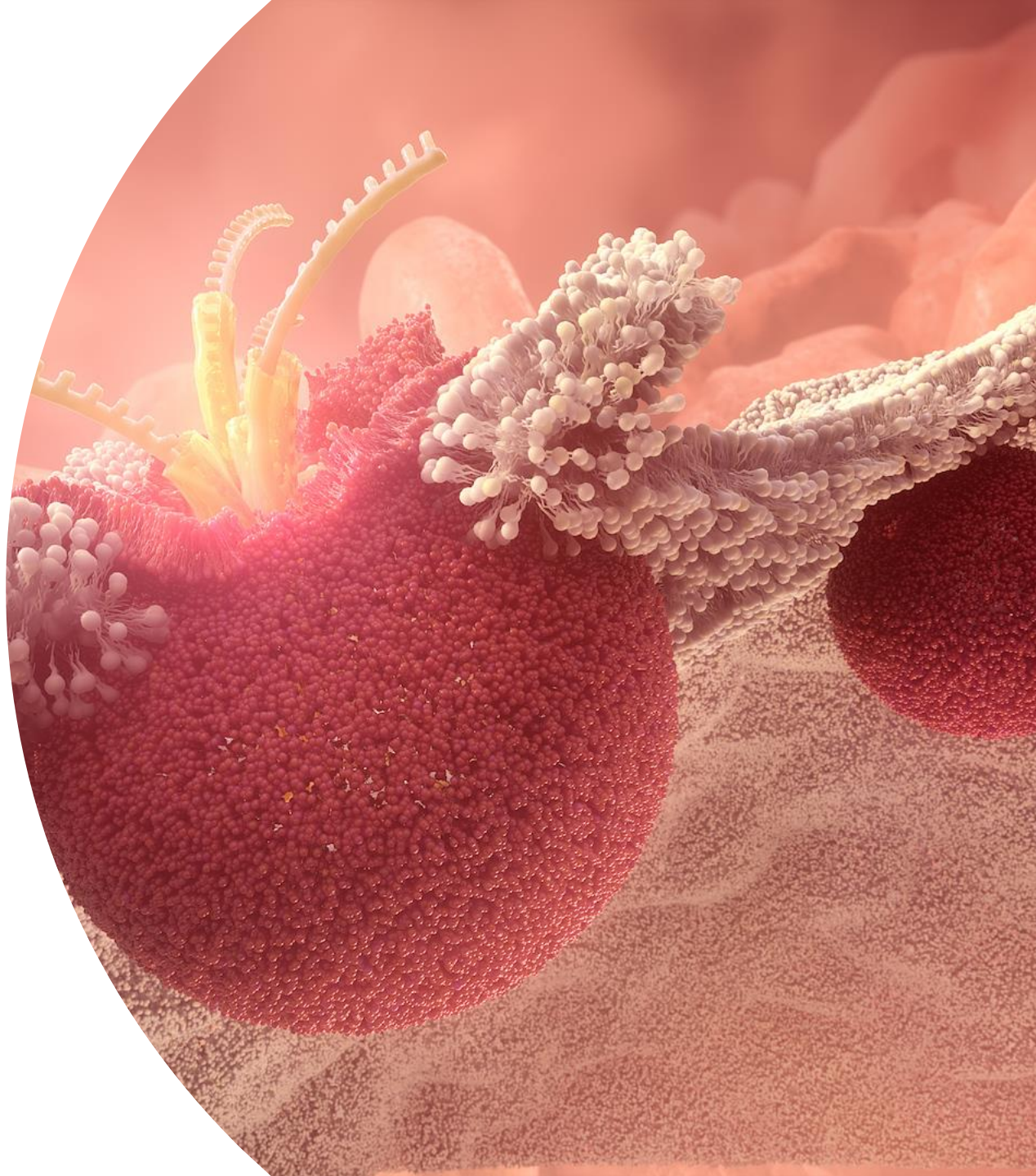
Intracellular Delivery - Status & Challenges

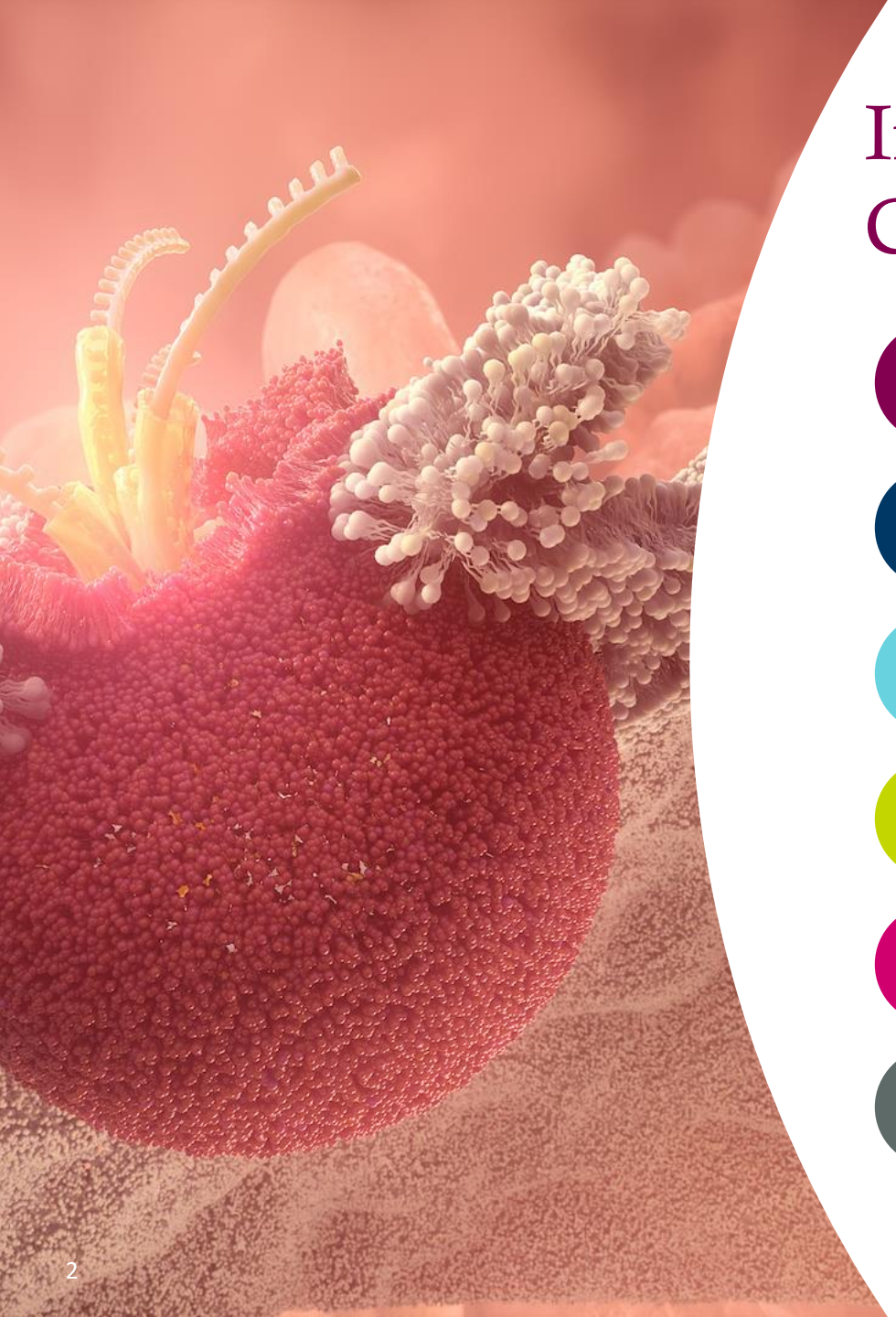
A Personal Perspective

Marianne Ashford - Senior Principal Scientist, Advanced Drug Delivery, Pharmaceutical Sciences, R & D, UK.

"Inside Out: Navigating Innovation in Intracellular Drug Delivery – Exploring Cutting-Edge Technologies" Workshop

14th May 2024

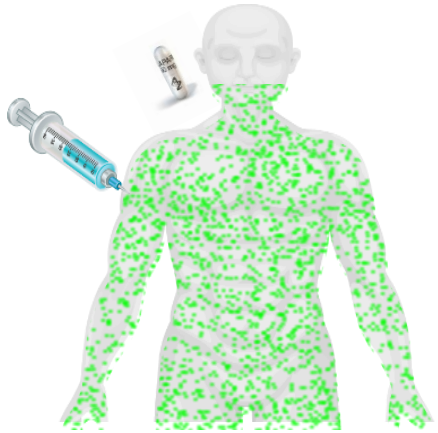
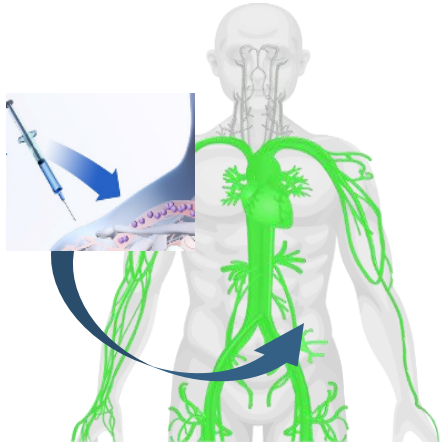
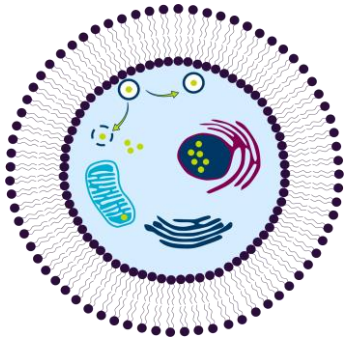




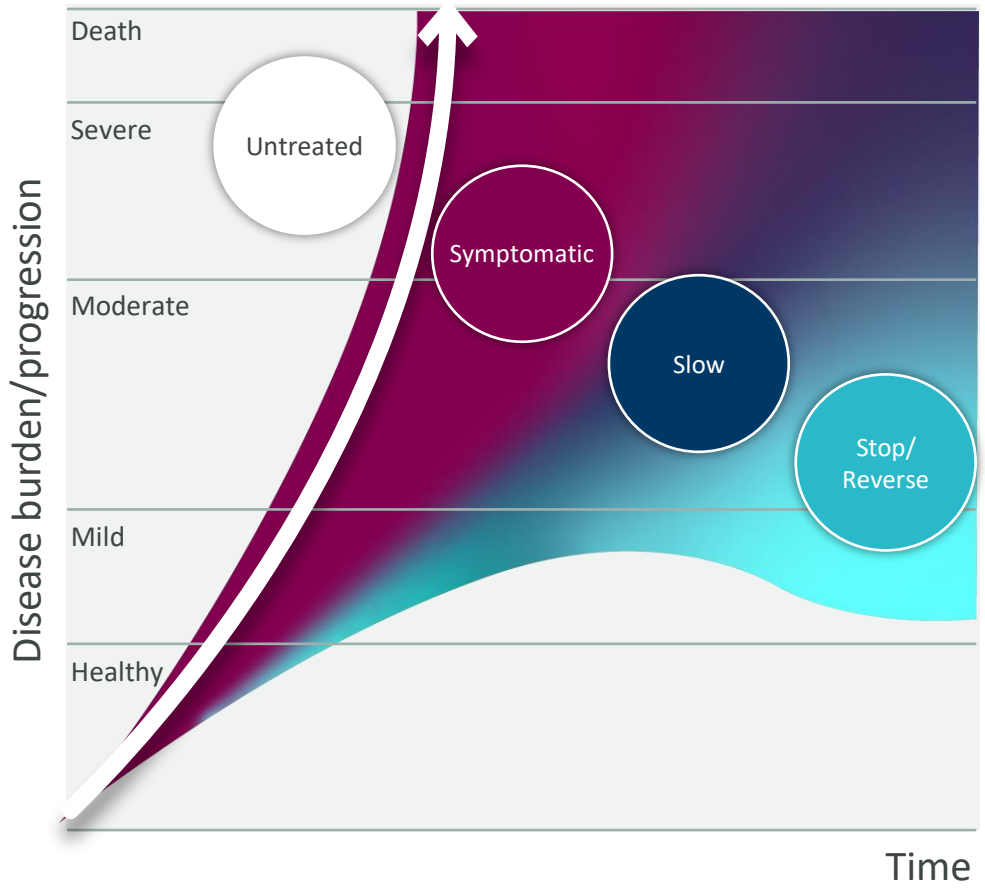
Intracellular Delivery –Status & Challenges

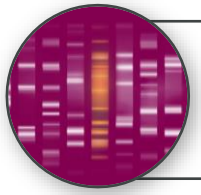
1. Introduction Drug modalities & Drug Delivery
2. Antibody Drug Conjugates
3. LNPs & RNA Medicines Status
4. Intracellular Delivery & Endosome Escape
5. Example Road Map to Clinic – LNPs & challenges
6. Other systems & reflections

Advancing Drug Delivery and Enabling New Targets

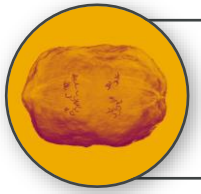
Delivery Type	Conventional Delivery	Targeted Delivery	Site Specific Intracellular Delivery
			
Therapeutic Modality	Small molecules Antibodies Peptides for extracellular targets Antisense oligonucleotides	Small molecules Nanoparticles/Antibody Drug Conjugates ASO/siRNA - Ligand targeted	ASO, siRNA microRNA mRNA Gene editing Cell Therapy

Addressing the biggest challenges in healthcare requires a shift from symptom management to slowing and stopping disease

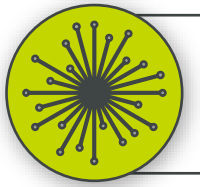




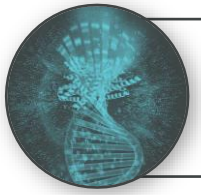
Target underlying biology



New drug modalities and combinations



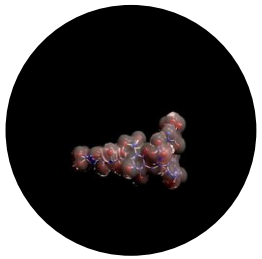
Precision medicine approach



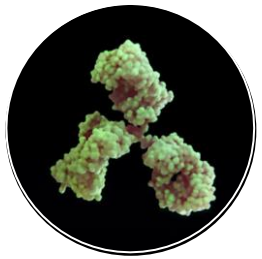
Earlier detection, diagnosis and intervention

Range of drug modalities to access next generation therapeutics

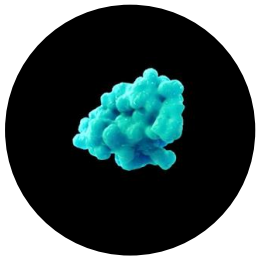
Traditional molecules



Small molecule

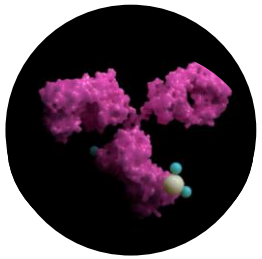


Monoclonal & bispecific antibodies



Therapeutic protein

Advanced antibodies



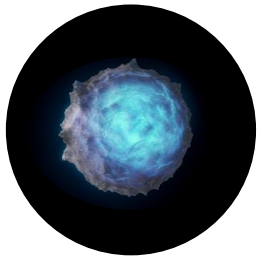
Antibody drug conjugate

Bispecific fragment antibody

Fragment antibody

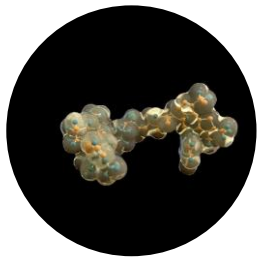
Viral/ cell based therapy

In vivo expressed biologics



Cell therapy

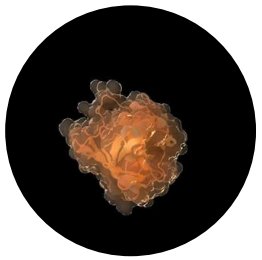
Advanced small molecules



PROTAC

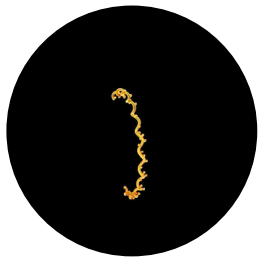
Peptide-based

Bicyclic peptide

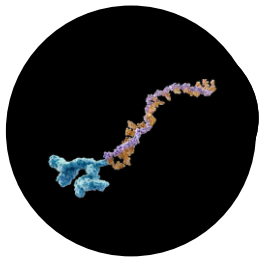


Anticalin ® protein

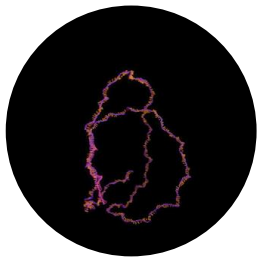
Nucleotide-based therapeutics



Antisense oligonucleotide

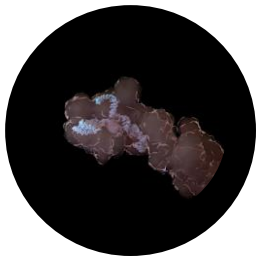


Oligonucleotide conjugate



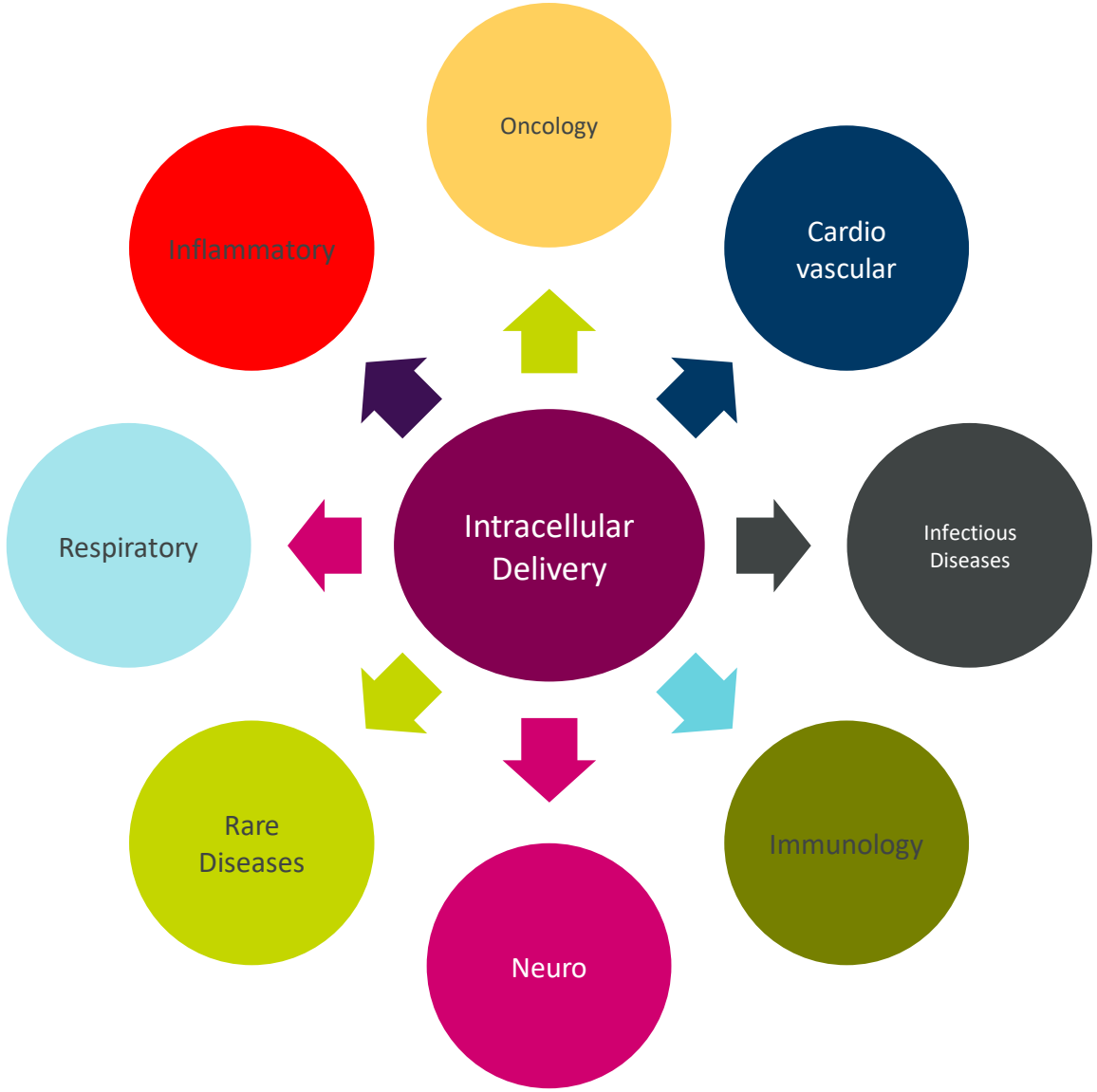
Modified RNA

DNA

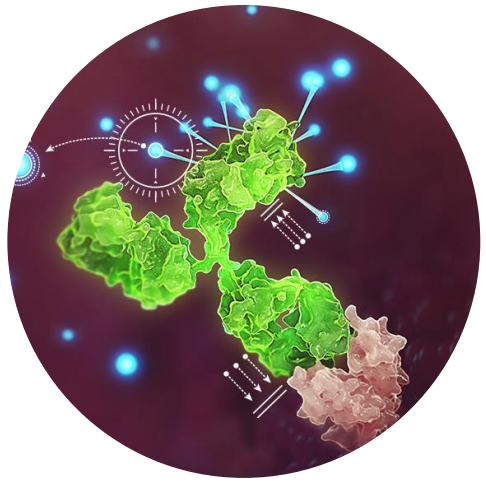


CRISPR gene editing

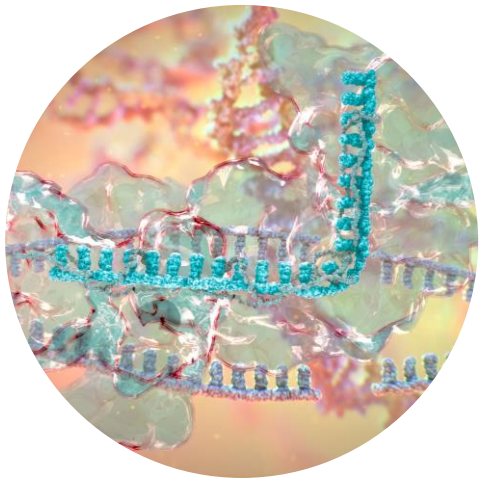
Improved Cell Specific Intracellular Delivery enables therapy for more Diseases



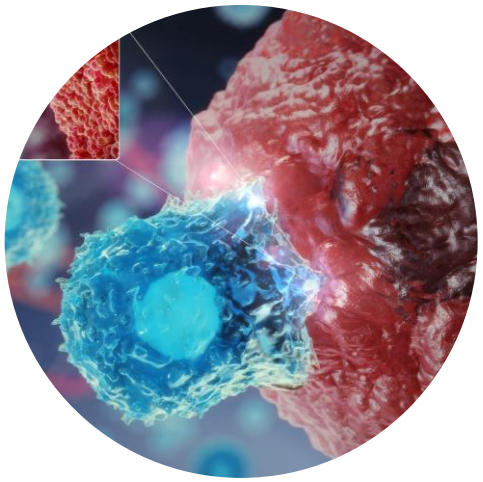
Efficient Intracellular Delivery is critical for Next Generation Therapeutics



Antibody Drug Conjugates
& other targeted systems



Gene Therapies

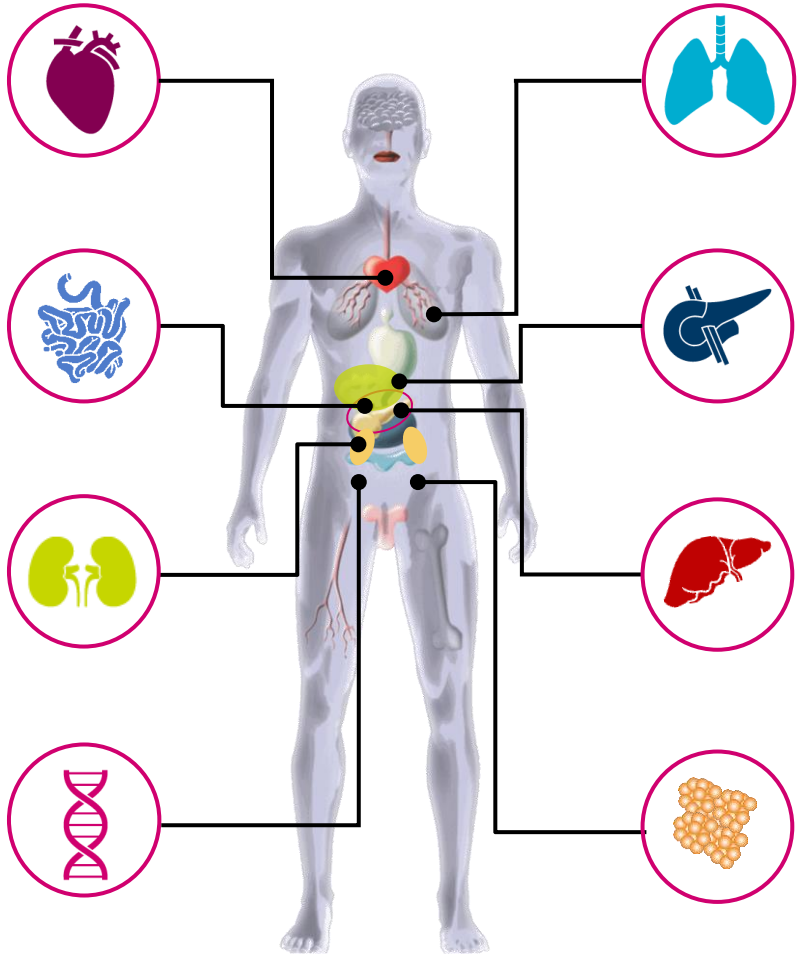


Cell Therapies
Moving to in-vivo CAR

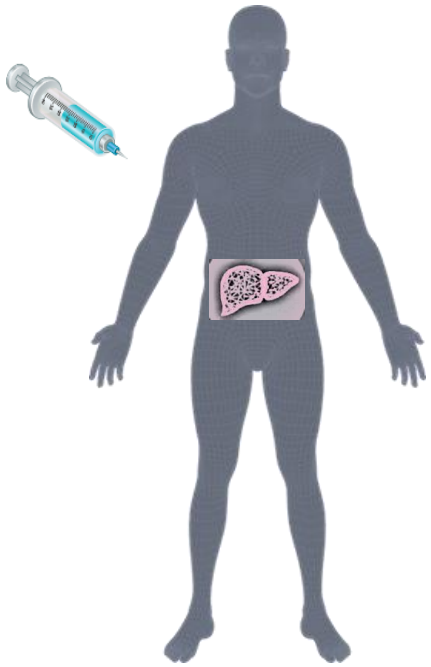
Targeted drug delivery enables modulation of targets in specific tissues and cells

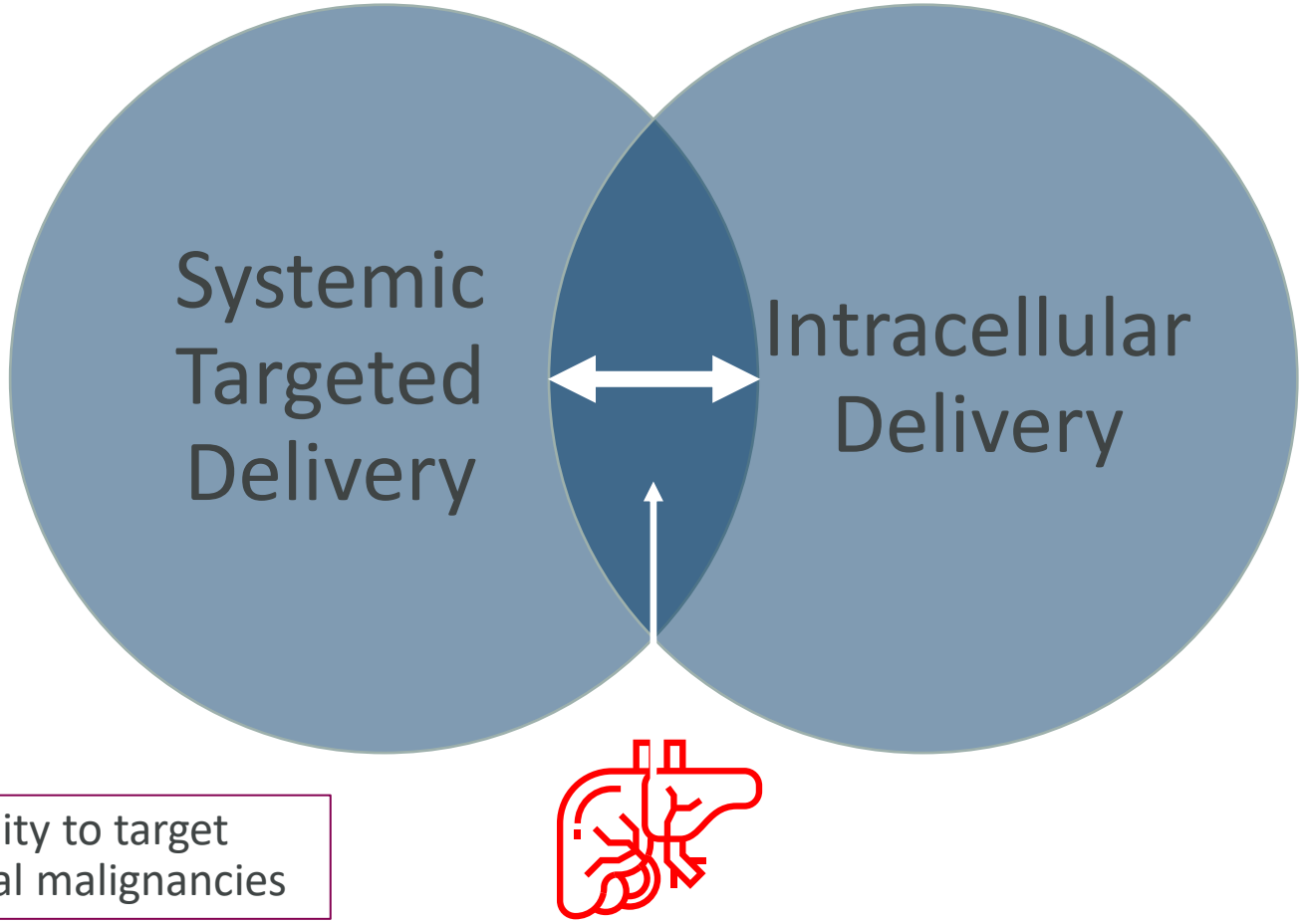
Targeted delivery will

- Improve therapeutic index
- Expand druggable target space
- Deliver new modalities
- Reduce dose & cost of goods



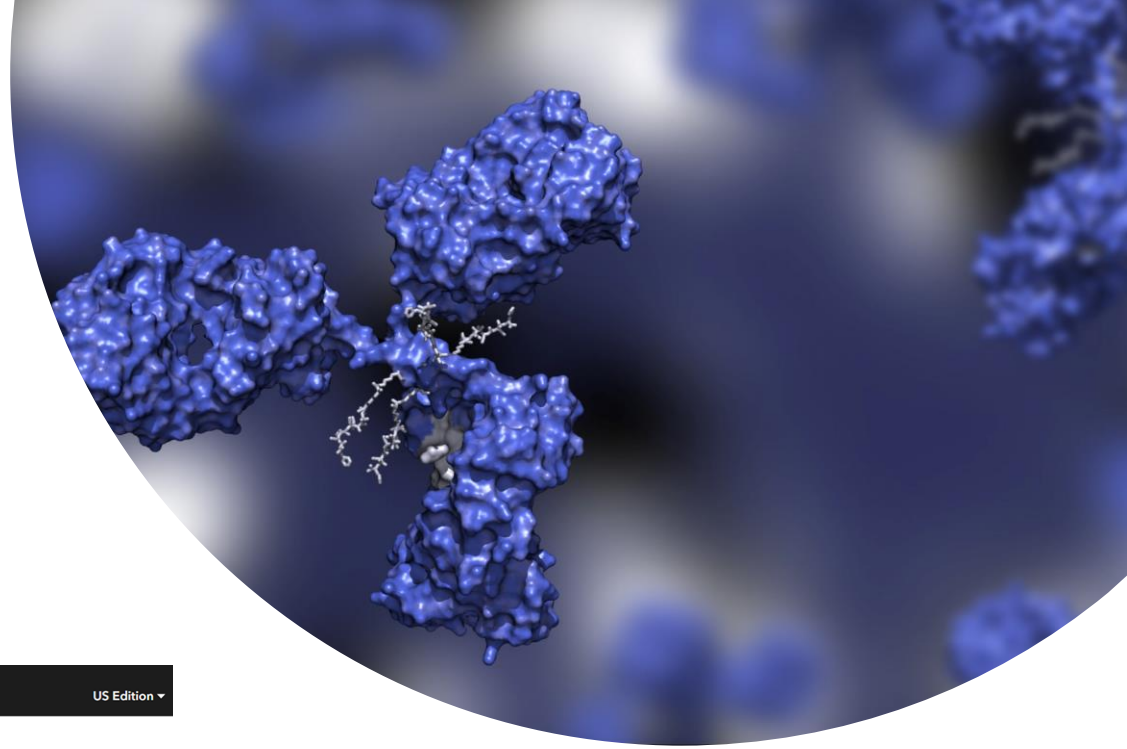
Delivery critical for expanding druggable target space





N.B Except Oncology - ADCs have shown ability to target both solid tumours (Her 2) and haematological malignancies

Antibody Drug Conjugates



- 15 Approved Products
- > 170 in clinical trials

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Opinion | Lisa Jarvis, Columnist

Targeted Cancer Drugs Finally Live Up to the Hype

The science around designing and testing smart-bomb chemotherapy has coalesced, reflected by billions of dollars in oncology dealmaking.

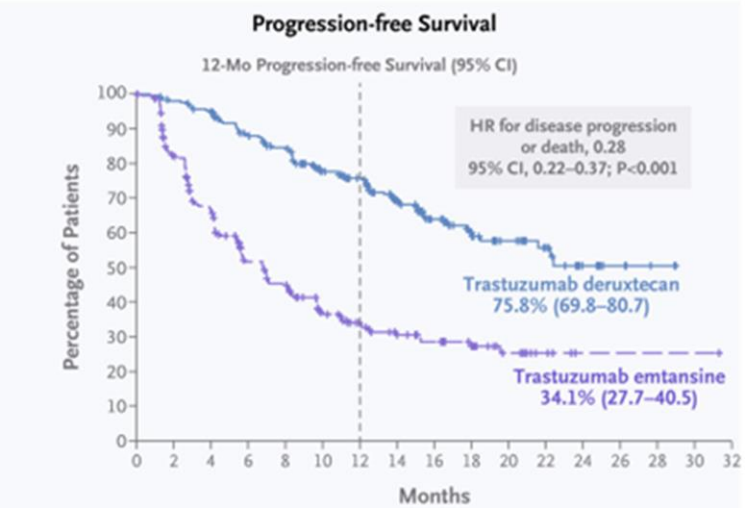
5 December 2023 at 12:00 GMT

nature reviews drug discovery https://doi.org/10.1038/s41573-023-00709-2

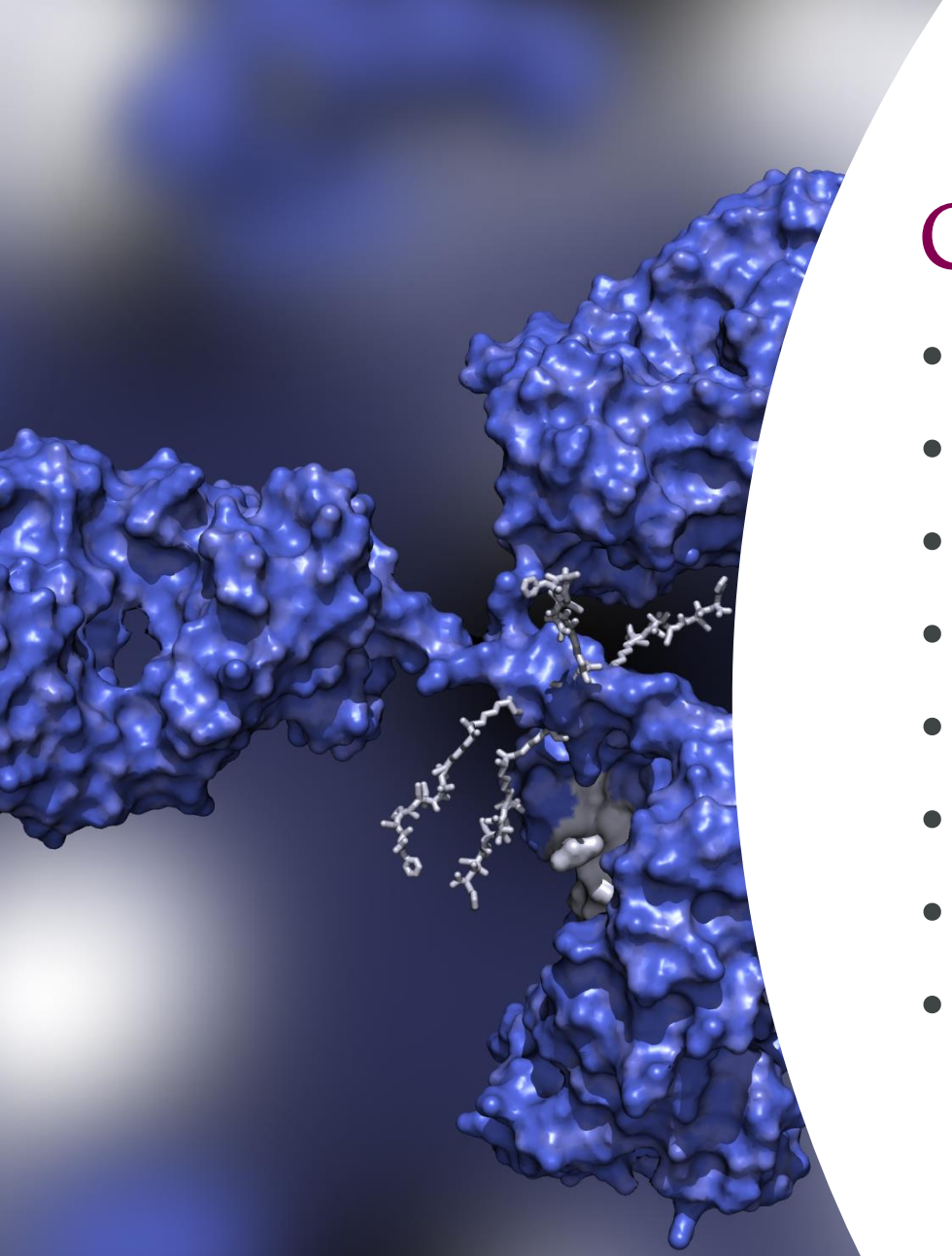
Review article Check for updates

Antibody–drug conjugates come of age in oncology

Charles Dumontet[1], Janice M. Reichert[2], Peter D. Senter[3], John M. Lambert[4] & Alain Beck[5]



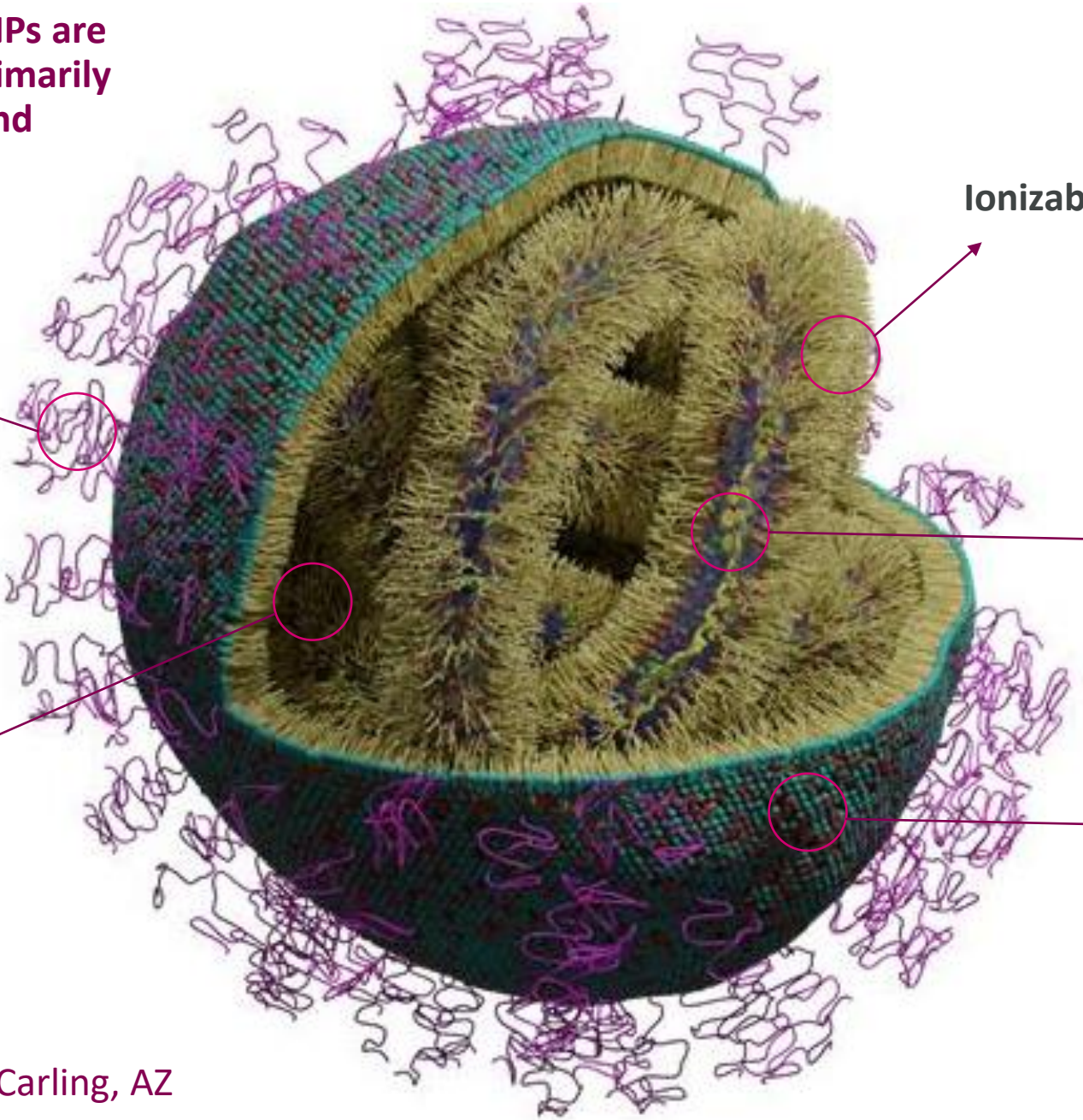
Cortes J et al,. N Engl J Med 2022; 386:1143-1154



Challenges of ADCs

- Limited number of payloads, linkers, antibodies
- Careful selection of payloads, linker, antibody,
- Challenge to increase Drug to Antibody ratio
- Toxicity which tends to be platform dependent
- Resistance
- Restricted to potent payloads
- Non-specific binding
- Tissue penetration

Lipid Nanoparticles, or LNPs are a drug delivery system primarily used for the protection and delivery of nucleic acids



Artwork credit: Carl-Johan Carling, AZ

Delivery Status of RNA Medicines with LNPs

Vaccines
Intramuscular delivery
Platforms – “plug & play”

Hepatic, Topical & ex vivo Therapies

Curative editing therapies (PCSK9, TTR, SCD)

Inhaled and locally delivered therapies in development

In vivo Extra – Hepatic Delivery

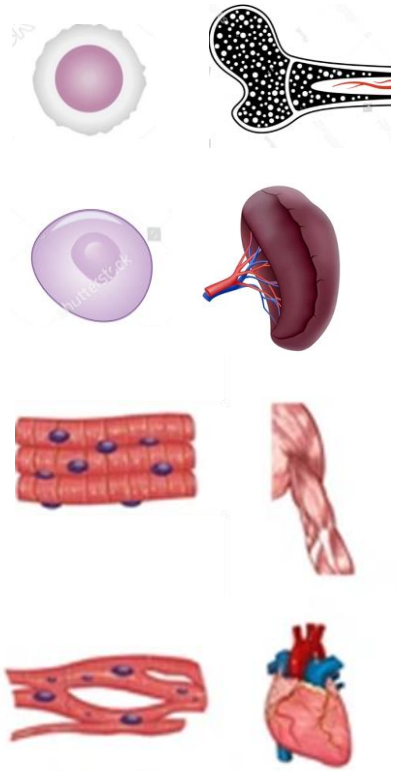
Bone Marrow

Immune Cells

Heart

Muscle

Brain …..



Progress with Nucleic Acid based Therapeutics

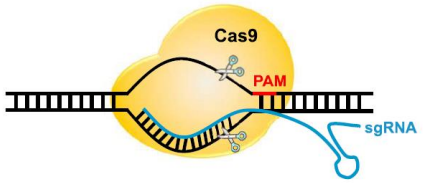
ASOs
siRNA

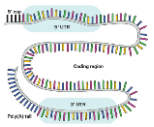
mRNA
short half-life, low expression

self-amplifying RNA
low dose, high expression, long half-life, size, immunogenicity

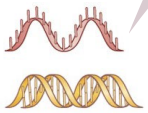
Circular RNA
long half-life, no capping/ poly A tail
Young platform

Gene editing tools



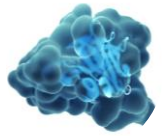


Gene of Interest Encoded Therapeutic



Gene of interest

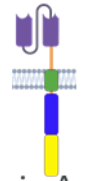
Vaccine Antigen(s)



Therapeutic proteins

Gene editing enzymes

Monoclonal Antibody

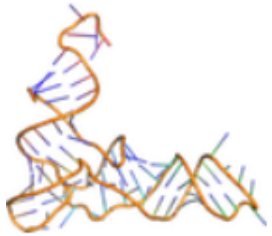


Chimeric Antigen Receptor

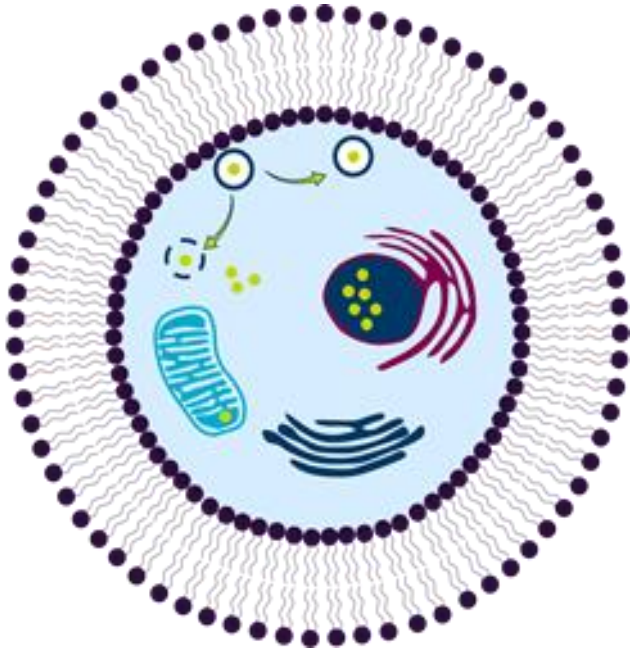
Challenges for new modalities – intracellular delivery & productive targeted delivery

Antisense oligonucleotides ~4kDa

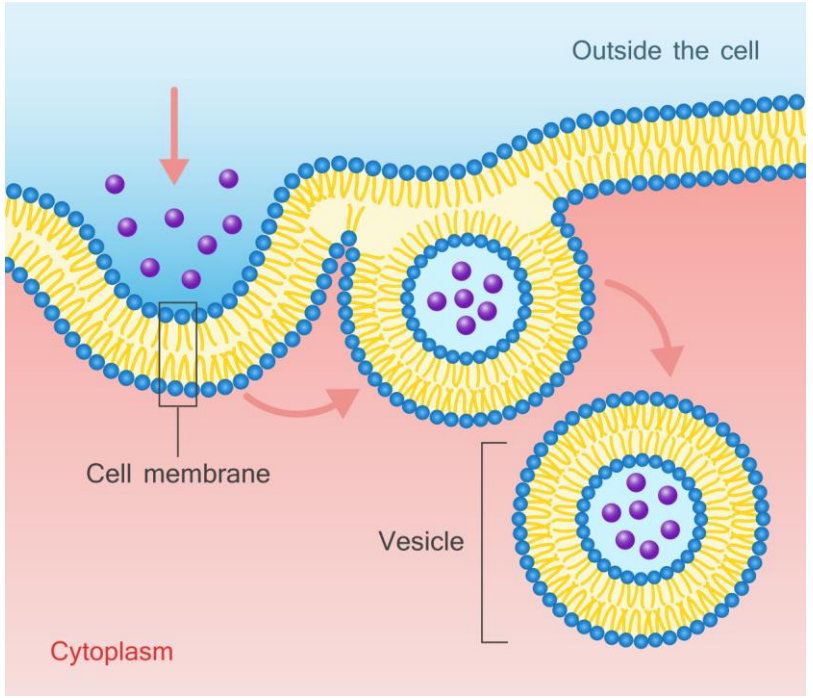
siRNA ~ 14kDa



mRNA 300-10,000 kDa

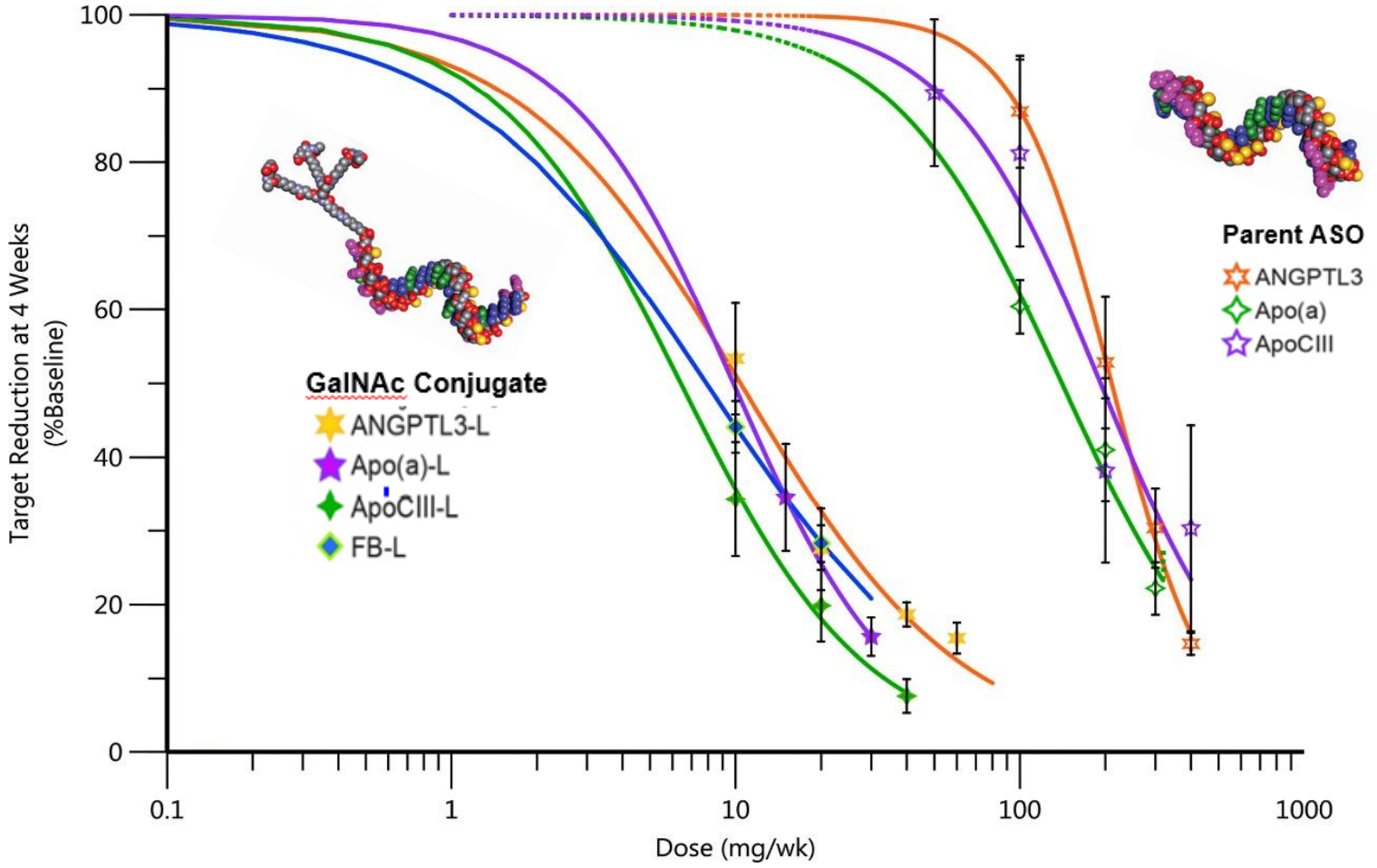


Endosome Escape is Critical



~ 1-2% endocytosed material successfully delivered to cytosol in many systems

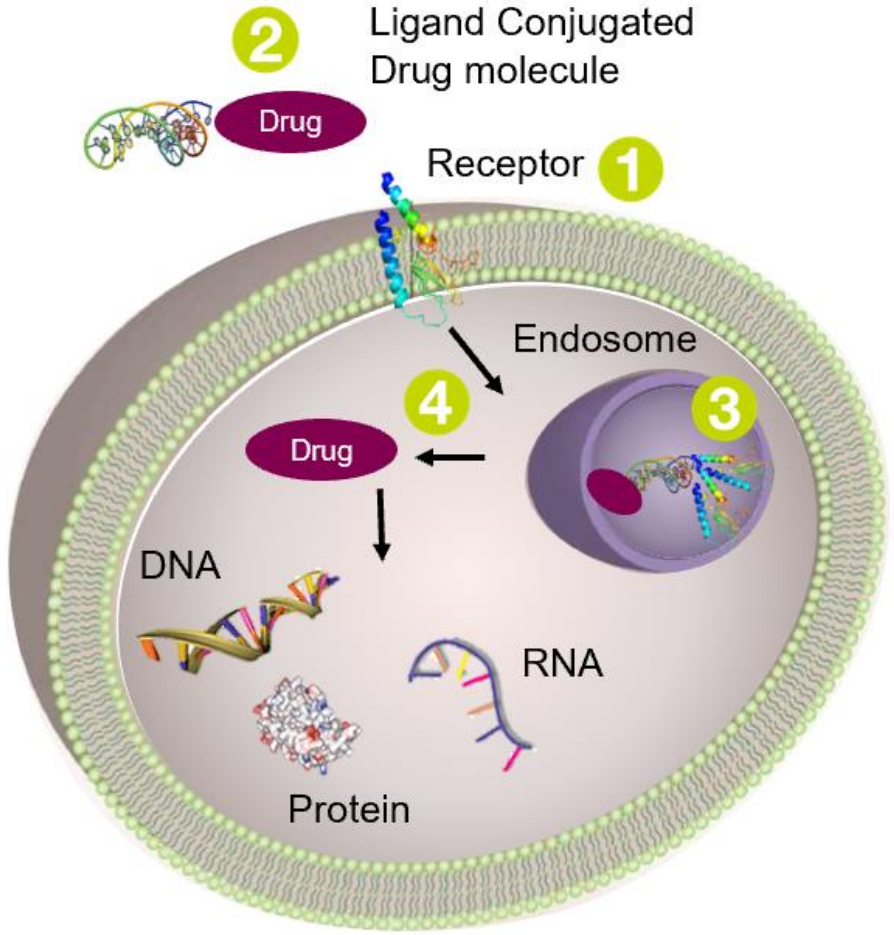
Targeted intracellular delivery of GalNAc conjugated ASOs achieve similar efficacy at 10x lower dose in healthy volunteers



Adapted from Crooke S. T. et al, (2019)

Important Processes for successful ligand mediated intracellular delivery

1. Requires specific or enriched receptor expression on target cell
2. Ligand/s that binds to the receptor with sufficient affinity and selectivity
3. Cellular uptake mechanism that internalizes drug cargo, ideally without activating signaling
4. Escape from endosome
5. Receptor should cycle efficiently between plasma membrane and endosomes



Efficient Endosome Escape still needs addressing

PERSPECTIVE

Endosomal escape of RNA therapeutics: How do we solve this rate-limiting problem?

STEVEN F. DOWDY

Department of Cellular and Molecular Medicine, UCSD School of Medicine, La Jolla, California 92093, USA

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Brief update on endocytosis of nanomedicines

Siddharth Patel[1], Jeonghwan Kim[1], Marco Herrera[1], Anindit Mukherjee[1], Alexander Kabanov[3,4,*], Gaurav Sahay[1,2,*]

[1]Department of Pharmaceutical Sciences, College of Pharmacy, Oregon State University

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Review

Open camera or QR reader and scan code to access this article and other resources online.

Delivery of RNA Therapeutics: The Great Endosomal Escape!

Steven F. Dowdy, Ryan L. Setten, Xian-Shu Cui, and Satish G. Jadhav

PNAS

PERSPECTIVE | BIOCHEMISTRY |

Endosomal escape: A bottleneck for LNP-mediated therapeutics

Sushmita Chatterjee, Edo Kon, Preeti Sharma, and Dan Peer Authors Info & Affiliations

Edited by Sangeeta Bhatia, Massachusetts Institute of Technology, Cambridge, MA; received June 6, 2023; accepted August 22, 2023

March 4, 2024 | 121 (11) e2307800120 | https://doi.org/10.1073/pnas.2307800120

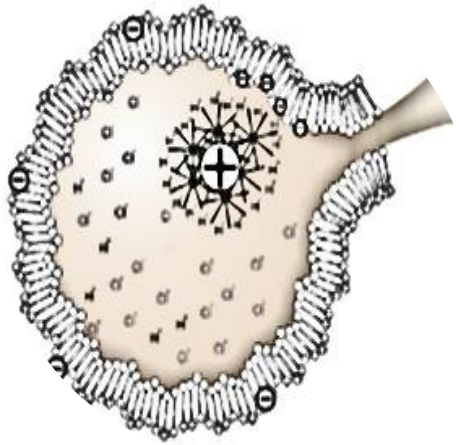
"requirement to develop robust and less complex methods to study endosomal escape"

Key Routes of Endosome Escape

Loss during natural acidification /endo-lysomal pathway & membrane destabilisation

Pore Formation

Persistent membrane destabilisation via cationic or fusogenic materials can result in pore formation



Rupture & Burst

Proton sponge effect increased cationic charge, create osmotic gradient & pressure leading to rupture

Membrane Fusion

Can result in cargo release to cytosol

Oligonucleotides

Small drugs - diffusion

LNPs

Adapted from : Marten et al ; Intracellular delivery of nanomaterials: How to catch endosomal escape in the act NanoToday(2014)

mRNA Loaded Lipid Nano-Particle – Overview Roadmap to The Clinic

Nano-particle Candidate selection

Roadmap to the clinic to optimize a lipid nano-particle (LNP) for a given tissue

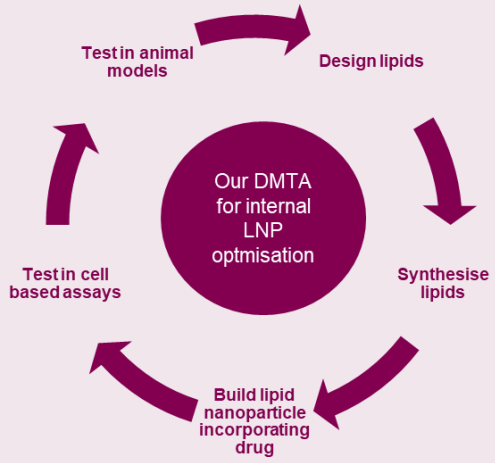
Ph1

Target Validation → Lead generation → Lead optimization → Preclinical

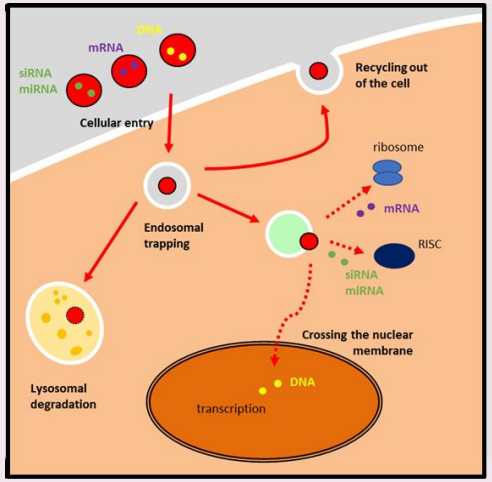
Designing the right LNP

Transforming it into a drug product

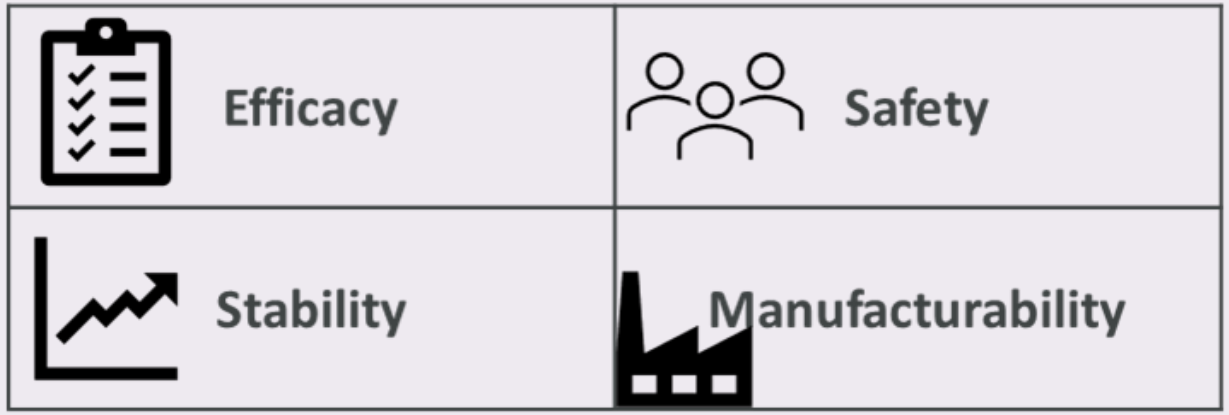
Focus on DMTA to improve safety and efficacy



Key challenge: increasing endosomal release



Desai et al 2019

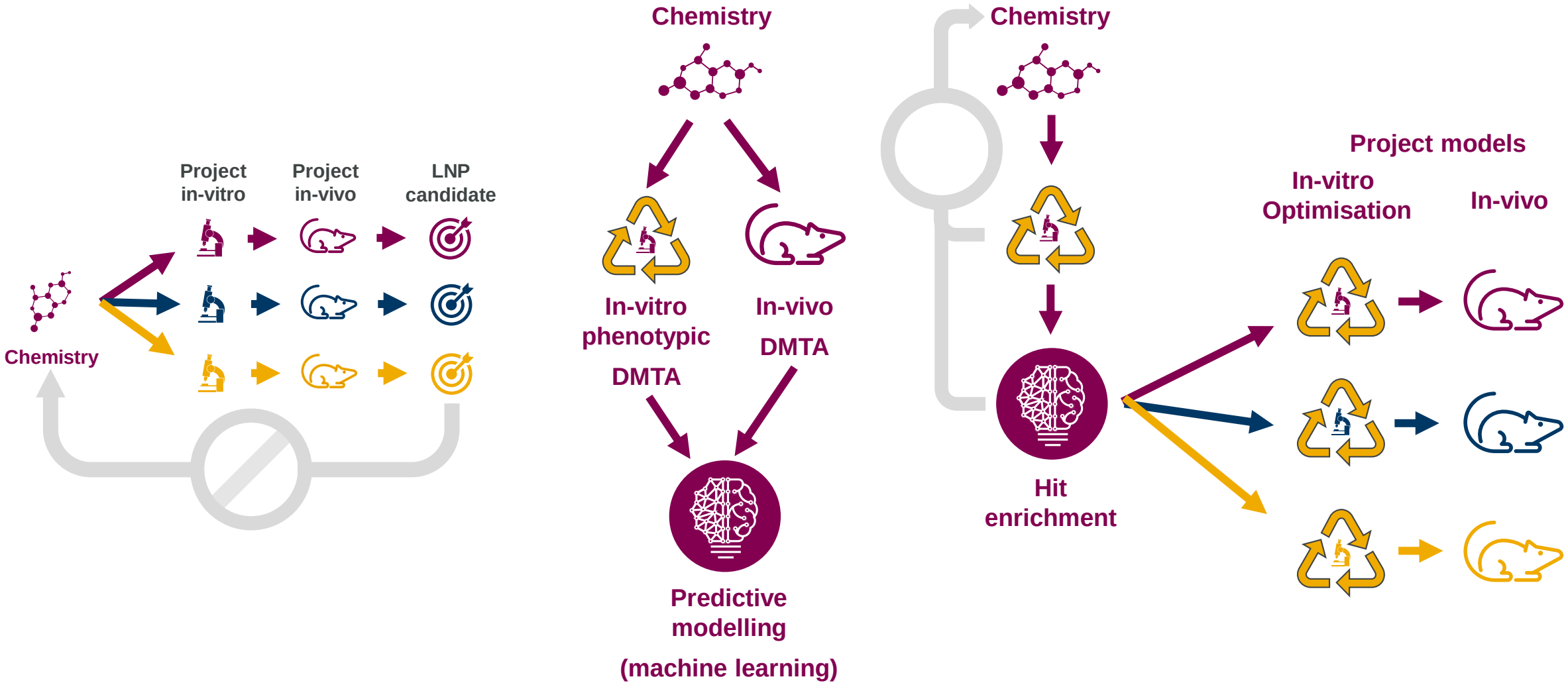


Discovery

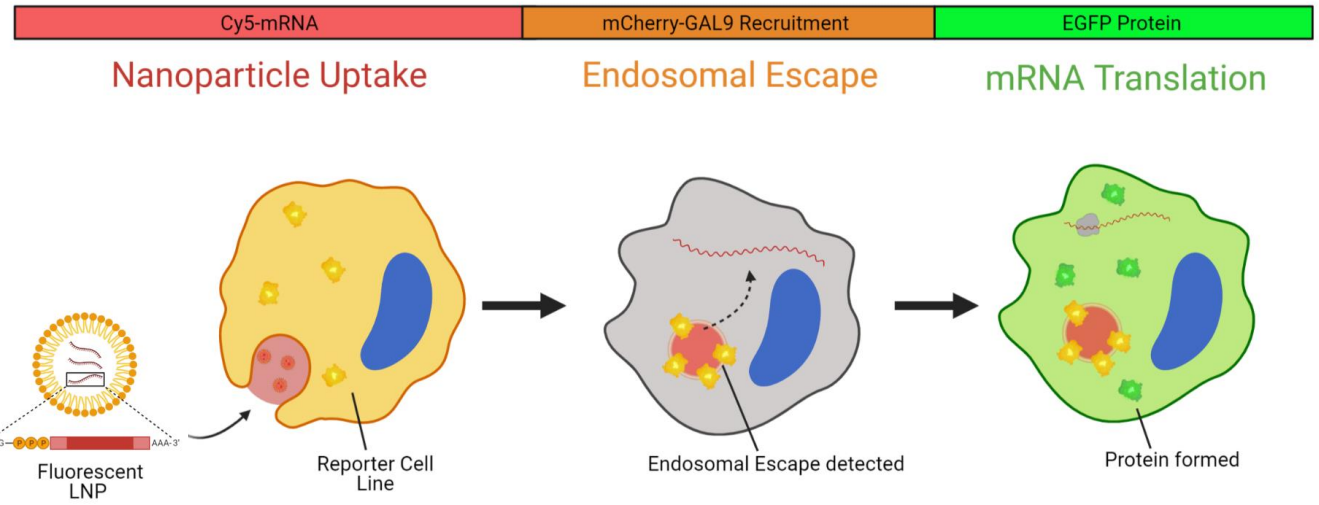
Focus on stability and manufacturability

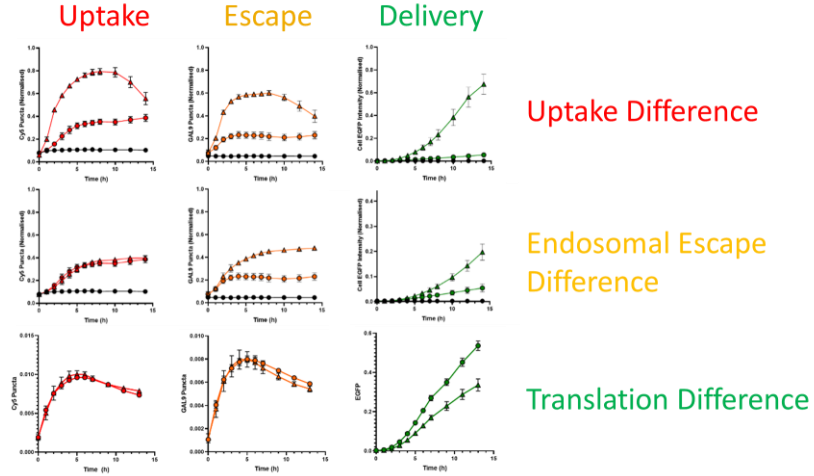
Development

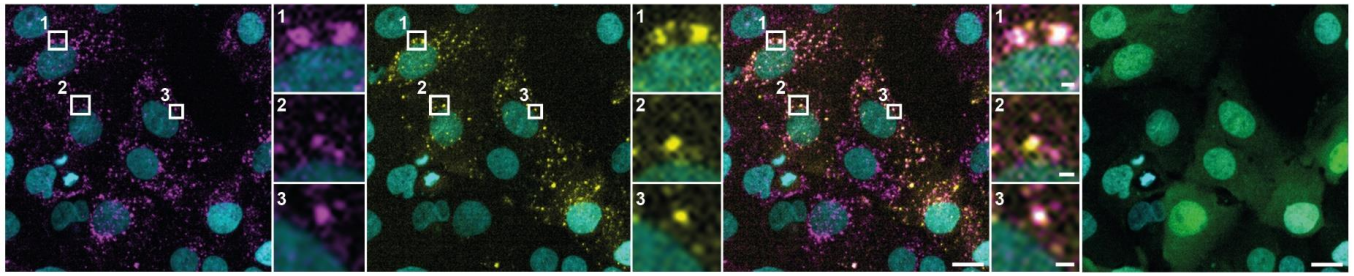
Nanoparticle screening needs to be more predictive – better IVIVC



NanoProfiler Imaging Assay to Understand Intracellular Delivery







Uptake Escape Merge Translation

A high-throughput Galectin-9 imaging assay for quantifying nanoparticle uptake, endosomal escape and functional RNA delivery

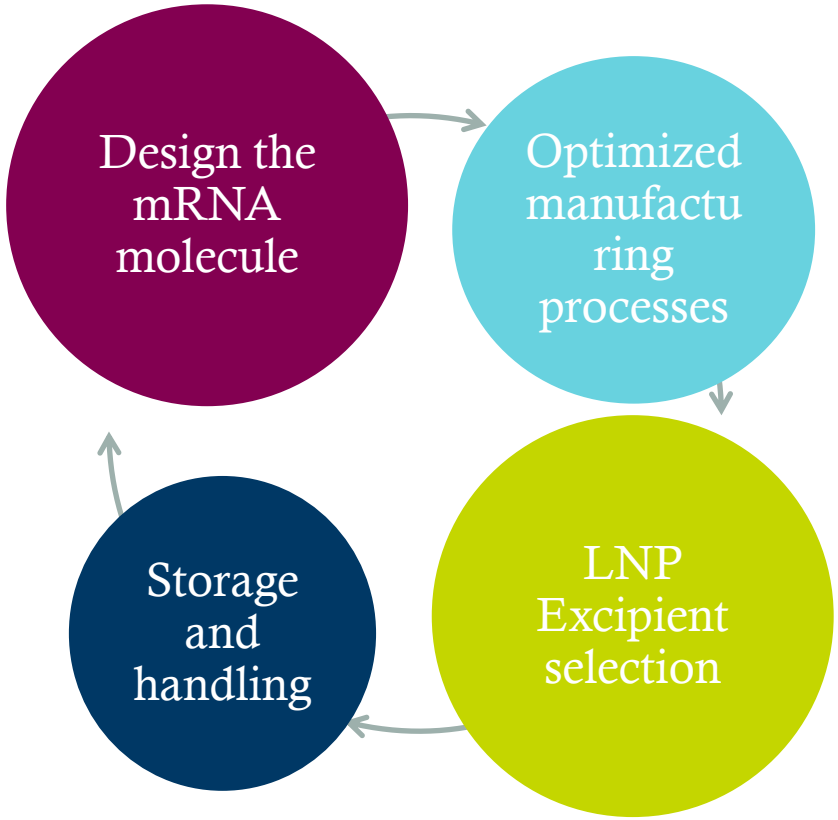
Michael J. Munson, Gwen O'Driscoll, Andreia M. Silva, Elisa Lázaro-Ibáñez, Audrey Gallud, John T. Wilson, Anna Collén, Elin K. Esbjörner & Alan Sabirsh

Communications Biology **4**, Article number: 211 (2021) | Cite this article

Development strategies for a stable mRNA LNP product

Design the mRNA molecule

- Modified nucleotides
- More GC
- More secondary structures
- Shorter mRNA
- Purity level of mRNA



Optimized manufacturing processes

- Type of process
- Time
- Temperatures
- mRNA concentration
- RNase free

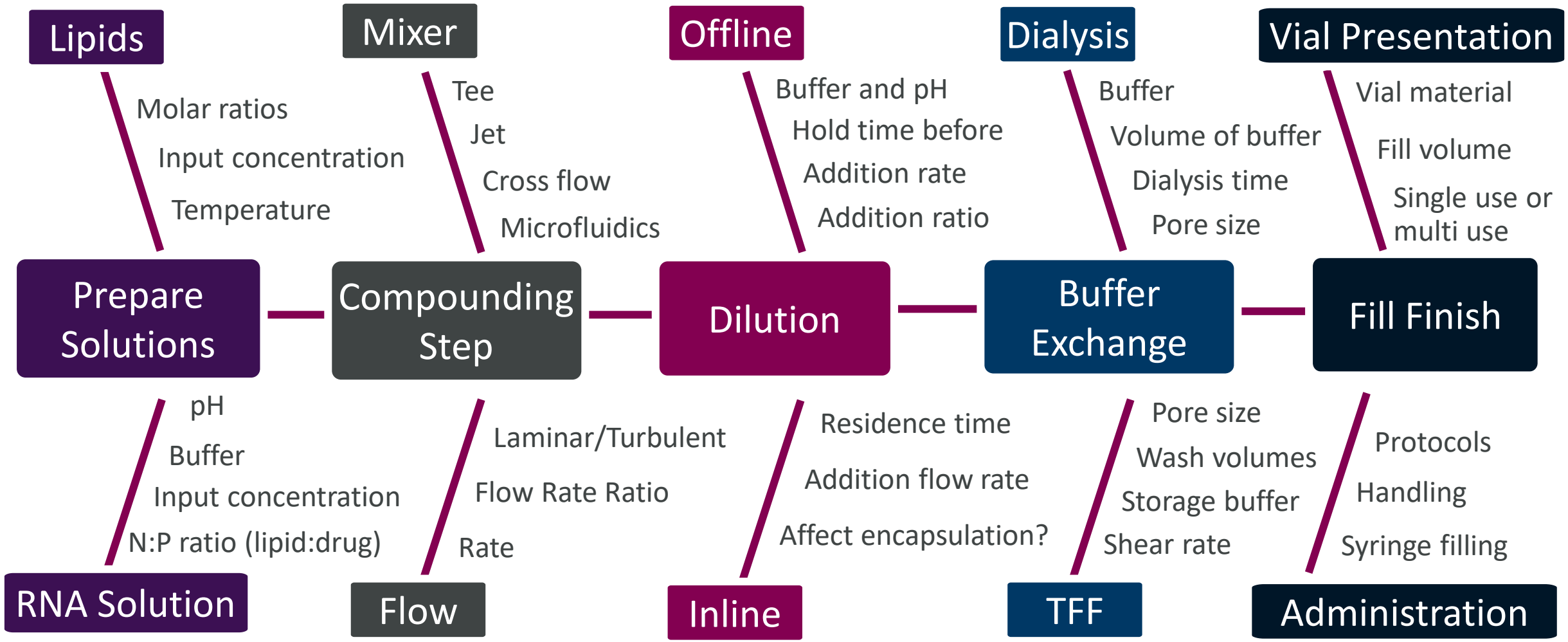
Storage and handling

- Long time storage at lower temperatures
- Removal of water
- Freeze drying
- Kit – based approach drug product

LNP excipient selection

- Purity of lipids
- Buffers
- Cryoprotectants
- Osmolytes
- RNase free

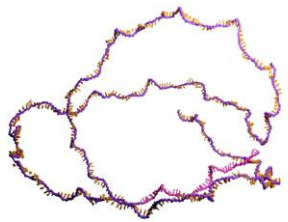
Lipid Nanoparticle Manufacturing Process



mRNA based therapeutics –New analytical challenges

Chemical properties of mRNA

- Susceptibility to degradation
- Large size > 300kDA and negative charge
- Polydisperse

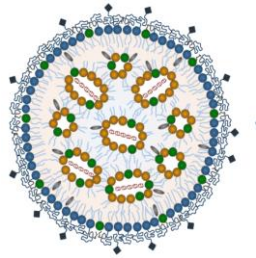


Methods for purity measurement

- Multiple methods are needed for separation of impurities
  - Ion-pair Reversed Phase Liquid Chromatography
  - Capillary electrophoresis
- Separation methods need to be stability indicating
- LC-Mass-spectrometry methods are an excellent complement to separation techniques
  - Requires mRNA digested into shorter fragments prior to analysis
  - Enable testing of Cap-less, Tail-less impurities and verification of Identity

Drug Delivery system

- LNP Lipid Nanoparticles–Delivery Solution for mRNA
- Complex analytical characterisation of the LNP



Requirement of potency assay !

Advances in Lipid Nanoparticles for siRNA Delivery *Pharmaceutics* **2013**, *5*(3), 498-507

There is more to intracellular delivery than lipid delivery…….

Theresa Reineke
Co-Founder

UNIVERSITY OF MINNESOTA

Liberate Bio

Liberate is using automation, in vivo high-throughput screening, and machine learning to accelerate discovery of novel extrahepatic delivery vehicles

GENEDIT
GENE THERAPY DELIVERED

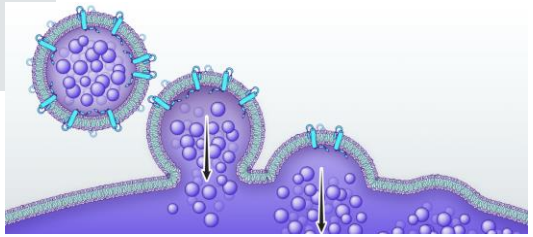
GenEdit Announces Multiyear Collaboration and License Agreement with Genentech to Develop Novel Nanoparticles to Deliver Genetic Medicines for Autoimmune Disease

January 23, 2024 / *in News* /

SOUTH SAN FRANCISCO, Calif., January 23, 2024 – GenEdit, Inc., a developer of genetic medicines that leverage its NanoGalaxy® platform for tissue-selective delivery, today

entos

Fusogenix proteo-lipid vehicle (PLV)

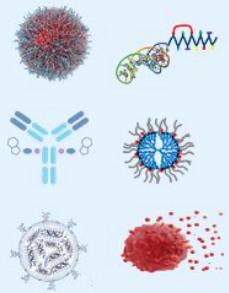


evox

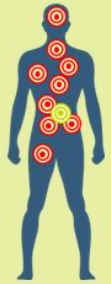
DELIVEREX® PLATFORM FOR ADVANCING EXOSOME-MEDIATED DELIVERY OF GENETIC MEDICINES

Capability build to design, develop and exploit specific intracellular delivery approaches to enable next generation therapeutics

Delivery tech. & toolbox

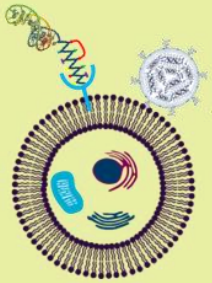


Biodistribution Imaging Modelling

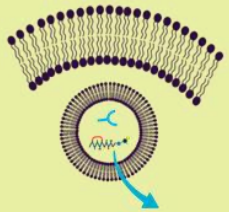


Cell type / sub-tissue localisation

Receptor/ cell binding



Internalisation Release Endosomal escape



Cell trafficking/ sub-compartments localisation

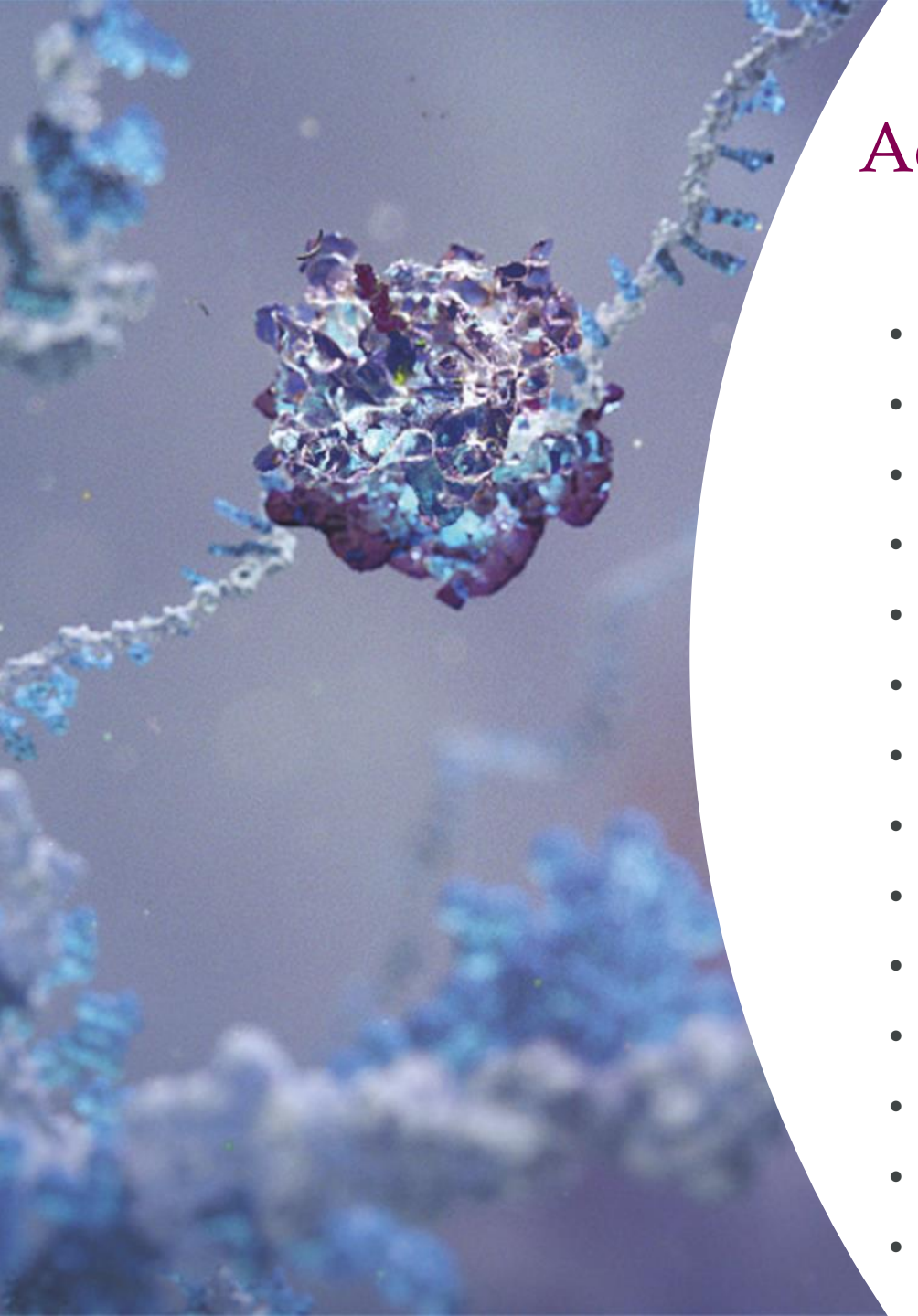
Pharmaceutical Development to ensure Product Quality

Robust Scale up & Manufacture

Advanced analytical characterisation

Regulatory Advocacy

Designing from concept to commercial product



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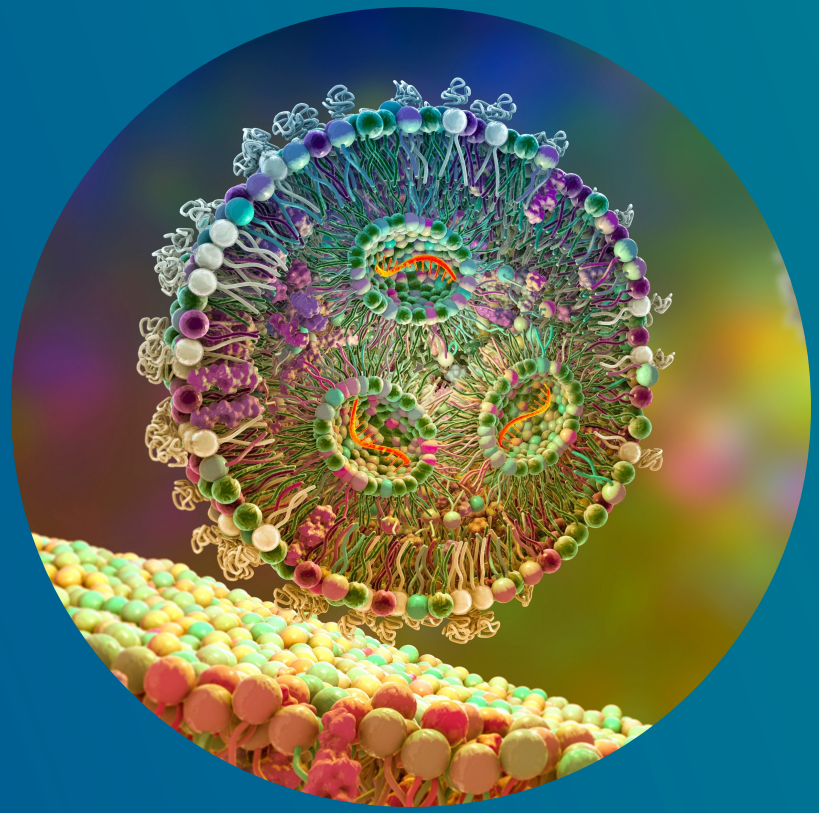
- Annika Pålsson
- Jessica Eriksson
- Kristina Friis
- Sara Pereira
- Bei Cheng
- Zimeng Wang
- David Ulkoski
- Al Sabirsh
- Lennart Lindfors
- Liping Zhou
- Advanced Drug Delivery team members
- Collaborators at Carnegie Mellon and Vanderbilt Universities

Introducing the Intracellular Drug Delivery Centre

Dr Juliana Haggerty
Head of Intracellular Drug Delivery centre



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Intracellular Drug Delivery Centre (IDDC)

- Centre of Excellence for companies/academics to access state of the art capabilities and expert support
- Design, formulation, characterisation and manufacture of nano-delivery systems (NDS) for multiple payloads and targets
- R&D programmes to solve challenges & flexible industry engagement model

CPI are leading this complex multi-partner programme

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CATAPULT Medicines Discovery

We are addressing critical scientific and commercial challenges

- in vitro models
- Thermostability and alternative delivery routes
- Payload transport and release in the body
- Delivery beyond the liver
- Prediction of immunogenicity, safety and adverse reactions
- Unclear pathways to product registration
- Supply chains and manufacturing
- Complex IP landscape and high licensing costs

R&D and business support work packages

Creating the baseline for future industry and academic projects

LNP formulation screening and characterisation

Enhanced methods to enable in vitro -in vivo correlation

Smart, next gen manufacturing

Supporting the ecosystem

IDDC Leadership Team

Dr Juliana Haggerty

Head of Centre of Excellence

IDDC Lead

Prof Yvonne Perrie

Chair in Drug Delivery within Strathclyde Institute of Pharmacy and Biomedical Sciences

Dr Neill Liptrott

Reader in Pharmacology and Immunocompatibility , Liverpool Nanotherapeutics Hub coordinator

Prof Robin Shattock

Professor of Mucosal Infection & Immunity

Dr Sarah Brockbank

Lead Scientist of External Drug Discovery

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Overarching technology focus areas

Screening & Formulation

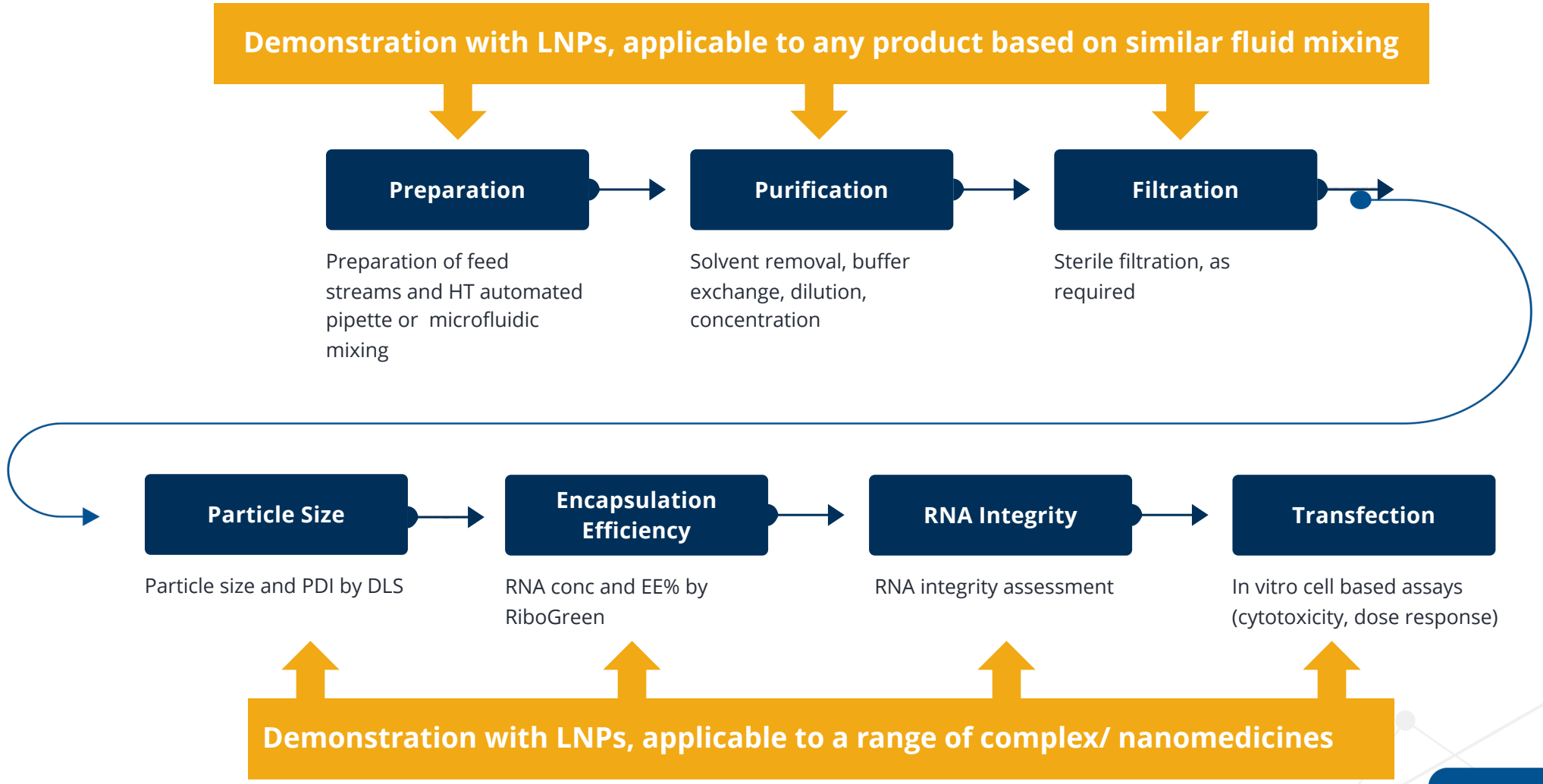
Manufacturing

AI for lipid design, novel lipid synthesis and screening	Automated HT formulation to screen lipid & formulation against multiple payloads	Cobot integration – lab of the future	HT Characterisation (basic physicochem & cell based tox and transfection)	In depth, advanced characterisation (in vitro, in vivo, advanced imaging)	Next gen LNP manufacture (digital, sustainable)	New integrated PAT, advanced process models, digital twins

Underpinning digital capability – upload data to cloud, apply advanced models to enable adaptive (intelligent) DoE, model development and deployment, simulation

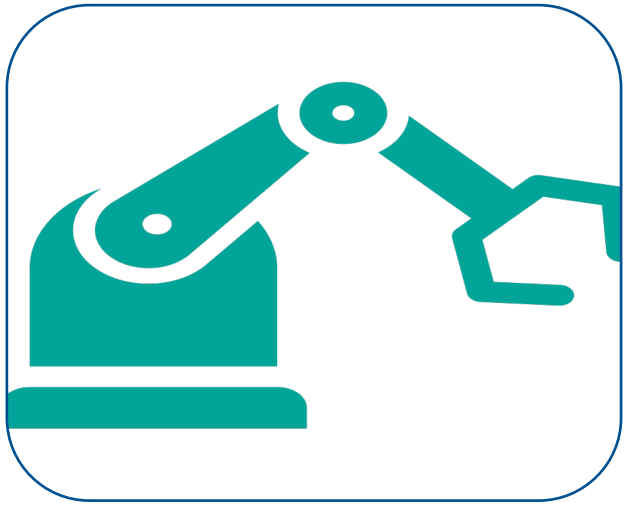
Creating a flexible platform for screening, characterisation and manufacture that is flexible and adaptable to answer multiple research questions (tune for thermostability, targeting, immunogenicity, test new PAT and models)

Building a HT Screening & Characterisation Platform



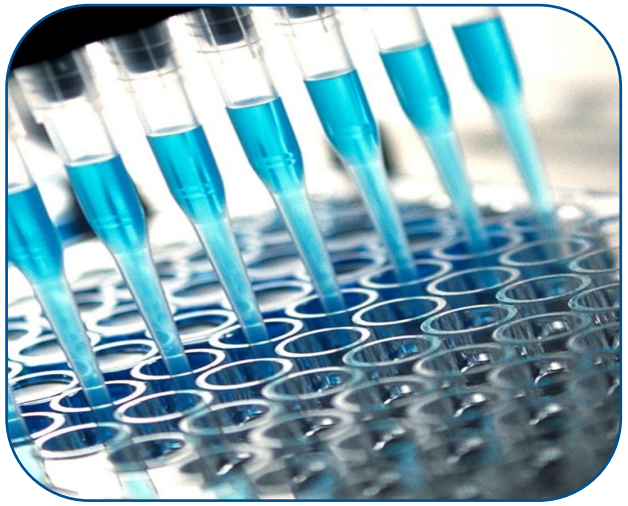
Digital Technologies to enhance screening and formulation

Demonstrating “Lab of the Future” Proof of Concept through a mobile, flexible and configurable approach to nano pharmaceutical product development



Utilise automation to increase productivity, reduce material requirements.

Phasing in of additional automation (collaborative mobile robot)



Screen:

payloads (different sized RNA)

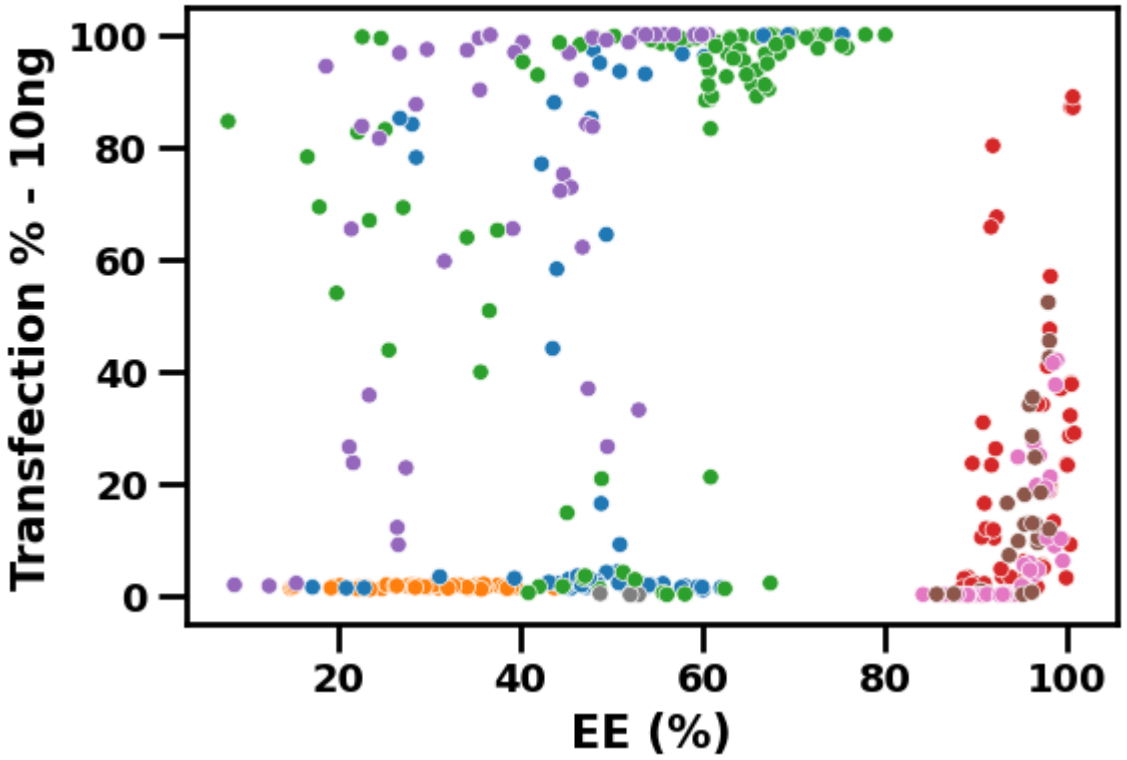
off the shelf and novel lipids

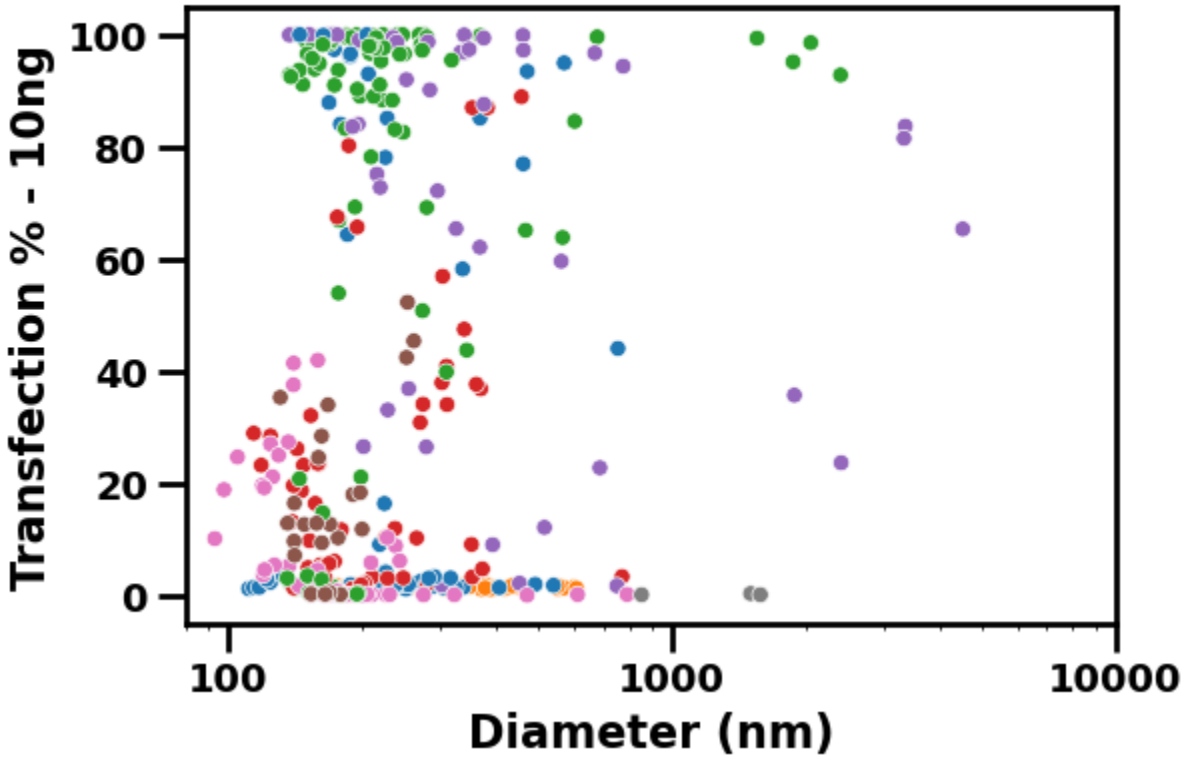
different formulations to create robust dataset

Analyse using AI software

Enables modelling, directed optimisation, and prediction from sparse datasets to reduce physical experiments

Lipid screening output: Example data





Each coloured dot represents a different class of lipid.

In these data formulation composition within each class was varied to generate a range of sizes and encapsulation efficiencies

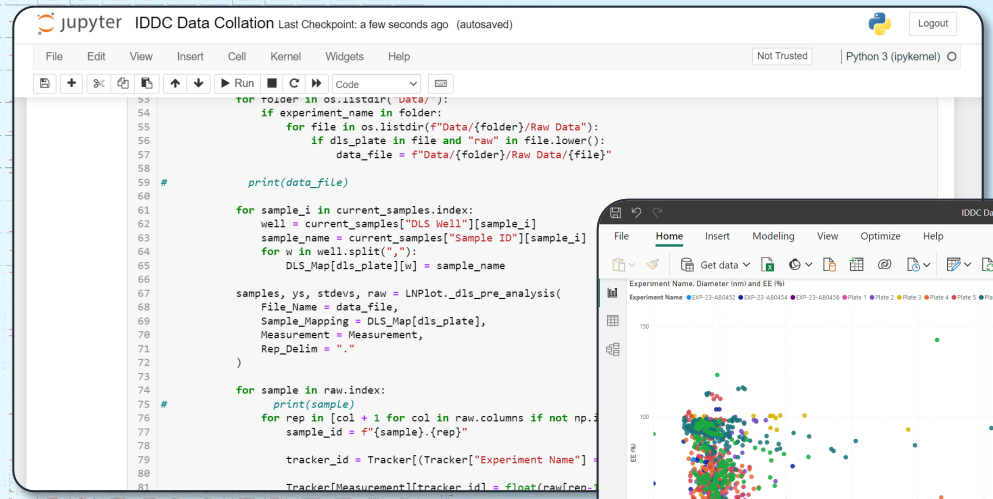
Complex Data

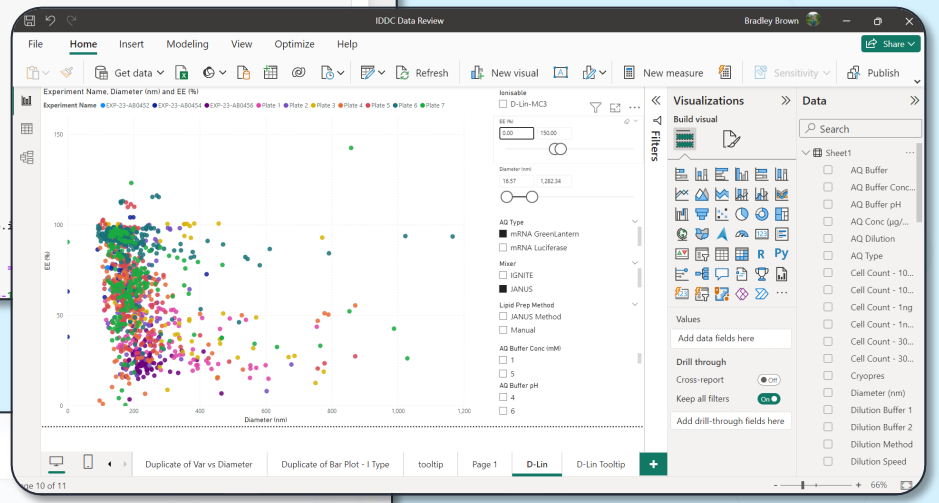
Problem

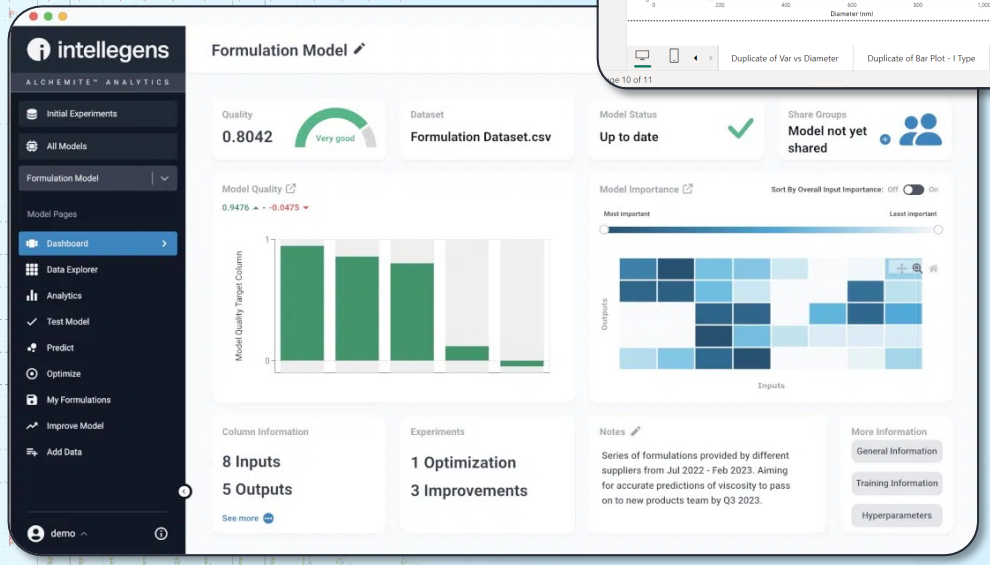
- Automation allows for large amounts of data to be collected
- Lots of variables to be investigated
- The dataset is highly multi-dimensional
- Trends can be difficult to spot

Solution

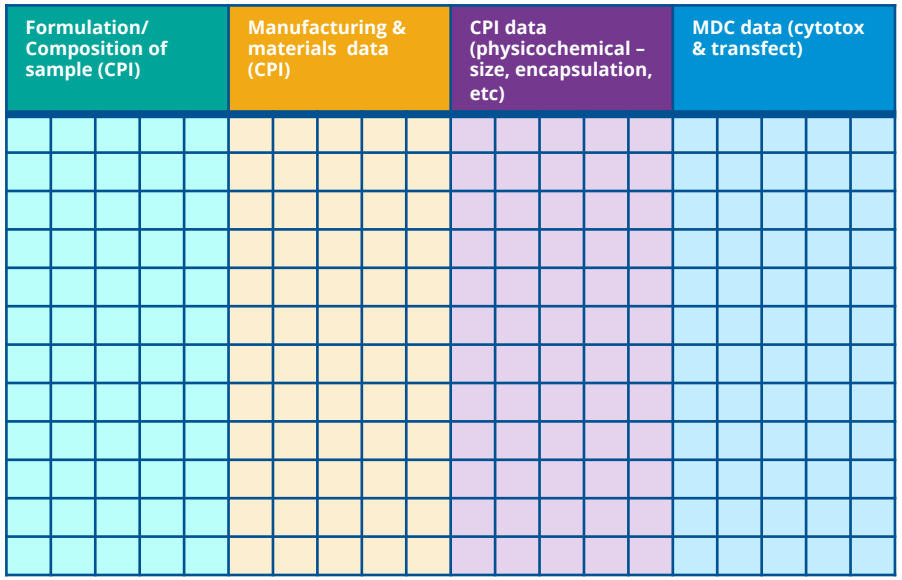
- Python for data handling
- PowerBI to quickly visualise data
- Alchemite (ML-enabled adaptive DoE) to find trends and interactions, make predictions, and guide experimentation







Phase 1 – Primary Screening



Select formulation

Select formulation

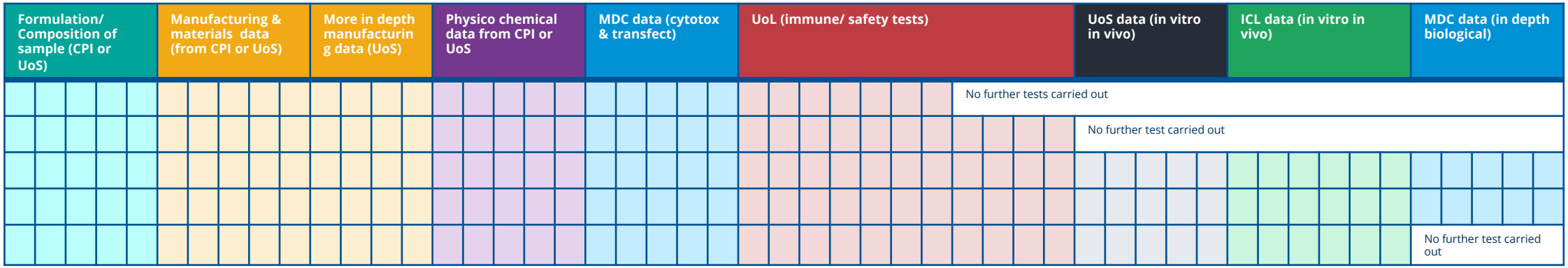
Select formulation

Select formulation

Select formulation

- Take down-selected formulations through to phase 2
- Make phase 2 formulations at larger scale, and carry out primary screen test plus in- depth tests
- Some formulations will be rejected part way through in-depth screening

Phase 2 – In depth Screening



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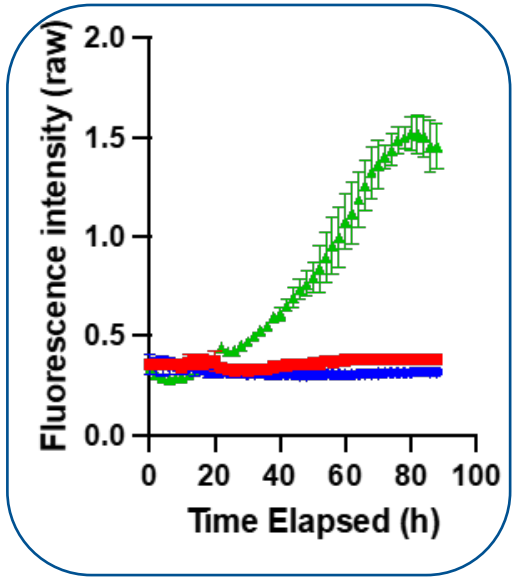
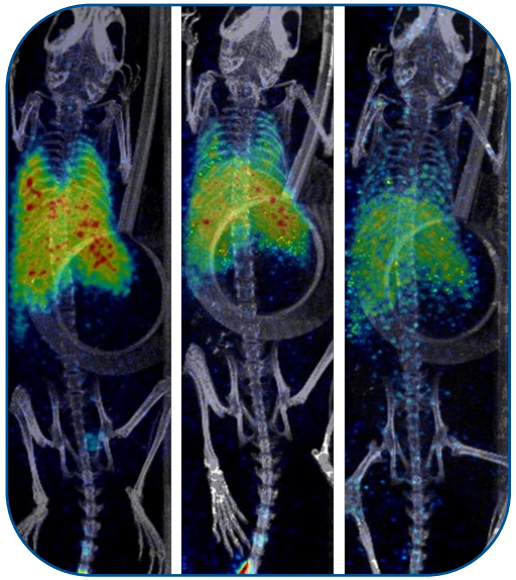
Advanced analytical platform for characterisation of LNP biodistribution and cellular delivery

In depth characterisation of tissue penetration, cell targeting and biological response

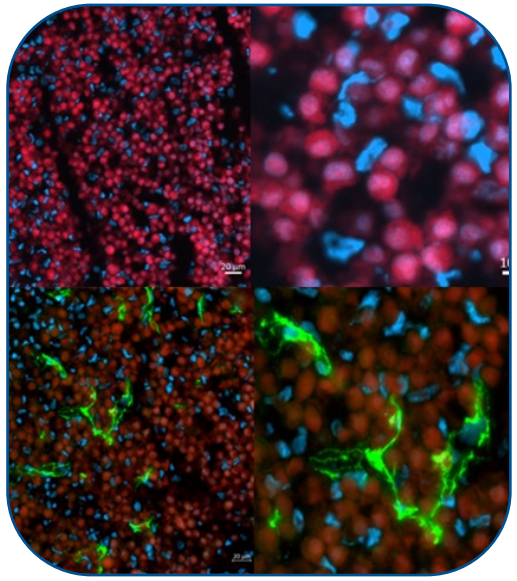


High content primary screen of novel lipids for biological activity

High resolution microscopy, to determine the extent and mechanism of LNP internalisation into the target cell



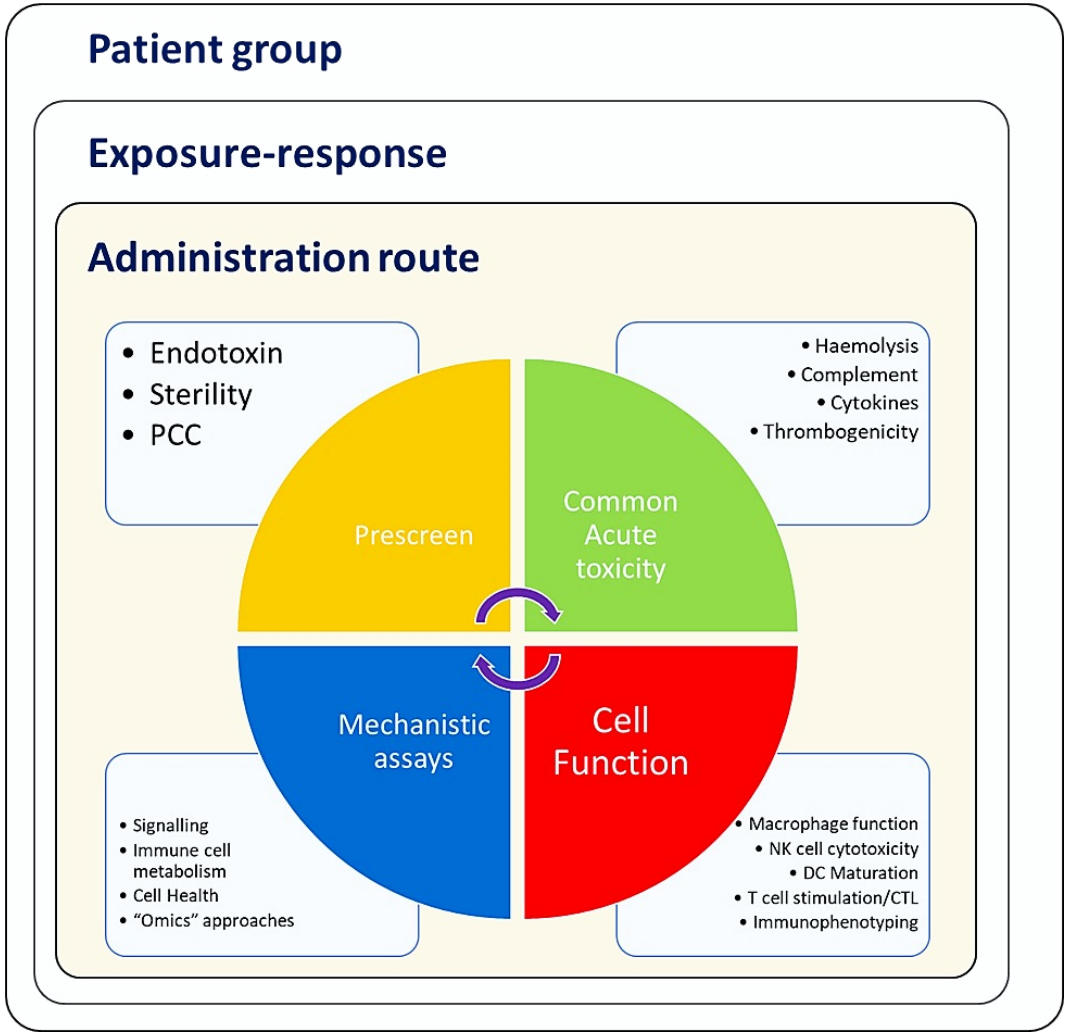
A comprehensive in vivo imaging capability utilising PET* to assess biodistribution and targeted delivery of LNPs in vivo



Ex-vivo analysis using immunofluorescence microscopy provides understanding of tissue penetration, cellular delivery and efficacy

PET: positron emission tomography

Analytical cascade for immunocompatibility assessment of complex medicines



Iterative cascade, tailored to the delivery system, taking into account key decisions such as **route of administration** and the **intended patient group**

- Pre-screen to assess possible bioburden and biological contamination in formulations
- Determine common acute toxicities from whole blood responses
- Assessing specific immune cell responses, utilising harmonised SOPs
- Mechanistic assays for bioenergetics and immunometabolism

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Advanced characterisation capabilities in IDDC

Physicochemical Characterisation

- Size and size distribution
- Encapsulation efficiency (& mass balance)
- RNA integrity

In vitro (cell)

- Cellular uptake and kinetics (high content imaging) in HEK293/HeLa using labelled LNPs
- Endosomal uptake and release in genetically modified cell lines (high content imaging)
- In vitro target engagement and effect, GFP expression or gene KD (Incucyte, qPCR or POE-mRNA)
- Cellular expression and innate profiling in targets for transduction including range of cells (muscle, antigen presenting, adipocytes, hepatocytes and epithelial)

Ex vivo

- Tissue penetration
- Cell type specific cellular uptake, delivery and efficacy, immunofluorescence microscopy)
- Ex vivo uptake, expression, innate profiling and target identification in human skin explant model

In vivo

- Biodistribution, PK and targeted delivery (PET)
- Animal models (incl xenograft or orthotopic tumour models/ brain tumours for BBB delivery) and routes of administration
- In vivo rodent studies of multiplexed RNA-LNPs, for tissue expression profiling

Immunocompatibility

- Complement activation via measurement of iC3b, sC5b-9, C4d, Bb (multi-plex, single-plex or proteomics)
- Whole blood, or PBMC, exposure to LNPs – release of immuno-mediators
- Platelet activation via multicolour flow cytometry
- Inflammasome activation in monocytes (primary, human)
- Basophil and/or mast cell activation and degranulation
- Anti-PEG antibody screening in “positive” AR individuals

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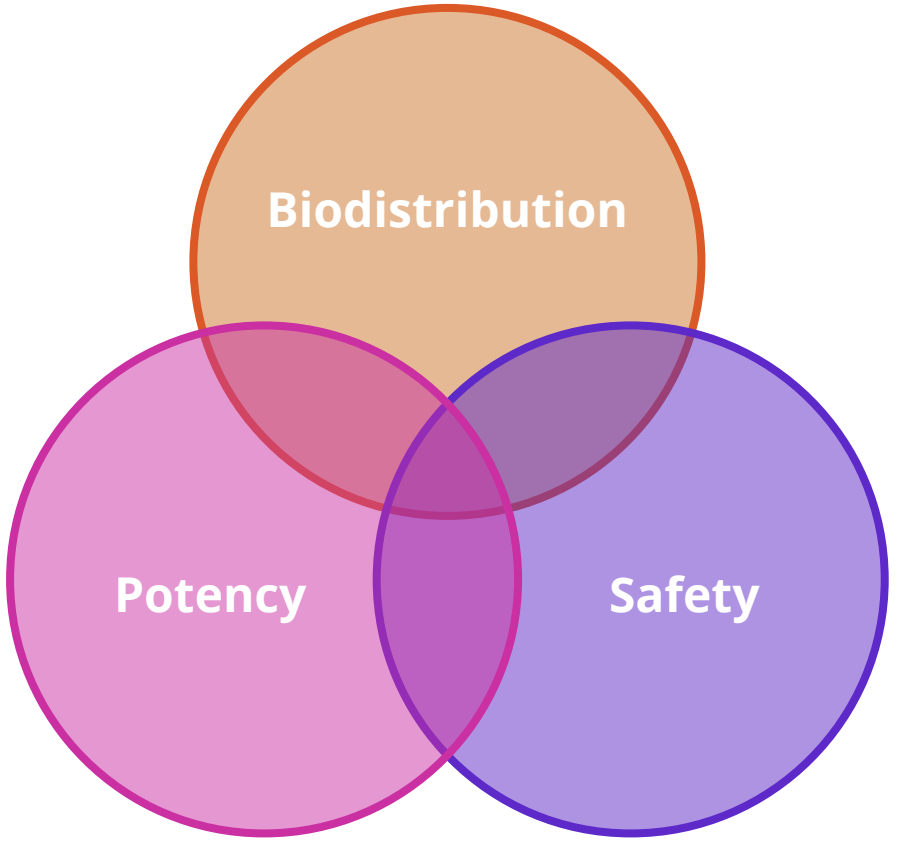
Enhanced methods, and models, to improve in vitro-in vivo correlation

Current in vitro models can fail to fully predict in vivo, human, responses to complex medicines.

IDDC has a dedicated work package to investigate the utility of advanced, human, cell models to understand key areas in the development of complex medicines.

Assays and models developed, will be assessed using standardised procedures and interlaboratory comparisons, amongst partners with the relevant experience.

Advanced capabilities will then be made available to the community, via the IDDC infrastructure.



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Supporting the Ecosystem

- Internal ECR Programme for skills development
- External training in development

Training

- Plan to publish regulatory road mapping tool

Regulatory

- Papers, conferences, workshops
- Whitepaper on targeting in planning

Dissemination & Engagement

- IDDC capability available via contract research

Contract Research

- Actively building additional collaborations
- Manufacturing, digital, characterisation and non-LNP focus

Collaboration