRNA vaccines towards the clinic.

Complex Medicines:
Understanding Safety and Efficacy

Project failure due to unacceptable safety, or a lack of clinical efficacy during development, remains an issue for many drug-development programmes.

Testing the safety of complex medicines follows the same principles as any medicine but is complicated by the potential for toxicities associated with multiple components in the test item.

As for any new medicine, target validation is required to build confidence in the biological hypothesis and is typically confirmed in animal disease models, where challenges can arise due to the formulation of complex medicines, understanding exposure and selecting suitable models to test targeted delivery.

In this session, a panel of experts described how to establish pre-clinical safety and efficacy data for complex medicines. Dr Jenny Worthington discussed how to plan an *in vivo* efficacy study for a complex medicine, while Dr Juliana Maynard used case studies to describe how imaging technologies can be used to determine whether the medicine has reached the target tissue. Richard Knight ended the session by outlining the three principles of safety "Target, Chemistry and Patient" which can be applied to any drug modality.

Complex Medicines:
Determining Efficacy *in vivo*

Dr Jenny Worthington, Co-Founder and Director of Science
AxisBio

Taking Complex Medicines *in vivo*

There are four main steps involved in the pre-clinical analysis of complex medicines, which are largely the same as those involved for standard molecules:

1. **Pharmacokinetics** (PK) of the complex medicine
2. **Biodistribution**
3. **Efficacy** studies with multiple doses
4. Dedicated **toxicity** studies

Pharmacokinetic Questions and Considerations

Consideration needs to be given to circulation, stability, and clearance. Questions such as differential blood flow rates; blood components that the complex medicine may encounter; stability of the complex medicine, not only *in vivo* but also for samples collection; and clearance route all need to be considered. In particular, for complex medicines *in vivo*, the need for expertise on sample collection, and the cost of bioanalysis, should be considered.

Efficacy Studies Consideration

Complex medicine efficacy studies require the same planning steps as those for small molecules, and multiple studies will be required. A good efficacy study will need the best model system and consideration needs to be given to adverse effects, dosing occasions and tissue collection.
For complex medicines, do not underestimate the quantity of material required for *in vivo* studies and plan for the manufacture and cost of a complex material prior to starting the studies.

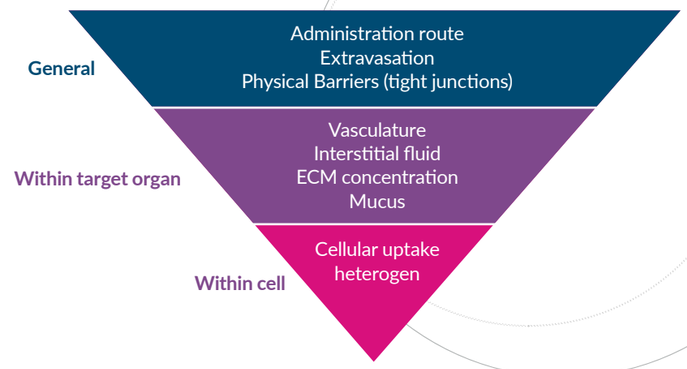
Toxicity Considerations

Do complex medicines have complex toxicology? It is very important that sufficient and appropriate tissue samples are taken from animal studies, in order to address any safety concerns.

In summary, collaboration with different experts to help guide the *in vivo* study work is essential.

Biodistribution Considerations

The graphic opposite highlights areas for consideration. Is biodistribution affected by the route of administration?
Are targeting moieties involved? Are there physical barriers to consider, such as tight junctions? Once within the target organ, how does the complex medicine move around organs with, for example, the presence of mucus and heterogenous tumours, and where vasculature is poor? Are the cells capable of taking up the complex medicine?



Dr Jenny Worthington, Co-Founder and Director of Science, Axisbio

Jenny Worthington gained her PhD from the University of Ulster, Northern Ireland, in the area of cancer gene therapy, and worked through postdoctoral positions before establishing a research team in prostate cancer pre-clinical research. She has used her background in cancer research and drug discovery to move into a commercial setting and has nurtured a team of scientists who provide clients with an excellent pre-clinical service portfolio.

Complex Medicines:

Physiochemical Characteristics of Complex Medicines
New and Novel Approaches to Understand the Pharmacokinetics of Complex Drugs

Dr Juliana Maynard, Lead Scientist - Pre-clinical Imaging
Medicines Discovery Catapult

Physiochemical Characterisation of a Complex Medicine

To facilitate rapid translation into clinical practice, the characterisation and tracking of complex medicines, from the systemic level through to the cellular level, is key. Establishing the relationship between *in vitro* and *in vivo* performance significantly increases the likelihood of clinical success.

MDC offers a broad range of capabilities and expertise in multi-parametric imaging modalities, in addition to having access to animal models and radiochemical and commercial tracers to support other companies with their drug development programmes.

- Home office PPL and radiological licence
- Access to animal models across disease areas
- Access to novel radiochemistry and commercial radiotracers
- Access to pre-commercial technologies
- Multi-model enhanced interpretation of functional and molecular data

Molecular Pathology

- Position Emission Tomography (PET)
- Gamma counting biodistribution analysis
- Computed Tomography (CT)
- High Frequency Ultrasound (HFUS)
- Bioluminescence/NIR imaging (MIS)
- Mass Spectrometry Imaging (DES, MALDI)
- Digital Spatial Profile (Nanostring DSP)
- Advanced Microscopy (super-resolution/confocal/multiphoton)
- Photoacoustic imaging (PIA)

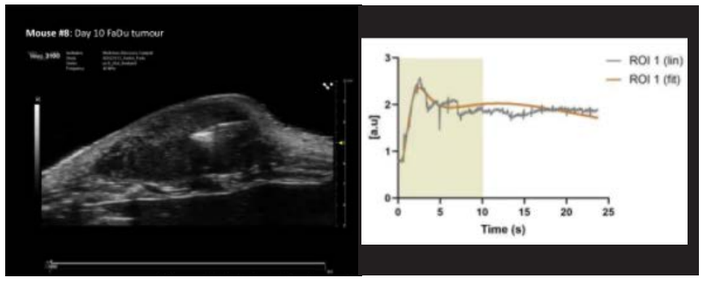
In Vivo/Ex vivo anatomical and functional imaging

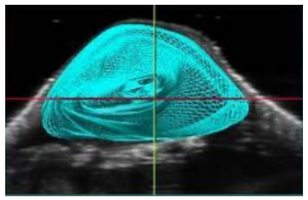
The following **case studies** highlight the support provided to other companies to help them further understand the pharmacokinetics, accumulation and distribution of their complex medicine.

Case Study 1
Xerion Healthcare

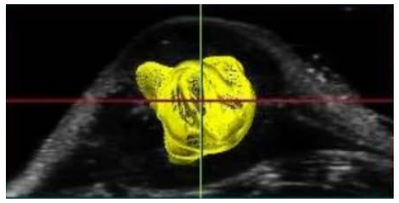
Xerion Healthcare has developed a nanomedicine with significant promise to transform radiotherapy which, whilst effective on cancer cells, also carries a dose-limiting toxicity to surrounding tissues.

Titanium oxide nanoparticles are an optimal size for cancer cell uptake, with the ability to give substantially increased doses of radiotherapy with less toxicity. MDC employed *in vivo* capabilities to determine the distribution and characterisation of the nanoparticles using high-frequency ultrasound to visualise the injected nanoparticles in the tumour and their dispersion. Micro CT was used on extracted tumour cells to visualise the nanoparticles flow in 3D.





Tumour volume - 200mm1



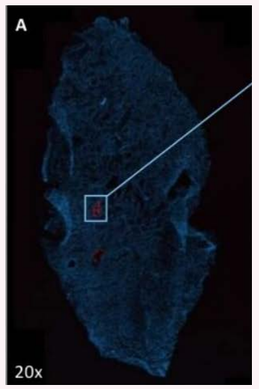
NP distribution - 46.1mm1

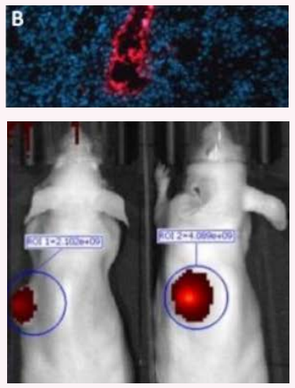
Case Study 2
Sixfold Bioscience

Sixfold Bioscience has developed a programmable oligonucleotide delivery system (PODS) to deliver RNA therapies to specific cancer cells. The PODS consist of a non-toxic RNA scaffold with a targeting ligand, specific to the disease cells, which carries the therapeutic cargo to the tumour. The modular design makes it possible to attach multiple functionalities, ensuring the delivery platform has the potential to work across different areas.

MDC was able to determine proof of concept for delivery, distribution and destination of the PODS. The PODS were labelled with Cy7 dye, injected intratumorally into a mouse model and the signal determined *in vivo* using near infrared imaging. *Ex vivo* examination using microscopy determined which cells the PODS entered, and the PK/PD characterisation was mapped using zirconium (Zr) POD labelled PK assessments.

Using imaging technologies, it is possible to assess the Proof of Concept (PoC) and efficacy of the therapeutic RNAs delivered by PODS in order to confirm tumour shrinkage, and compare this to other delivery mechanisms, platforms and standards of care.



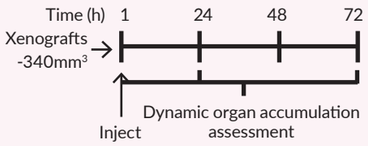


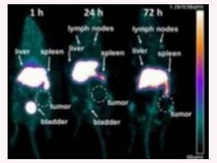
Case Study 3
University of Leeds

The University of Leeds has been working with therapeutic ultrasound-triggered microbubbles to deliver cytotoxic drugs to cells and minimise cytotoxic effects. MDC supported this work with characterisation and Proof of Concept studies. PET imaging demonstrated improved tumour accumulation of the labelled microbubbles *in vivo*, and the liver and spleen accumulation also determined the clearance method. Further imaging validated the mechanism of action and the potential of the platform.

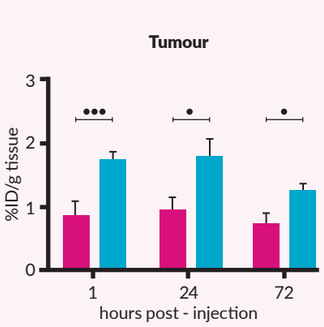
Imaging offers immense potential for drug delivery platforms and non-invasively measures the pharmacokinetics to help drive complex medicines more rapidly into clinical development.

- VEGFR2 LE-SN3B (“Zr-Iposome) 0.4mgkg SN3B n=15
- thMBs+T (“Zr-Iposome on MB+US Trigger) 0.4mgkg SN3B n=15





LE-SN38
thMBs+T



Dr Juliana Maynard, Head of Translational Imaging, Medicines Discovery Catapult

Juliana Maynard has a PHD in neuroendocrinology from the University of Edinburgh and works with a wide range of molecular imaging modalities, including PET, SPECT, CT and ultrasound. Previously, Juliana was Head of Imaging Services at Alderley Imaging and worked at AstraZeneca for nine years.

Complex Medicines:

Do Complex Medicines Present Different Challenges from a Safety Perspective?

Dr Richard Knight, Director and Co-Founder
ApconiX

Is Safety More Complicated for Complex Medicines?

Safety is underpinned by understanding the biological implications, characterising the hazards, and assessing the risk to patients. The more components there are to consider in the test item, the more complex and complicated this becomes.

Safety evaluation is a well-regulated environment with many guidelines available for non-clinical safety studies. Safety assessments should consider the risks that are raised by the novel agents and what data would be helpful in putting that risk in the context of the clinical trial.

Target, Chemistry, and Patient Framework

This framework can be used to identify the key risks for any programme, including complex medicines. On-target pharmacology, selectivity, secondary pharmacological activity of unintended targets, genotoxicity and ADME properties, such as metabolites and routes of excretion, all need to be considered. It is also important to understand the patient context and whether the specific patient population can tolerate the novel therapeutic.

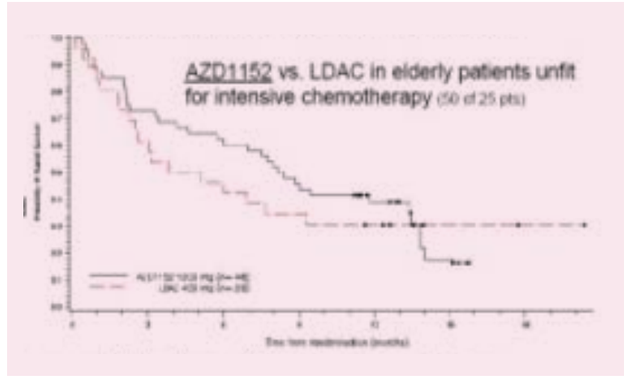
This approach - target, chemistry, and patient - is just as relevant for complex medicines such as nucleic acids, which may be highly selective for target, but have known toxicities associated with their chemistry.

Safety Case Study

AstraZeneca developed an Aurora B kinase inhibitor called AZD1152 with clinical benefit in AML. However, delivery was via a seven-day continuous IV infusion, leading to restricted use with on-target bone marrow toxicity. The development of a slow-release nanoparticle led to localised accumulation and improved clearance, which in turn allowed weekly dosing and a better safety profile.

Encapsulation of the drug in the nanoparticle delivered improved efficacy at a lower dose intensity and reduced toxicity.

As critical attributes were changed to optimise the product, new safety studies were required to characterise the risks with the new product. Despite the altered toxicity profile, overall tolerability was good, and the compound is currently in Phase II studies.



Similarly, nanoparticle delivery is being explored by Blueberry Therapeutics for localised delivery of the antifungal drug Terbinafine, to treat resistant skin infections. Terbinafine has been delivered orally since the 1980s and can cause systemic toxicity. There is a wealth of clinical safety data available for the active drug and also the Nanocin carrier molecule. In this instance, the regulatory agencies accepted the recommendation that no further pre-clinical safety studies on the combined product would be of value. This treatment has now successfully progressed to Phase II. The principles of safety for Target, Chemistry and Patient can be applied to any drug modality.

Physical and Chemical Composition
- Release-controlling polymer
- +/- targeting ligand
- Drug loading
- Counetr-ion
- Particle size distribution
- Surface charge
- Drug release rate

Production process
- Process aids
- Emulsion composition
- Particle formation
- Process controls (pressure, temp. etc)
- Purification

Critical material attributes
- Block architecture
- PEG and PLAM
- Homo-PLA free PEG
- Residual monomer, solvent

Size: ~100 ± 30nm

Drug load ~15%

Similar *in vitro* Release profile

Potential Critical Quality Attributes
- Surface characteristics
- Drug release rate/profile
- Particle size and PDI
- Morphology
- Free drug concentration

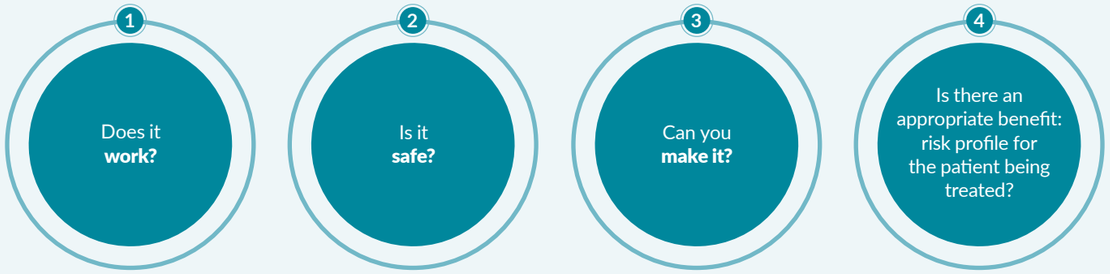
Dr Richard Knight, Director and Co-Founder ApconiX

ApconiX provides nonclinical safety consultancy and ion channel laboratory services, and are based at Alderley Park. Prior to starting ApconiX in 2015, Richard was Senior Director in Safety Assessment at AstraZeneca, with more than 25 years of project experience. He has worked across multiple therapy areas involving small molecules, biologics, proteins and oligonucleotides and has been involved in bringing over 35 new candidate drugs into clinical trials, as well as six to market.

Complex Medicines:

Complex Medicines: Ready for the Clinic / Scaling-up for Success

For any medicine, including complex medicines, four key questions need to be answered before it can gain regulatory approval:



In this final session, a panel of experts described preparation for the clinic, how to formulate and scale-up the medicine and how to navigate the regulatory process.

Dr Claire Patterson outlined the advantages of good formulation and the attributes that are important for biological performance. Emily Port and Graham Worrall then described the innovative and specialised techniques required in the scale-up and manufacture of complex medicines - both talks stressed the need for methods of formulation and manufacture to be in place much earlier in the drug discovery programme than for conventional medicines.

Finally, Prof. Alan Boyd concluded the webinar series by summarising the needs of regulators and how the challenges for a complex medicine may not be the Phase III clinical trial but the manufacturing process.

Complex Medicines:

The Advantages of Good Formulation

Dr Claire Patterson, Senior Principal Scientist
SEDA Pharmaceutical Development Services

Specific Formulation Development Challenges for Complex Medicines

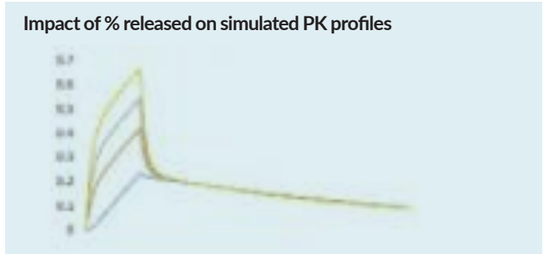
Most complex medicines are administered intravenously, intramuscularly, subcutaneously, or directly into a tumour, and the formulation can play varying roles. For instance, the formulation can be a fairly simple delivery vehicle, in which the active pharmaceutical ingredient (API) is solubilised or dispersed. Or it can be a much more complex carrier system, where the formulation is crucial for delivery to the site of action.

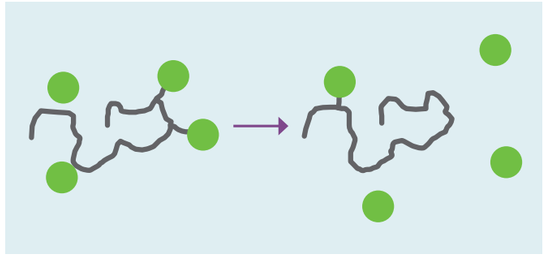
Understanding the attributes of the formulation is key to understanding the biological performance of the product and its manufacturing reproducibility; are particle size, aggregation potential, structure/conformation, surface functionalisation, and release of the API comparable in each batch?

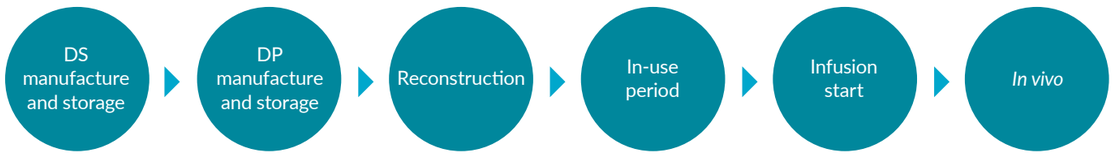
Typical Formulations and Data to Consider

Drug conjugates, including antibody drug conjugates, polymer conjugates or dendrimers, are typically formulated as a sterile solution for injection in a vehicle containing components; this helps to ensure stability, solubility and biocompatibility.

Complex medicine formulation attributes must be characterised early in the development process as they are critical to product performance from a safety and an efficacy perspective. Characterisation techniques can be complicated and are often non-compendial.







e.g. SITUA method

A key challenge can be developing methods able to discriminate between a drug that is conjugated versus that which has been released. API release needs to be considered from manufacture to the point of administration. It is critical that the API is not released prematurely in the formulation vehicle, prior to administration. In addition, the behaviour of the drug conjugates *in vivo* also need to be understood. For instance, how do size and surface properties impact biodistribution and when and where the free drug is released. Both of these can have implications on the efficacy and the safety of the product.

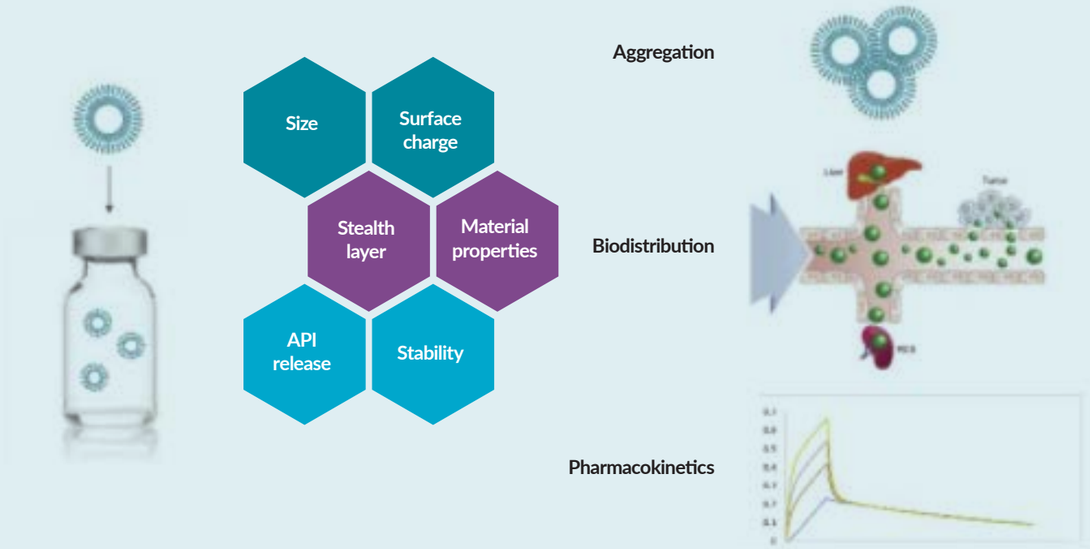
Aggregation can be an issue for complex medicines, where some may undergo irreversible aggregation and concomitant loss of biological activity or increased immunogenicity. The formulation should be designed to maximise colloidal stability and assays developed to detect aggregation.

More complex drug delivery systems, such as liposomes, polymeric nanoparticles and liposome nanoparticles (LNPs) all encapsulate the drug to provide protection from degradation in the biological environment, or to help it cross biological barriers such as cell membranes but must also release the API at the target site of action. As a result, an understanding of the impact of formulation attributes on biological performance is critical.

With conventional medicines taken orally, formulation can only impact the rate and extent of oral absorption. However, formulation attributes for complex medicines can also impact absorption, distribution, metabolism, and elimination of the API.

The key is to understand the attributes of the formulation, which may be important for the biological performance of the medicine, and the fact that this needs to be in place much earlier in the drug discovery programme than for conventional medicines. These attributes must be monitored and controlled within acceptable ranges as the project moves from research through to clinical development and scale-up in order to maximise the chances of success.

Potential critical quality attributes of Nanomedicines



Dr Claire Patterson, Senior Principal Scientist, Seda Pharmaceutical Development Services

Claire Patterson obtained a Master of Pharmacy (MPharm) and a PhD in Pharmaceutics from the Universities of Nottingham and University College London, respectively. Claire is an experienced biopharmaceutics scientist, having spent 12 years at AstraZeneca, with roles in early and late-stage product development, linking *in vitro* to *in vivo* product performance. Seda provides pharmaceutical development and clinical pharmacology services and consultancy to the pharma and biotechnology Industry and Claire's current focus is on subcutaneous and complex parenteral biopharmaceutics.

SEDA Pharmaceutical Development Services

Complex Medicines:
Overcoming the Challenges of Scaling-up a Complex Medicine

Graham Worrall and Emily Port, Team Leader
CPI

Nanomedicines and Challenges in Formulation and Manufacturing

Interactions with biological systems are highly dependent on the nanomaterial characteristics and the manufacturing process. Scale-up requires stable, critical quality attributes to ensure a consistent biological performance from bench scale to clinical scale.

Two stages are involved in the scale-up process for complex medicine. The first is the **system self-assembly**, which is a combination of two streams to form nanoparticles using a mixing device. **Scale-up considerations** include stability of the nanoparticles once the two streams are combined, volume, flow rate, and flow rate ratio and inputs, all of which can change the characteristics of the final product.

System self-assembly

Scale-up considerations

Parameter	Small scale	Large scale
Volume	20 mL	10 L
Flow rate	12 mL/min	115 mL/min
Flow rate ratio	Constant	
Inputs	Constant	

The second stage is **downstream processing**, where the product is essentially cleaned. Solvent and impurities are removed, and the volume is reduced to a specific concentration. As in the first step, volume, flow rate of the process, and pressure, need consideration, as well as filter surface area and filter pore size. Throughout scale-up, success is defined by making sure the characterisation results are consistent, including particle size, polydispersity index, zeta potential, loading and API release.

Additional **scale-up considerations** include pre-experiment safety assessments. This includes ensuring that during the experiment there is the capability to handle large volumes and that after the experiment there is efficient clean down to avoid any cross-contamination between batches, and appropriate waste disposal.

Examples of advancing complex medicines production at CPI include the development of a unique test facility to support microfluidic scale-up of 'difficult to produce' nanomedicines, the development of new manufacturing methods and improving supply chain coordination for nanopharmaceuticals.

It is essential that the effects of any changes made to parameters of scale-up on the actual product are understood, in order to ensure the biological performance of the product remains the same.

Downstream processing

Scale-up considerations

Parameter	Small scale	Large scale
Volume	20 mL	10 L
Flow rate	50 mL/min	1 L/min
Filter surface area	100 cm^2	5000 cm^2
Filter pore size	Constant	

Emily Port, Team Leader, CPI

Emily Port received a Chemistry MChem from the University of York. Before CPI, she worked in the formulation team at the Unilever Deodorants Global Design Centre in Leeds, where she developed personal care products. Since joining CPI in 2017, Emily has mainly worked on nanopharmaceutical projects, with a particular interest in the scale-up of encapsulation of nucleic acids into lipid nanoparticles.

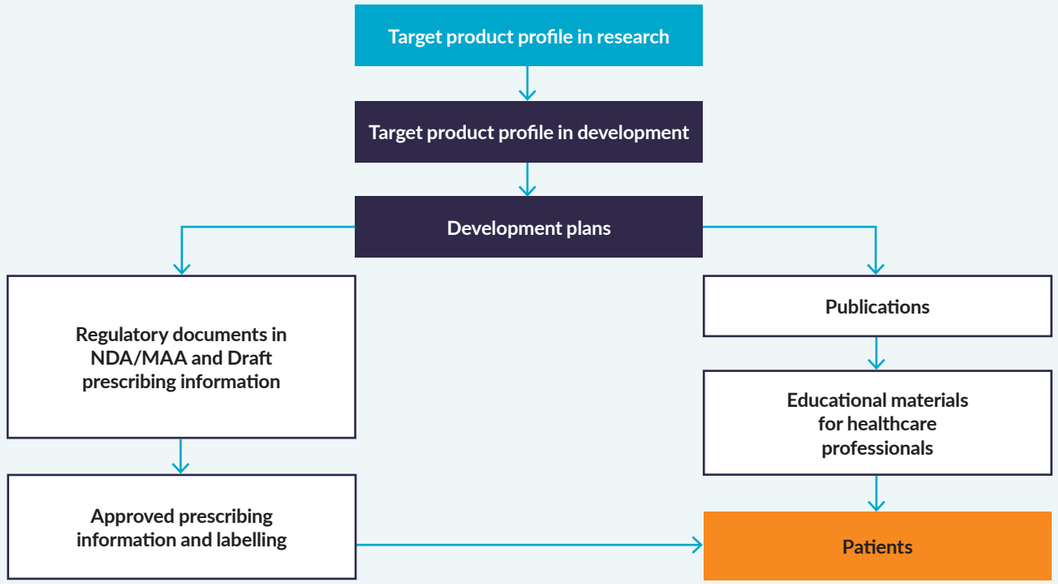
Complex Medicines:
Everything You Want to Know About Development But Were Afraid to Ask

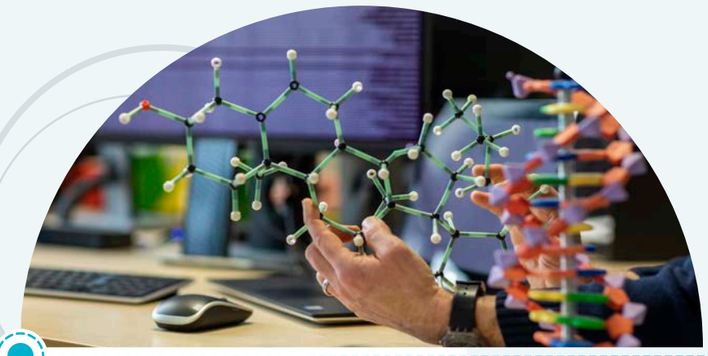
Prof. Alan Boyd, Fellow and immediate Past- President of the Faculty of Pharmaceutical Medicine
Boyds

For any medicine, including complex medicines, four key elements are required for Government and regulatory approval: consistent manufacturing quality, safety and tolerability, demonstration of beneficial therapeutic effect and the benefit: risk profile, i.e. is it appropriate for the disease being treated?

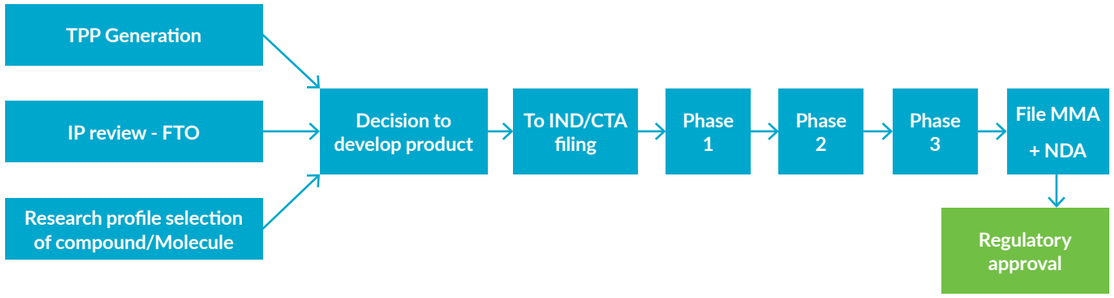
The Drug Development Process

Drug development is a complicated process, so knowing what the desired outcome is for the drug is vital to the whole process. This can be done using a target product profile (TPP).





The development plan is driven by the TPP and will include the generation of all the essential data required for the regulatory bodies, as well as the information needed to design the clinical trials – manufacturing and chemistry, pre-clinical data, pharmacokinetics and toxicology.



The above schematic gives a basic guide to drug development – including complex medicines – and the main stages to consider. From the initial research, TPP, product patent, scaling to a large manufacturing process and assessment in *in vitro* and *in vivo* studies and pre-clinical safety. From this, the investigational new drug (IND) filing can be made, and the clinical programme designed.

The process can be adapted for certain products or routes of administration, which may have implications for complex medicines. For instance, where a medicine is directed to tumour tissue or cell types in terminally ill patients, the First in Human (FIH) study will be in patients without the early phase studies in healthy volunteers.

Challenges in the Process

Unlike conventional medicines, the rate-limiting step for complex medicine production is not the phase III clinical trial results but the actual manufacturing process. When developing a complex medicine, ensure that the challenges are predicted. Indeed, expect these problems and prepare with scenario planning and by learning from medicines ahead in the clinic.

When developing a regulatory strategy, include *in vitro* and *in vivo* data to demonstrate efficacy, what the tox programme will look like, the plans for manufacturing and plans for the FIH study.

Regulatory guidance should be sought early in the development process, including advice from local and international organisations such as the Medicines and Healthcare products Regulatory Agency (MHRA), the European Medicines Agency EMA and the FDA. But also make good use of regulatory guidance documents, as regulatory support and advice are even more important for complex medicines.

For many complex medicines aimed at patients with rare diseases, take advantage of any special provisions, e.g. orphan drug status, but also SME status and early access to medicines initiative.

Overall, no matter what is being developed, it needs to be manufactured to a consistent quality. The efficacy and safety needs to be proven, and the benefit: risk profile needs to be established.

Prof. Alan Boyd, BSc, MB, ChB, FRSB, FFLM, FRCP, FFPM, Fellow and immediate Past- President of the Faculty of Pharmaceutical Medicine, Boyds

Alan Boyd is a graduate in Biochemistry and Medicine from the University of Birmingham. Following postgraduate training, he joined the pharmaceutical industry at Glaxo and then ICI/Zeneca, eventually becoming Global Head of Medical Research for the company. In 1999, he became the R&D Director for Ark Therapeutics in the UK, specialising in the development of gene therapies.

In April 2005, Alan founded Boyd Consultants to support early-stage life science companies, and help universities translate research ideas into medicines. Boyd Consultants now works with a wide range of organisations across Europe, North America and Japan.

Glossary

ABBREVIATION	MEANING
ADCs	Antibody Drug Conjugates
ADME	Absorption, Distribution, Metabolism and Excretion
AML	Acute Myeloid Leukaemia
API	Active Pharmaceutical Ingredient
CD8	cluster of differentiation 8
CryoEM	Cryo-electron Microscopy
CT	Computerised Tomography
DAR	Drug Antibody Ratio
DLS	Dynamic Light Scattering
DODAB	Dioctadecyldimethylammonium Bromide
DOPE	Dioleoylphosphatidylethanolamine
E7	Early gene 7
FFF	Field Flow Fractionation
FIH	First-In-Human
GFP	Green Fluorescence Protein
GMP	Good Manufacturing Practice
HER2	Human Epidermal growth factor Receptor 2
HPV	Human Papillomavirus
IND	Investigational New Drug
LCMS	Liquid Chromatography - Mass Spectrometry
LNPs	Lipid Nanoparticles
MDa	MegaDalton
MoA	Mechanism of Action
mRNA	Messenger Ribonucleic Acid
PD	Pharmacodynamics
PET	Positron Emission Tomography
PK	Pharmacokinetics
PODS	Programmable Oligonucleotide Delivery System
RNA	Ribonucleic Acid
SEC-MALS	Size Exclusion Chromatography for Multiple Angle Laser Light Scattering
siRNA	Small Interfering Ribonucleic Acid
SPECT	Single Photon Emission Computed Tomography
TPP	Target Product Profile

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