Medicines Discovery Catapult

MDC Connects: A Guide to Drug Discovery

Reshaping Discovery Together

CATAPULT Medicines Discovery

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About **MDC Connects**

An important part of the work of Medicines Discovery Catapult (MDC) is our focus on connecting the UK drug discovery community.

In the Spring of 2020, the world was in the grip of the COVID-19 pandemic and much of the scientific community went into lockdown. Conferences and meetings were cancelled and many of the scientists were working remotely. MDC decided to connect the community by running a series of weekly webinars: MDC Connects.

These were sessions to educate, inform and advise the community on the drug discovery; and the sessions were aimed at small companies who are developing their own medicines. For this series we focused on preclinical research with an emphasis on small molecules and starting with:

- What makes, and how to identify, a good target
- Methods to identify the chemical compound which would become the drug
- How to optimise that drug
- How to test it works in models of disease
- And how to demonstrate that it would be safe to dose in humans

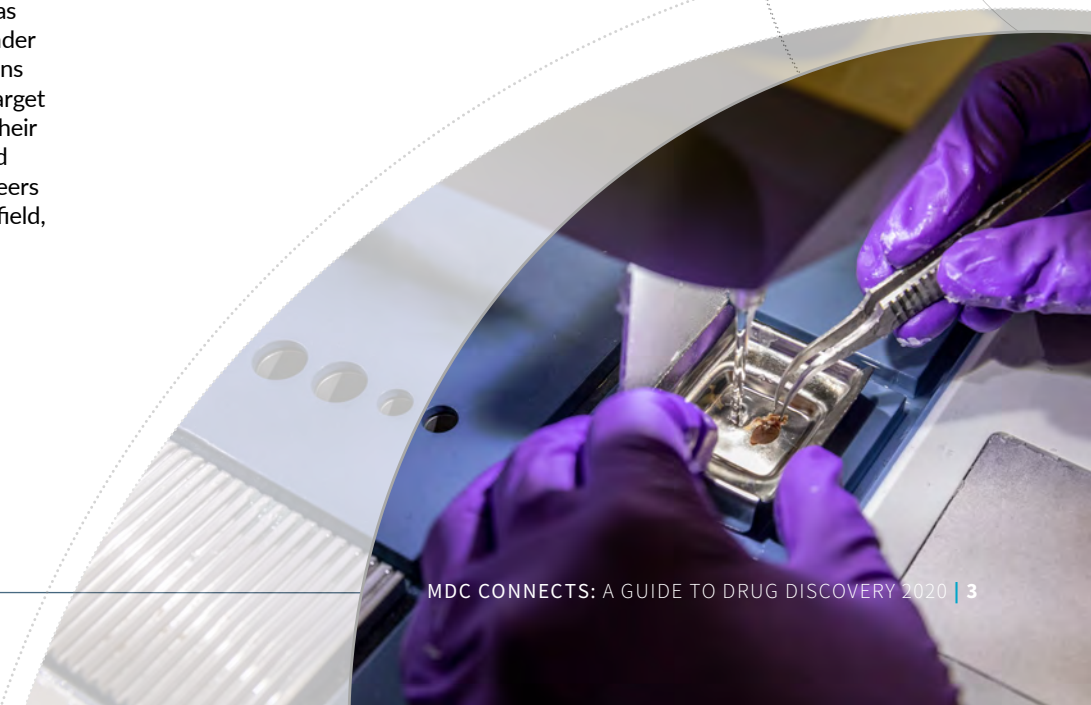
We invited experts from our partner CROs together with experts from within MDC to deliver the sessions. In total, the series consisted of 9 weekly webinars, which we believed would span the lockdown period. Each webinar was delivered by 3 experts totalling 26 speakers from 17 companies.

The MDC Connects webinar series was fantastically well received with just under 1,000 people registered for the sessions from the UK and beyond. While our target audience was companies developing their own medicines, our delegates included students, scientists starting out in careers in medicines discovery, experts in the field, keen to keep in touch.

For this guide we've written up a summary of each presentation from the webinars, creating a great resource of modern drug discovery knowledge. None of this would have been possible without the community supporting us, and we'd especially like to thank the partners who took part in MDC Connects. Please take a look at their websites and get in touch if you think they can help you.

Thank you, and enjoy the MDC Connects: A Guide to Drug Discovery!

Sarah Brockbank
Medicines Discovery Catapult



1 | Identifying the Target

Lack of efficacy is one of the most important causes of failure in clinical trials. Therefore, identifying the biological target for a drug discovery project is one of the most important decisions the project team will make. The safest and most potent molecule will still fail if a team is working on the wrong target for the disease of interest.

In the first webinar, we considered what makes a good target and how we identify them. Davide Gianni described the importance of selecting the right target and the approaches used by AstraZeneca, and John Overington showed how informatics and data mining demonstrates which targets and target classes have the best chance of success.

View the recording and slides for the first webinar
Identifying the Target

Target discovery at AstraZeneca: discovery, identification, and novel targets

Both the pharmaceutical industry and non-industry researchers spend a lot of time analysing why there are high attrition rates in the drug development process. Attempts to reduce the number of efficacy- and safety-related failures in the drug discovery phase led AstraZeneca to developing the 5R Framework:

1. Right target
2. Right tissue
3. Right safety
4. Right patients
5. Right commercial potential

Designed to help guide successful drug discovery, implementation has seen an increase in success rate of 15%, however lack of efficacy was still a root cause of drug discovery failure. This led to the realisation that an effective target discovery platform was critical to reduce the attrition rate observed in clinical studies.

Combination of innovative screening technology and translatable biological models

Genetic linkage to disease and model translatability are critical features associated with reduced clinical efficacy. The two driving concepts to building an integrated target discovery platform are therefore a translatable model, and genetic target validation to identify, prioritise and validate novel drug targets. Genomic data and CRISPR gene editing have enabled the identification and validation of new drug targets.

External collaboration with the Innovative Genomics Institute (IGI) who create CRISPR inhibition and CRISPR activation libraries and assess DNA damage response, the CRUK Functional Genomics Centre, who run pooled genome wide screens for oncology and identify mechanisms of resistance, and an established collaboration with the Karolinska Institute in Stockholm, who assess secretome libraries from human cells, supports the discovery of new drug targets and the target discovery platform.

Target discovery using CRISPR libraries

The development of a CRISPR-Cas9 DOX system has increased the relevance of screening and precision and helped minimise off-target effects with the use of an insulator, doxycycline.

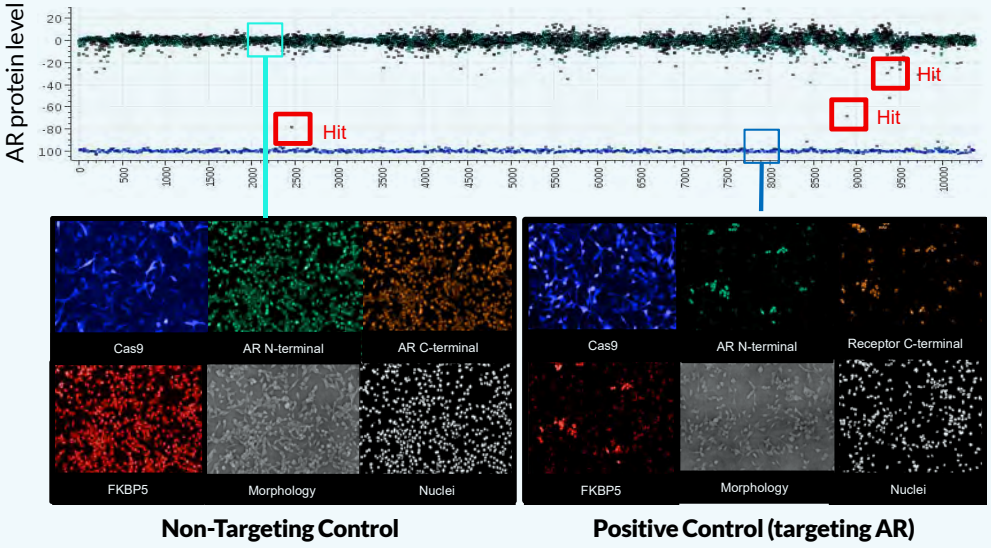
The technology has been demonstrated through the generation of cell models and characterisation of multiple prostate cancer cell lines induced to express CAS9, using genome-wide CRISPRn and CRISPRa array libraries. The androgen-receptor is a key drug target in prostate cancer and the use of a high content, biological assay has allowed staining for the androgen receptor and proliferation and the key target genome androgen receptor FKBP5.

At AstraZeneca, we have developed a target discovery platform using highly validated genome-wide CRISPRn and CRISPRa libraries that delivers new target opportunities for the AstraZeneca portfolio with an increased chance in the clinical development programme.

Identification of regulators of AR stability to identify novel targets for CRPC

RZ'>0.8

Well data across primary screen: positive controls (blue), negative controls (green) & library gRNAs (black)



Non-Targeting Control **Positive Control (targeting AR)**

Strong opportunity to impact AZ portfolio

Screening across multiple PC cell lines expressing clinically relevant AR variants

Identification of known & novel regulators

- Epigenetic Modulators
- E3 Ubiquitin Ligases
- Co-regulators
- Regulators of Transcription
- Splicing Factors

Current status:

- Confirmed and ranked hit list undergoing target validation

About the author

Dr Davide Gianni, *AstraZeneca*

Davide is Associate Director Functional Genomics in Discovery Sciences, BioPharmaceuticals R&D at AstraZeneca and is responsible for leading a team of scientists to deliver new therapeutic opportunities for AstraZeneca's therapy areas of interest. Davide joined AZ from Boehringer-Ingelheim in 2015 where, acting as a Research Laboratory Head, he has led a team of scientists aimed at identifying and validating novel target opportunities for Oncology. Earlier in his career, he conducted his postdoctoral studies at The Scripps Research Institute in La Jolla (California) where he focused his research activities on deciphering the contribution of Reactive Oxygen Species (ROS) in mechanisms underlying human diseases including cancer, neurodegeneration and cardiovascular disease.

AstraZeneca

AstraZeneca is a global, science-led biopharmaceutical company that focuses on the discovery, development and commercialisation of prescription medicines, primarily for the treatment of diseases in three therapy areas - Oncology, Cardiovascular, Renal & Metabolism, and Respiratory & Immunology. Based in Cambridge, UK, AstraZeneca operates in over 100 countries and its innovative medicines are used by millions of patients worldwide.

Target Identification with informatics and data mining

What is druggability?

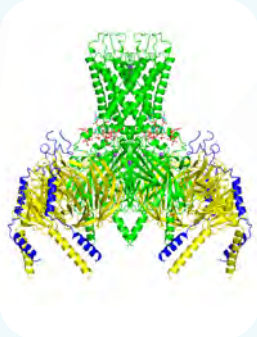
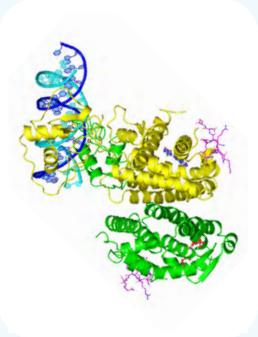
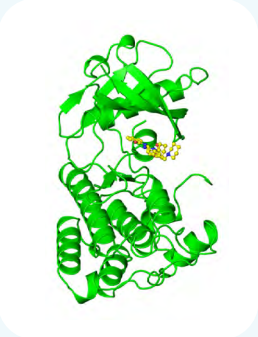
Central to the drug discovery process is the identification of a suitable efficacious target, and the ability of a novel drug-like compound to bind to and modify that target. A successful drug target needs to demonstrate two key properties — 1) it has to have a site capable of binding drug-like molecules, i.e. a druggable site, and 2) it has to have a causal link to a disease process. Historical drug targets have both of these properties by definition, and analysis of their features can help guide and derisk future drug discovery.

Druggability usually runs in families

The curation and analysis of databases of known drug targets have allowed them to be classified into protein families, within which are four main target classes of privileged 'druggable' families - Rhodopsin-like GPCR ligands, ion channels, nuclear receptors and protein kinases. Approximately 53% of historical drug targets and 70% of approved drugs modulate one of these 4 targets, so investing in screening technologies, in compound libraries and in expertise around the system biology and signalling of these proteins supports the drug discovery process, alongside the use of informatics to gather data on the desired target.

Use of these databases, i.e. the ChEMBL database, shows known drug targets such as GPCR ligands, yield a good return on investment - 18% of compounds published in lead optimisation studies are GPCR ligands, while 30% of approved drugs on the market are GPCR drugs. So whilst identification of a novel drug target is scientifically fascinating and exciting, discovery productivity will be lower with a significant investment in time and resource required as the explicit cost of this higher novelty.

Privileged Historical Target Families

Rhodopsin-like GPCR PDBe: 3sn6	Ion channels PDBe: 4kfm	Nuclear receptors PDBe: 3e00	Protein kinases PDBe: 4foc
			
22% of drug targets **33%** of small mol drugs	**12%** of drug targets **18%** of small mol drugs	**6%** of drug targets **17%** of small mol drugs	**13%** of drug targets **2.4%** of small mol drugs

Over 53% of all targets and 70% of drugs modulate these four target classes

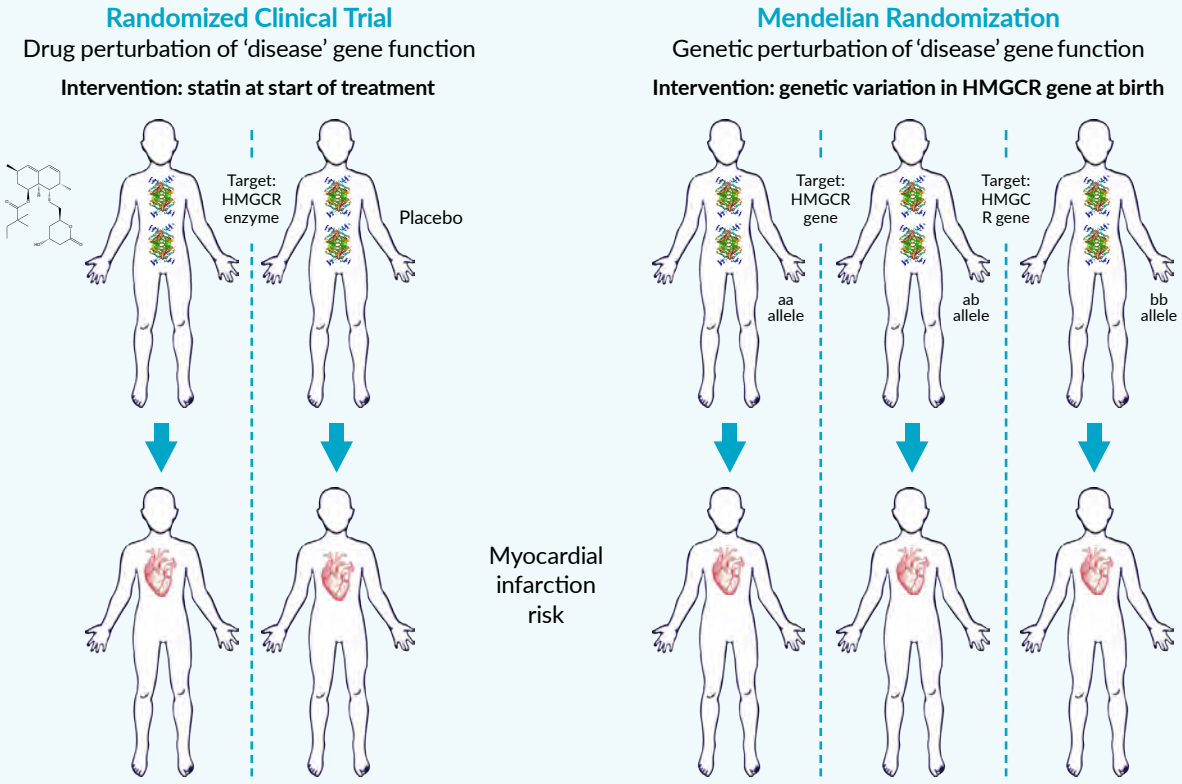
Foresight via genetics

Whilst knowledge of target druggability is essential, so is the efficacy component. Mendelian randomisation can provide evidence of the causal relationship between the target and disease and provide a way of anticipating the likely success of a target. The real benefit of pre-validating the success of these targets is it can be done prior to large scale, expensive phase 2 trials.

Using the resources available

When considering drug targets for analysis, triage and so forth, the use of online resources can support these decisions. Examples include Open Target - a collaborative project between several industry partners, the EMBL-EBI and the Sanger Institute who publish a richly curated and integrated collection of data. Illuminating the Duggable Genome (IDG) is a global project whose aim is to identify and provide information on less well studied proteins within commonly drug-targeted protein families. Finally, the CanSAR platform at the Institute of Cancer research provide data on somatic diseases.

Mendelian Randomization in target validation



About the author

Professor John P. Overington, *Medicines Discovery Catapult*

In 2017 John joined the Medicines Discovery Catapult as CIO, where he leads the development and application of informatics approaches to promote and support innovative, fast-to-patient drug discovery in the UK through collaborative projects across the applied R&D community. He was involved in the development of the medicinal chemistry database StARLite - the precursor to ChEMBL. More recently, the work extended into large-scale patent informatics with the Open patent database SureChEMBL. John has a degree in Chemistry from the University of Bath and a PhD from Birkbeck College, London. He is a visiting professor at UCL and the University of Manchester.

2 | Hit Identification

The identification of high-quality leads is an important step in the discovery of a new drug, but in order to achieve a relevant starting point there are several factors to consider in the design of the assays, the reagents and the compound collection.

In the second webinar, Trevor Askwith outlined the important factors to consider when embarking on a hit identification programme and the development of a screen, the importance of quality protein, a quality compound collection and good assay design.

Gary Allenby provided examples of assays with case studies, focusing on cell-based assays and taking a look to the future describing the use of complex assays at this early stage.

And finally, Andrew Pannifer described the importance of building and then maintaining a compound collection against which to screen.

View the recording and slides for the second webinar
Hit Identification

HIT ID screening— understanding why your target is key

A critical starting point for a drug discovery programme is the Hit identification of a high-quality compound translatable to an optimisation programme, and delivery into the drug candidate. This relies on an integrative and comprehensive medicines research platform comprising chemistry, protein science and assay biology. These provide a screening cascade to identify the most druggable domains, identify where the Hits are binding, and help increase the success of a project leading to the development of a drug.

High HIT rate

To identify a good quality compound and good quality Hits, Domainex utilises the LeadBuilder virtual screening platform. By using the information on the target protein or the ligand of interest, it utilises structural information or homology models and screens up to 1000 compounds. The interrogation of different virtual libraries and the NICE library consisting of commercially available compounds enables rapid Hit expansion.

The fragment library is a highly curated diverse library of 1,300 fragments which are rule of three compliant and contains a large number of 2D and 3D fragments which can be run in parallel. All these components ensure all the Hits from the screen are good quality compounds that translate to a good chemical starting point.

Providing a high-quality, cost-effective service

Critical to good quality protein production is a high-quality quality control of the protein for purity, activity, compound binding. Domainex utilise different analytical techniques to ensure this is met using processes such as a multiple step chromatography.

Domainex uses microscale thermophoresis, which is a solution-based system to assess the protein, that requires exceptionally low protein samples, typically 5–15 nM concentration in about 5 ml. It has the advantage of focusing on the right protein. It also uses multiple different labelling strategies, directed by the structural information gathered about the target. This enables the delivery of proteins in their near physiological states, providing the opportunity to identify Hits in a method that is more likely to be translated into the drug at the end of the project.

An integrated approach

Domainex uses an integrated suite of drug discovery services to support emerging biotechnology, pharmaceutical companies, and translational research to identify and develop a drug candidate. Not only does it provide access to high-quality Hits, it can also support with protein production and technology to confirm binding, measure binding thermodynamics and confirm structural biology to identify optimal conditions and determine and characterise 3D structures.

About the author

Dr Trevor Askwith, *Domainex*

Trevor is a Group Leader in assay biology with over 10 years' experience of small-molecule drug discovery research. Prior to joining Domainex, Trevor held the role of Head of Assay Development and Screening in the Drug Discovery Group at UCL, where he ran a number of successful Hit ID for novel targets emerging from the university. Before joining UCL, Trevor was a Senior Research Fellow in the Drug Discovery Centre at the University of Sussex where he helped to establish the facility as well as develop and run cell-based and biochemical screening assays to support Welcome Trust and CRUK funded projects. Trevor obtained his PhD from the University of Birmingham working with Prof Martin Stevens where he studied the mechanisms of taurine depletion in diabetic neuropathy.

Domainex

Domainex is a high quality, fully integrated drug discovery service company based near Cambridge, UK serving pharmaceutical, biotechnology, academic and patient foundations globally. They offer a tailored range of biology and chemistry services from a single location, taking our clients from target nomination to delivering pre-clinical candidates. Approximately 80% of their scientists have PhDs and have over 10 years of average industrial experience across a number of therapeutic areas.

Cell-based screening: Old dogs with new tricks

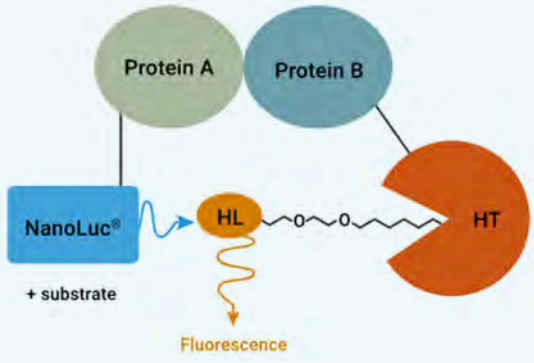
Aurelia Bioscience are a pre-clinical contract research organisation specialising in the development of bespoke assays and discovery-based projects for clients. The company offers assay development to detect protein-protein interactions in living cells using technology such as the NanoBRET target engagement assay, cell culture using 3D cell-based screening applications and magnetic electrospun micro-fibre technology, the study of protein degradation using proteolysis targeting chimeras (PROTAC) and protein detection and characterisation using Western Assay System (WES/JESS).

Protein-protein interactions in living cells

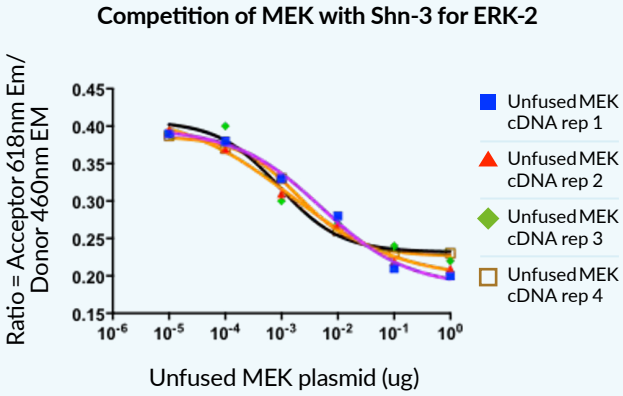
NanoBRET technology studies protein-protein interactions in living cells, using Bioluminescence Resonance Energy Transfer (BRET). A study investigating the interaction of Schnurri-3, a regulator of adult bone formation, with ERK-2 used 2 different vectors. One vector contained Schnurri-3 and NanoLuc®, a luminescence enzyme with a high efficiency to produce photons, and the 2nd vector contained ERK-2 plus HaloTag®, a fluorescent ligand. Fluorescence was observed when the two proteins came into close proximity. The NanoBRET assay screened for inhibitors of the Schnurri-3-ERK interaction and the ratio of the NanoLuc signal to fluorescence signal was determined. This was validated using MEK within the cells which competes for interaction and if over-expressed will decrease the fluorescence signal.

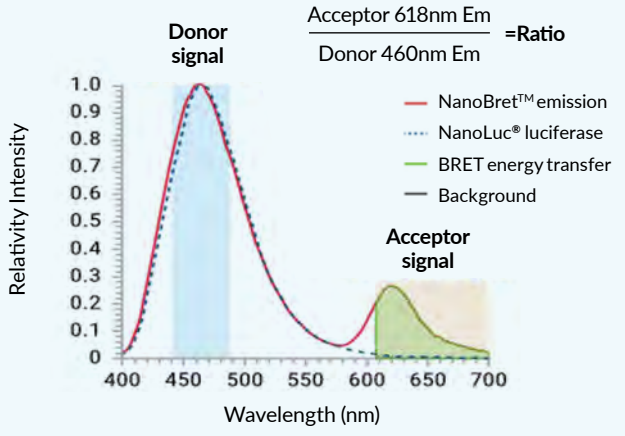
NanoBRET - Protein:Protein Interactions

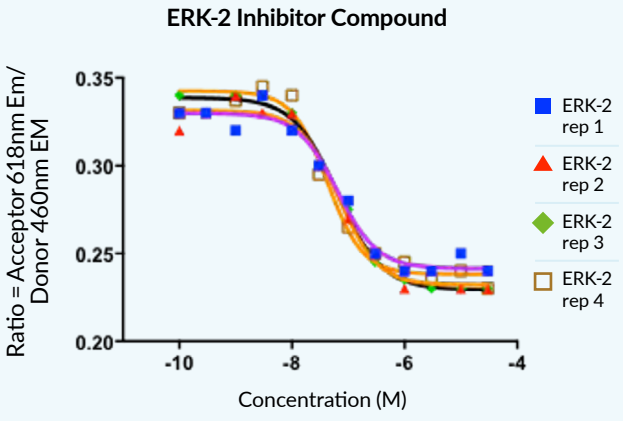
Target = Schnurri-3 interacts with ERK-2: Schnurri-3 is thought to regulate bone formation. Schnurri-3 suppresses ERK phosphorylation of GSK-3ß leading to suppression of ß-catenin. Theory – block Schnurri – ERK interaction you remove the brake and allow bone formation – Therapy - oesteoporosis



HT: HaloTag® NanoBret™ 618 Ligand **HL:** Halo Tag® protein





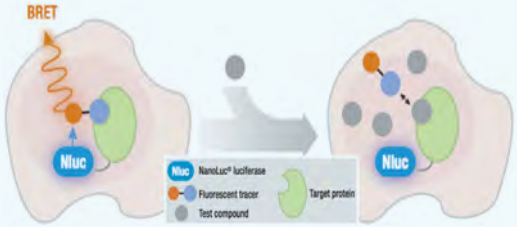


Based on these results, a high throughput screening assay was developed and 50,000 compounds were screened which identified a smaller number of compounds that interacted with Schnurri-3, blocking its binding to ERK-2.

NanoBRET Target Engagement

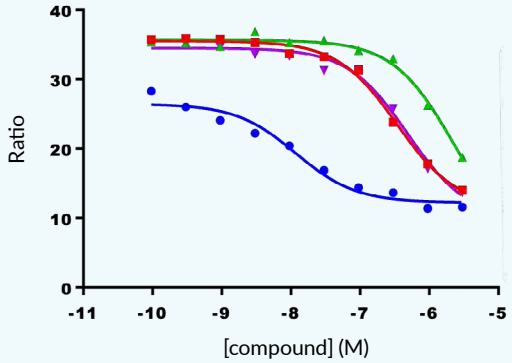
NanoBRET Target Engagement is a competition assay between a fluorescently labelled tracer and a kinase of interest, the interaction occurs in living cells. The full-length kinase is expressed in the cell and interaction takes place at a physiological ATP and physiological pH enabling the association and dissociation rates of the compound within the cells to be observed. The use of a labelled tracer activates fluorescence when in close proximity to the NanoLuc enzyme expressed either N- or C-terminal of the kinase protein, to produce a signal. This signal will decrease if a competing compound is introduced, and the tracer is no longer in close proximity to the NanoLuc enzyme.

Kinase screening using Target Engagement

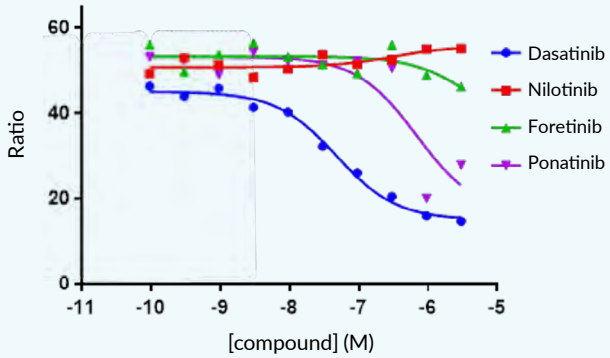


- For a Med Chemist success is driven by potency – but what about residency time?
- How long does a compound need to be bound in order to have an effect
- How can we perform residency time experiments

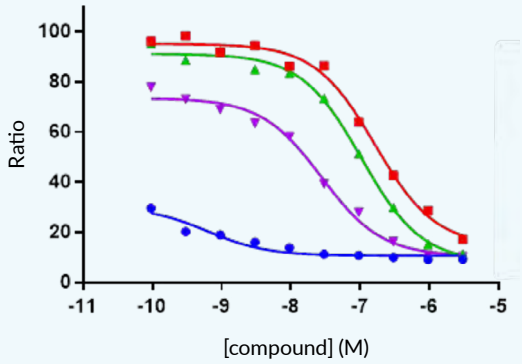
Effect of inhibitors on ABL kinase



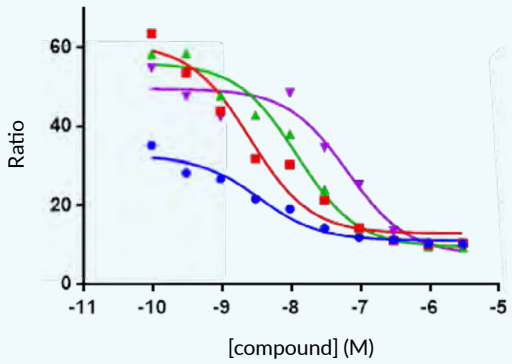
Effect of inhibitors on FGR kinase



Effect of inhibitors on EPHAS kinase



Effect of inhibitors on DDR kinase



Binding activity of each compound was determined in living cells. Cells were transfected with each of four kinase; ABL, FGR, EPHA8 and DDR-1. Cells were treated with exemplar kinase compounds including dasatinib, nilotinib, foretinib and ponatinib as a dose response for each compound competed against a fixed concentration of fluorescent tracer K4.

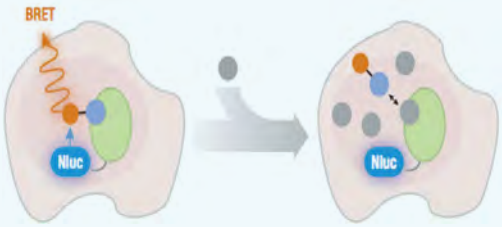
Adhere cells to a material that can be moved between wells – no washing, just change of plate

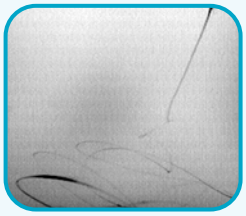
The assay also demonstrates both cellular permeability and potency of the compounds.

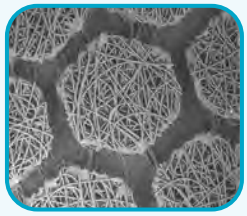
3-D cell-based screening

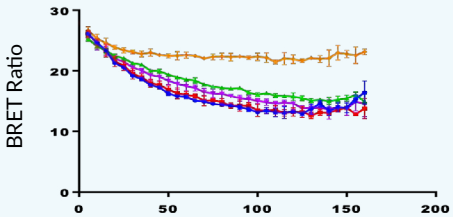
To investigate the residency time of how long a compound needs to be bound to a target to have an effect, an electrospun scaffold material has been developed onto which cells are seeded. The scaffold is magnetic allowed movement of an adherent monolayer of cells from well to well i.e. wells containing the tracer or wells containing compound. These can then be placed directly into the plate reader. The resulting graph shows time vs. the BRET ratio and the different compounds compete off the receptor at different rates.

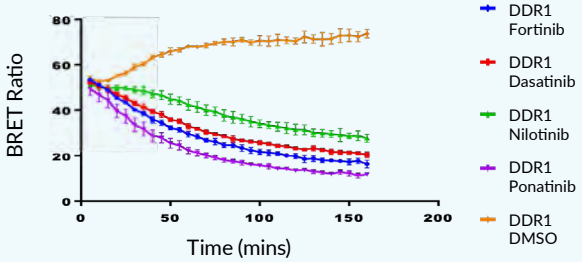
Changing the Paradigm – Move cells plate to plate

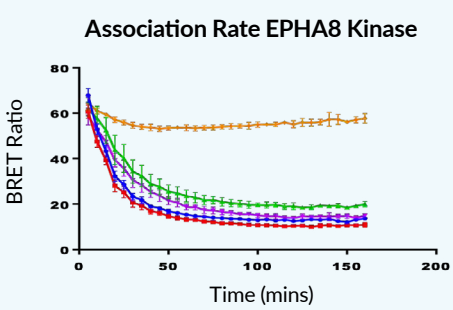


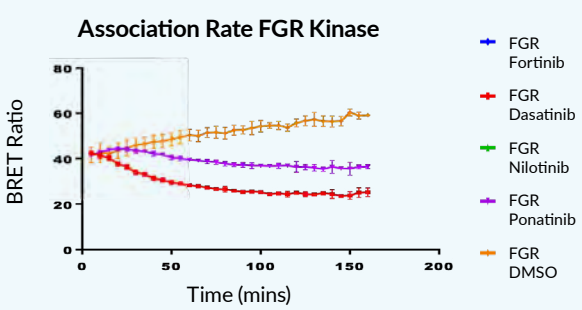


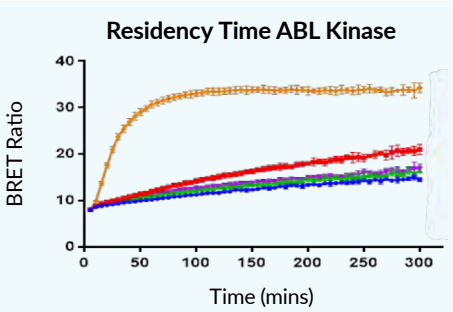


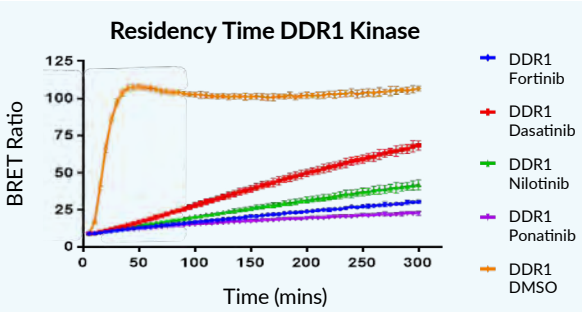


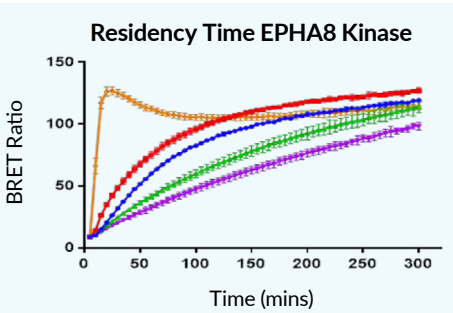


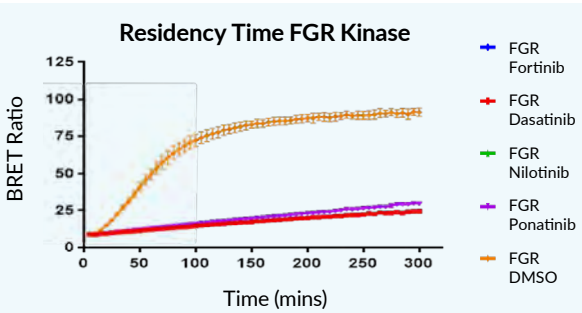










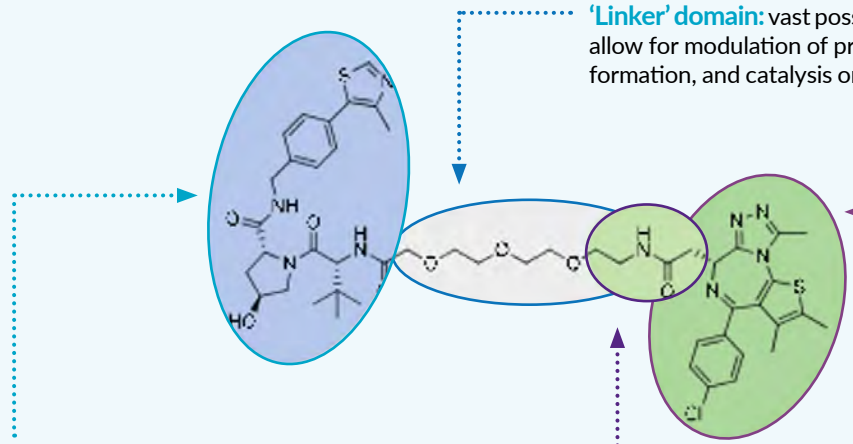


Proteolysis targeted chimeras (PROTAC)

PROTAC uses a ubiquitin-proteasome system to induce protein degradation at the proteasome. The technology uses a small chimeric molecule, one end selectively binds to the protein target of interest and the other end binds to an E3 ligase, held together with a linker. The E3 ligase ubiquitinates the protein, targeting it for degradation. Once degradation is in progress the PROTAC molecules dissociates and can be reused. An advantage of this system is it degrades the target protein rather than inhibits it, making it an excellent technique to study undruggable proteins.

Following treatment with PROTAC, high-throughput, capillary based, automated Western blot, WES and JESS are used to measure protein degradation, Both techniques are fully automated, the cell ligase/protein of interest is separated based on molecular weight, immobilised with UV and subjected to immunoprobe by aspirating the primary antibody, to generate a luminescence read out.

Degrader Molecule



'Linker' domain: vast possibilities for chemistries that allow for modulation of properties, ternary complex formation, and catalysis on a case-by-case basis.

Target-binding: covalent, orthosteric, and allosteric ligands for targets are known; specific biophysical principles underpinning what is required for effective degradation must be defined on a case-by-case basis.

E3-ligase binding: known examples include specific ligands for ß-TRCP1 MDM2, clAP, xlAP1 VHL, and cereblon. The best ligase for any given target must be defined empirically.

Exit vector: trajectory out of target protein binding pocket; can impact degrader properties and control ternary complex formation.

About the author

Dr Gary Allenby, *Aurelia Bioscience*

Gary is CEO and founder at Aurelia Bioscience where he leverages over 30 years of pre-clinical drug discovery experience and a number of scientific papers in leading journals to aid the discovery of new chemical entities. After gaining his PhD from Edinburgh University in 1990, Gary joined Hoffmann La Roche (USA) to investigate retinoid pharmacology and the role of nuclear hormone receptors in foetal development. He then went on to work in the Lead Generation Section of GSK (UK) in 1995, gaining expertise in the development of cell-based assays within the CNS disease area for high throughput screening as well as novel drug discovery assay technologies and automation. After leaving GSK, Gary spent over 10 years at AstraZeneca (UK) before founding Aurelia Bioscience in 2011.

aurelia bioscience

Aurelia Bioscience is a UK-based Contract Research Organisation (CRO) specialising in bioassay development, pharmacological profiling and high throughput screening. They use cutting edge assay technologies to effectively and reliably move novel compounds or biologics through the multiple stages of early drug discovery.

Targeted compound libraries

Drug development is a complex process. In target-based drug discovery, the identification of a target (typically a protein) can emerge from academic or commercial research using a combination of genetic association studies, together with approaches such as gene knockout/knock-in. Where available, tool compounds can also be used to increase confidence in the target.

Discovering chemical start points for drug discovery programmes that modulate the activity of the target has typically started with a high throughput screen, comprising hundreds of thousands to millions of compounds. This is very resource intensive, requiring large libraries and robotic infrastructure. Targeted libraries comprise fewer compounds (typically tens of thousands) enriched with compounds likely to modulate a particular target class, or have some other desirable property (such as CNS penetrance). By using fewer compounds in the screen, hits can be discovered faster and more cost-effectively.

Targeted libraries in hit identification

Most commonly, libraries can be directed towards particular target classes, such as kinases, serine proteases or bromodomains. They can also comprise molecules directed towards specific locations in the body, such as the CNS. Libraries may also be targeted to act via specific mechanisms such as including reactive moieties able to covalently modify the target – a method used successfully with kinases and serine proteases. Transition state analogues have also received recent attention.

What makes a good library?

The aim of a targeted library is to include as many bioactive compounds as possible from the full library in as small a subset as possible. Enriching collections for bioactivity can be done using informatics-based approaches such as conventional similarity searches or with machine learning models built on bioactivity databases. Great care must be exercised with these approaches; a highly efficient way for a machine learning algorithm to maximise the number of bioactive molecules in the selected subset is to pick unselective or frequent-hitting molecules that do not represent useful startpoints for drug discovery. Prefiltering for frequent hitters based on prior behaviour in screens where possible and using substructural filters is a critical step. Physicochemical properties are typically used for CNS-directed libraries while physicochemical "whole molecule" descriptors have also been found useful in target class-directed libraries. These descriptors are less biased towards explicit substructural features exemplified in the training set.

Future developments

Targeted libraries need to balance increasing the likelihood of finding hits, leaving room for serendipity to discover new chemotypes and minimising the number of promiscuous molecules. Application of structure-based and physicochemical property-based approaches hold the potential to balance chemotype bias intrinsic to fingerprint methods. Increasing the sophistication of filtering prior to selection using machine learning approaches to identify undesirable molecules is also likely to increase in importance.

About the author

Andrew Pannifer, *Medicines Discovery Catapult*

Andrew is Head of Cheminformatics at Medicines Discovery Catapult. After a PhD in Molecular Biophysics at Oxford University, mapping the reaction mechanism of protein tyrosine phosphatases, he entered the pharmaceutical industry in 2002. Firstly, at AstraZeneca and then at Pfizer, he performed structure-based drug design and crystallography, and in 2010 joined the CRUK Beatson Institute Drug Discovery Programme to start up Structural Biology and Computational Chemistry. In 2013 he moved to the European Lead Factory as the Head of Medicinal Technologies to start up cheminformatics and modelling and also to work with external IT solutions providers to build the ELF's Honest Data Broker system for triaging HTS output.

3 | Structural approaches for drug discovery

Structural biology is a vital tool in the drug discovery pipeline. Proteins are the targets for most marketed drugs, and so uncovering the molecular structure of the biological target to high resolution, researchers can directly visualise the interactions of the target with ligands and compounds, information vital to structure-based drug design.

In this webinar we discussed protein production, structural approaches and how structural data may be exploited to support chemistry. Derek Ogg described the ins and outs of generating good quality protein suitable for structural studies with particular reference to X-Ray crystallography. Rebecca Thompson showed how the emerging field of CryoEM can be applied to generate structural information for a wide range of targets, and Martin Slater described how structure based and ligand-based software tools can be used to virtually discover, design and optimise compounds.

View the recording and slides for the third webinar
Structural approaches to drug discovery

Proteins, X-ray crystal structures and how to get them

Proteins are the targets for most marketed drugs today, and high-quality purified proteins are required for the drug development process. Proteins are also important biological therapeutics, so the ability to produce high quality proteins is essential.

What is involved in protein production?

Highly expressed proteins can often be readily obtained from natural sources; however, proteins that are only produced in small quantities within cells are required to be generated recombinantly. This often involves making a synthetic, codon-optimised gene for the protein of interest, cloning it into a plasmid expression vector and then transfecting into cultured cells to express the recombinant protein. Common expression systems include E.coli, baculovirus/insect cells or mammalian cells, each with their advantages and disadvantages.

Success in obtaining the required protein often requires careful engineering of the protein constructs to be used, for example, is the full-length protein required or only a particular catalytic/functional domain, are post-translational modifications important for function/stability etc. Such considerations are also used to select the right expression system for the target protein in question. Addition of affinity tags (e.g. His6, Flag) can simplify purification whilst use of protein fusion partners (e.g. MBP, SUMO) can increase protein solubility.

Protein purification is then normally performed using a range of liquid chromatography technologies including affinity chromatography, ion exchange chromatography and size exclusion chromatography.

It is very important to monitor and determine the quality of the protein being produced and this is done using a variety of methods. SDS-PAGE gels give an indication of purity and approximate molecular size, absorbance at 280nm estimates protein concentration, analytical size exclusion chromatography provides the molecular size of the protein or complex in solution. Mass spectrometry gives an accurate molecular mass that can be used to confirm the identity of the purified protein. A functional assay, if available, is useful to determine if the purified protein retains its expected activity.

Protein structure determination by X-ray crystallography

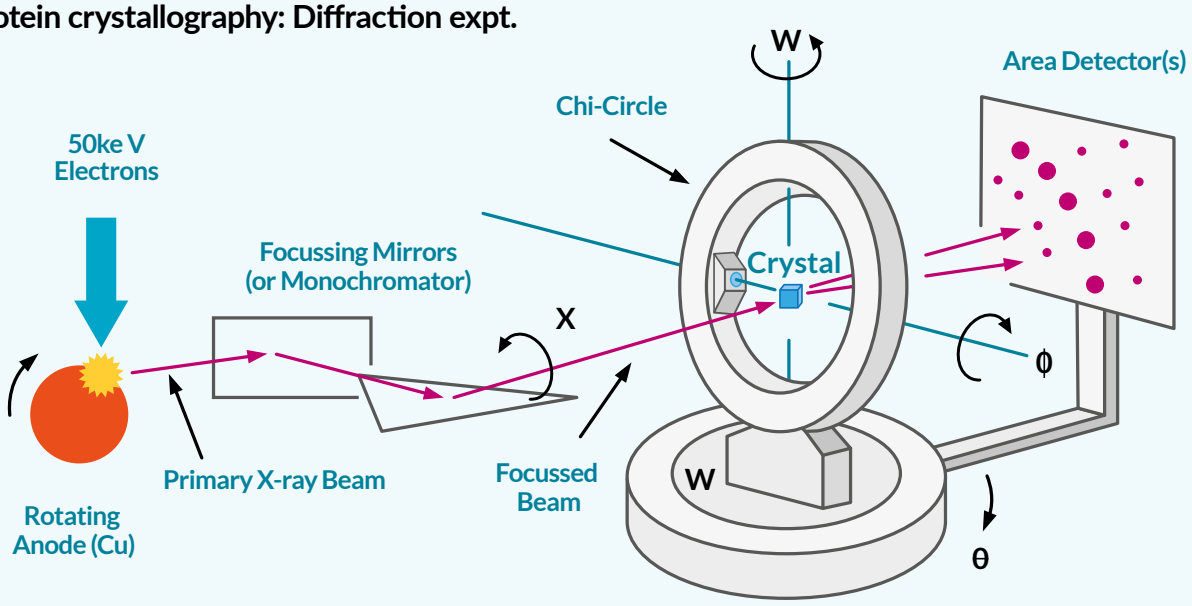
Knowledge of protein structure is important as it informs on protein function and can enable the rational chemical design of drug molecules. One common method used to determine the atomic structure of proteins is X-ray crystallography. This relies on the ability of proteins to form crystals that can diffract X-rays. However, as there is currently no way to predict in advance the optimal conditions under which a particular protein will crystallise, it requires an empirical process where large numbers of different combinations of precipitants, buffers, and salts are incubated together with the protein of interest to identify which conditions will produce suitable crystals. Such crystallisation screening is often carried out by robotic systems in 96-well plates using vapour diffusion methodologies.

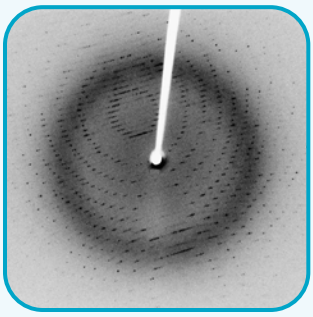
Once crystals have been successfully obtained, data is collected by exposing the crystals to intense X-ray beams, often at specialised X-ray synchrotron facilities. The diffraction data typically consists of a large number of images of the X-ray diffraction pattern that are obtained as the crystal is rotated in the X-ray beam.

Knowledge of the position and intensity of each diffracted spot or reflection in such images is generally not sufficient to reconstruct the protein's electron density within a crystal. Information on the relative phase angle of each reflection is also required and this can be determined using a variety of methods. Molecular Replacement relies on the availability of similar/homologous protein structures.

(sequence ID of >30%) to use as an initial model. If no homologous structures are available, then Multiple Anomalous Dispersion (MAD) methods can be used in which native methionine residues are replaced with selenomethionine during protein expression. The resulting small differences in diffraction intensities are then used to calculate the required phase information.

Protein crystallography: Diffraction expt.

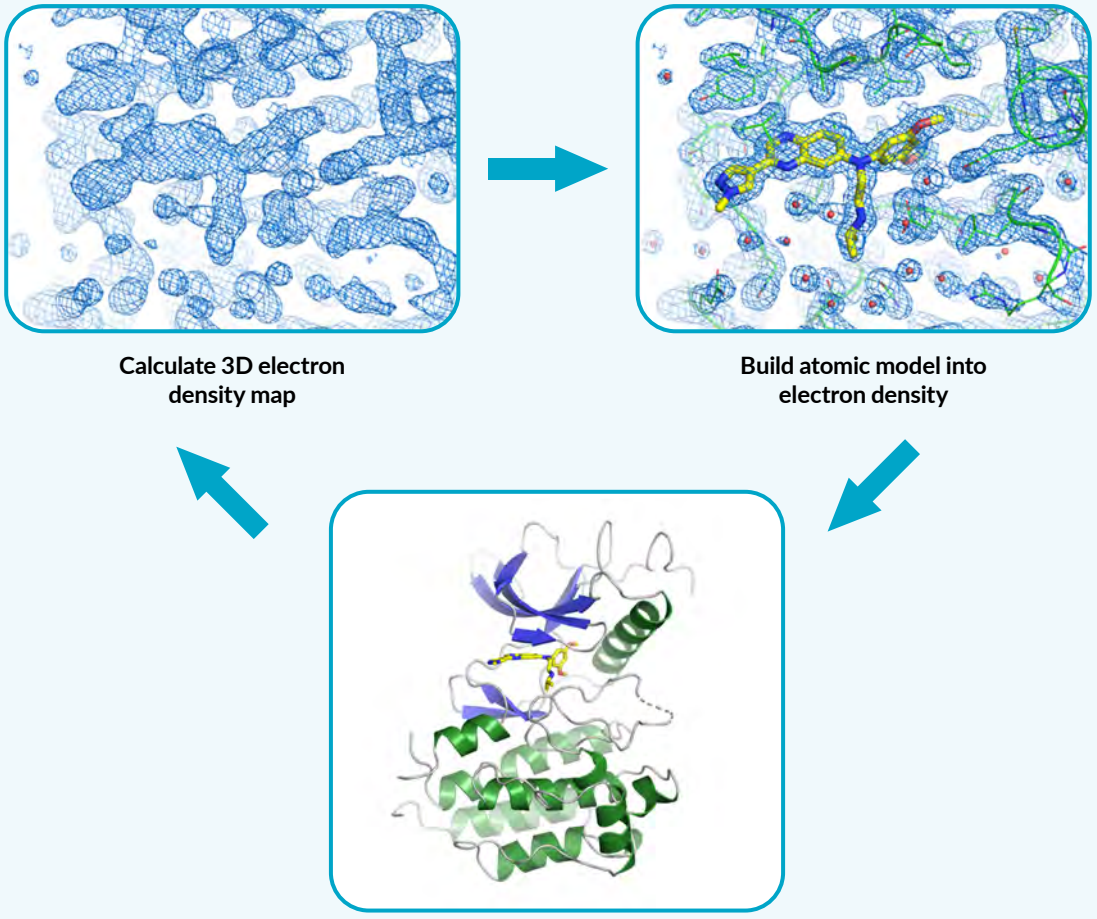




4-Circle Gonoimeter (Eulerian or Kappa Geometry)

- Generally the only information obtained from a diffraction image is: i) the **position** & ii) the **intensity** of the diffraction maxima ie. spots or reflections.
- This is not sufficient information to reconstruct the electron density within the crystal.
- Also requires **phase angle** of each reflection – but this is not directly observed in diffraction images.
- This is the so called "Phase problem".

Protein crystallography: Electron density to model



Once initial estimates of the phases are obtained, electron density maps of the protein within the crystal can be calculated and visualised using computer graphics. This allows a model of the protein to be built which can then undergo cycles of refinement against the experimental data to improve the model and provide even better phases until there is convergence.

Just as with purified proteins, it is important to monitor and determine the quality of the protein models that are generated.

Protein structures determined by X-ray crystallography should be explained by the experimental data e.g. have low Rfactor & Rfree values. The quality of the experimental data itself should be assessed by monitoring its resolution, completeness, redundancy, signal-to-noise, and merging statistics. Finally, protein models should also make stereochemical sense. This can be judged readily by examining Ramachandran plots to ensure that the amino acid backbone phi/psi angles fall within expected limits.

About the author

Dr Derek Ogg, *Peak Proteins*

Derek is Chief Scientific Officer at Peak Proteins. He carried out his PhD in Biophysics at the University of Leeds. After postgraduate studies he moved to Sweden to work as a protein X-ray crystallographer with number of biotech and pharma companies including Pharmacia and Biovitrum as well as the Structure Genomics Consortium in Stockholm. On his return to the UK he worked for ten years at AstraZeneca, Alderley Park before joining Peak Proteins as CSO in 2015.

PEAK PROTEINS

Make the molecules that matter

Optimising drug discovery

With the increasing pressure to drive efficiencies in the drug discovery process, innovative approaches using computational chemistry are delivering proven results, particularly in the areas of lead identification and optimisation. However, these techniques require expertise that may not be available in-house. There is an increasing trend to outsource computational chemistry in order to benefit from the advantages it delivers in terms of insight into the biological activity and interactions of molecules across a range of target classes, enabling the identification of new candidates that would otherwise have been overlooked. Outsourcing also makes computational chemistry methods affordable and accessible for smaller research organisations.

The process

When working on your project we become your scientific partner and share your goals and challenges. It is our business to ensure you achieve your project milestones.

By removing obstacles you gain a fresh perspective, save time and money and can improve discovery performance. Cresset Discovery Services has access to the best of breed ligand-based and structure-based software solutions developed by Cresset. Cutting edge methods, that are not yet available commercially, are also used. At all times our goal is to provide the best possible methods to deliver outstanding results. Whether virtual screening, broadening and protecting your IP position, managing your procurement process, finding a chemical starting point, or bridging resource gaps, Cresset Discovery Services has proven expertise to take your business to the next stage.

About the author

Dr Martin Slater, *Cresset*

Martin is Director of Consulting Services at Cresset Discovery Services. He studied medicinal chemistry at the Universities of Leeds and Huddersfield. In 1997 Martin joined the then start-up company BioFocus where, as Senior Research Fellow, he underpinned the SoftFocus library brand with the development of innovative chemogenomic tools and the design of over 40 commercially successful protein targeted libraries. Martin pioneered the use of Cresset's field-based technologies for targets including GPCRs, Kinases, Ion channels and Proteases for library generation and de-novo ligand design. Martin joined Cresset in 2011 as Director of Consulting Services.

cresset
discovery services
make the molecules that matter

Cresset Discovery Services delivers computational solutions to biological problems to pharmaceutical, biotechnology, agrochemical, flavour and fragrance companies. Their CADD experts provide an understanding into the properties and behaviours of chemical structures and proteins for the design of new, small molecules, ensuring molecular discovery is easier and more efficient.

Where can CryoEM be positioned in Medicines Discovery?

Cryo-electron microscopy (cryoEM) is a powerful structural biology technique that can be used to study a range of different macromolecular complexes and questions in medicines discovery.

What is cryo-electron microscopy

Recent advances in both software and hardware in cryoEM mean we can now routinely resolve structures for a whole range of different macromolecular complexes, to resolutions of around 3 angstroms.

CryoEM can be used for structure determination of potential targets, to directly examine the location of a small molecule, or examine binding of antibody or non-antibody binding proteins. Even where there may be heterogeneity (both in terms of compositional and conformational heterogeneity), cryoEM is often able to generate high quality structural information.

Advantages of cryo-EM

Typically, single particle cryoEM can enable structure determination of proteins and macromolecular complexes of ~100 KDa up to 1000s of KDa. CryoEM can provide structural information on large macromolecular complexes with many subunits or complexes with disordered regions, where other structure determining techniques such X-ray crystallography might not be suitable.

CryoEM is also useful for protein complexes where it is difficult to generate sufficient volumes or concentrations for other structural techniques such as X-ray crystallography, as cryoEM requires lower volume and concentrations of material.

How it works

The first stage is to generate a high-quality protein preparation. Negative staining electron microscopy can be used as an initial quality control step to provide an indication of purity, heterogeneity, aggregation, and degradation, to give a good indication of how the sample will behave in single particle cryoEM analysis.

The sample can then be prepared for cryoEM via vitrification. Approximately 3 µL of the macromolecular complex is applied to a cryoEM grid. A thin film (10-100nm) of liquid containing the specimen of interest is then formed and immobilised by plunge freezing into a cryogen such as liquid ethane to rapidly to a vitreous state.

The cryoEM grid is then screened to ascertain if the quality is good enough for a full data collection, This process can be manual, or automated with a small dataset collected. Initial results from a small dataset can be obtained in as little as 30 minutes using new image processing pipelines, which are entirely automated.

During full data collection, the optimised grid is placed into a Titan Krios microscope for data collection, which takes between 24 and 72 hours to generate raw data. We can then use single particle averaging techniques to generate a high-resolution three-dimensional structure of our protein or macromolecule of interest.

CryoEM opens the doors to structure-based drug design approaches to be applied to previously intractable targets. CryoEM is well suited to the analysis of membrane proteins, and other complexes which are hard to work with. Overall, cryoEM is a powerful tool for structure based drug design and medicines discovery.

The University of Leeds can offer expert support and equipment access in cryoEM. We support the full pipeline of structure determination by cryoEM, from sample quality control and preparation, to data collection and full analysis. We also offer comprehensive training opportunities. The facility sits within a wide network of world-class research facilities within the University, enabling us to take on complex and multi-faceted challenges in medicines discovery.

About the author

Dr Rebecca Thompson, *Astbury Biostructure Laboratory*

Rebecca is the Head of Faculty Biological Sciences Research Facilities and Deputy Director Asbury, University of Leeds. In this role she oversees the management of the facility, which includes two state-of-the-art Titan Krios microscopes. Her current research interests span cryo-electron microscopy 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.

ASTBURY BIOSTRUCTURE LABORATORY

The Astbury Biostructure Laboratory (ABSL) is the electron microscopy facility within the Faculty of Biological Sciences, University of Leeds. We operate state-of-the-art equipment for transmission electron microscopy, with two world leading ThermoFisher Scientific Titan Krios electron microscopes equipped with direct electron detectors. The facility offers sample preparation equipment and staff expertise to support users in preparing and imaging a wide range of biological specimens, from macromolecular complexes to cells, tissues and organisms. They also work with non-biological specimens.

4 | Optimising the Compound

For a molecule to be worthy of entering preclinical development it needs to have the desired biological activity, as well as DMPK properties and a safety profile appropriate for the targeted therapeutic indication. In this phase of the drug discovery process biologists and chemists work to optimise the properties of the compounds by utilising computational modelling, chemical reactions and synthetic transformations and a suite of biological *in vitro* assays and *in vivo* models.

In this webinar we focussed on the methodologies used to establish and optimise the DMPK properties and biodistribution of the molecules. Firstly, Al Dossetter described how multiple data sources can be used to improve the properties of compounds using in silico drug design and how this method can lead to a good quality candidate drug in fewer iterations. Richard Weaver outlined examples of DMPK properties of the molecule, how they are assessed, and common mistakes made, providing illustrations of how the issues can be resolved. Finally, Juliana Maynard described how imaging technologies can be used to establish the biodistribution and accumulation of the compounds *in vivo*.

View the recording and slides for the fourth webinar
Optimising the Compound

In-silico drug design: What to do, what not to do

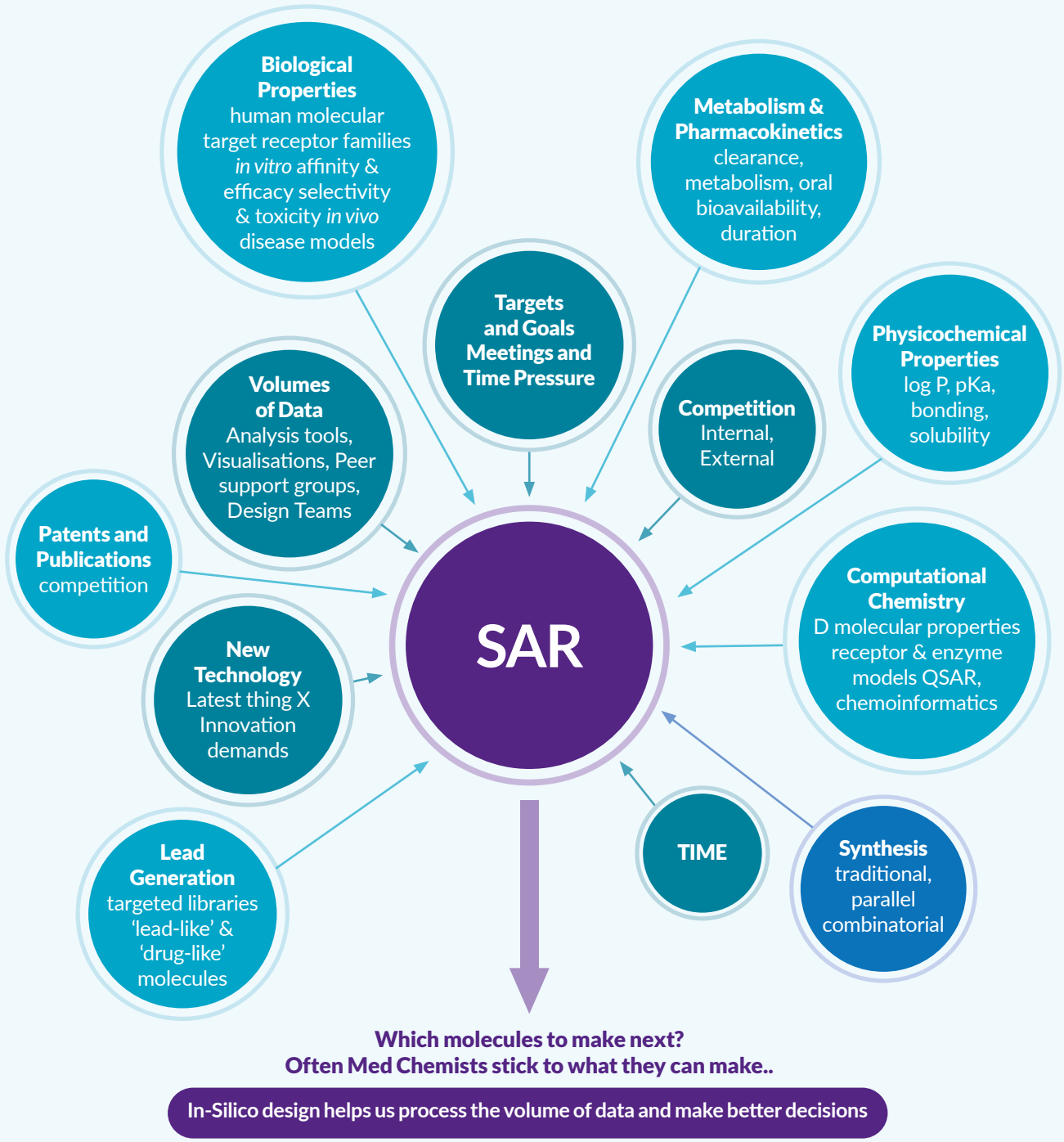
MedChemica is an in-silico drug design AI company with a suite of software and databases for drug design - they also support research projects such as the COVID moonshot project, alongside antibacterial and oncology projects.

Why use in-silico drug design?

In-silico techniques help analyse the data available unbiasedly, refine compound design and achieve the outcomes for a good quality candidate drug in fewer iterations.

2D computational methods assist in processing the vast amount of data available in an unbiased way to support better decisions on which molecules to progress and allows ranking of generated ideas.

Medicinal Chemist's Toolbox



What to do

It is key to obtain as much quality information on the compound as possible from various sources i.e. SAR from literature, patents, and structures, and use mathematical models to automate structure-activity decisions such as Free Wilson analysis. This determines the contributions each of the substituents or structural elements make to the activity/potency of the parent molecule. A more sophisticated, automated method is permutative matched molecular pair analysis (MMPA) which compares the relationships and properties of 2 very similar molecules.

What to watch out for

2D plots can provide detailed information but care should be taken to use the same scale on the x/y axis and that data are measured on a continuous scale. Limitations of 2D models should be considered - descriptions, atoms, or group patterns are used to encode molecules, models are only as good as the dataset from which they are generated, and every prediction is liable to errors.

3D design/structure-based drug design can be based on single molecule structures, conformational and torsional analysis, protein/ligand structures, docking and scoring and force field/ scoring. 3D design can include free energy perturbation, quantum mechanics, and molecular dynamics.

Structural modifications, size and lipophilicity are all important in compound development. The lipophilicity of a molecule can be estimated using calculated lipophilicity (ClogP). This should fall between 1-3 on the LogD/P scale, too polar or too lipophilic can generate problems with the compound.

Key to a solid understanding of the area are literature references. MedChemica provides a library of references which can be found at: www.medchemica.com/bucket-list/

In addition, the company currently has a rare opportunity with a community project searching for SARS COV-2 inhibitors of one of the proteases. The project can be followed on Twitter @covid_moonshot.

About the author

Al Dossetter, *MedChemica*

In 2012 Dr Al Dossetter co-founded MedChemica centred around the technology of Matched Molecular Pair Analysis (MMPA) as a method of accelerating medicinal chemistry. Previous to MedChemica Al gained his PhD from Nottingham University and after post-doctoral research at Harvard University joined AstraZeneca (AZ). He spent 13 years in medicinal chemistry spread across oncology (hormonal and kinase inhibitors), inflammation (OA and RA, enzyme inhibitors and GPCR targets) and diabetes (obesity, GPCR and enzyme inhibitors), delivering multiple projects and candidate drugs.

MedChemica

CREATING A STEP CHANGE IN MEDICINAL CHEMISTRY

MedChemica are domain leaders in SAR knowledge extraction and knowledge-based design and have built an end-to-end Artificial Intelligence (AI) platform (MCPairs) for drug, agrochemical and material compound design. The user interfaces are geared for direct use by chemists and were designed and built using more than 10 years' experience in the field. These software and databases not only reduce the time and cost to critical compounds and experiments, but also increase the quality. MCPairs Enterprise is available to license, with support and training packages, for larger companies. For biotechs, foundations and university researchers MCPairs Online is available. With a modern web browser, users can have secure access to all of MedChemica's AI system and databases of knowledge.

Optimising ADME and PK properties: Common mistakes made and how to identify and resolve the key issues

XenoGesis is a pre-clinical CRO based in Nottingham, UK who offers experimental *in vitro* and *in vivo* DMPK/ADME studies, bioanalysis and pharmacology, alongside physiologically based PK modelling, human PK and dose prediction. This talk focused on four key areas where mistakes are often made:

- What is the point of optimising *in vitro* clearance?
- The importance of the unbound drug concentration
- Bioavailability is the key to the developability of a drug
- DMPK is an essential component of an integrated drug discovery programme, not a siloed after thought

What is the point of optimising *in vitro* clearance (CLint)?

Intrinsic clearance is not always seen as a method worth optimising for many reasons. Often a poor correlation is seen between CLint vs *in vivo* clearance and as a result the assay is not always used.

Basic science can be applied to improve the correlations on a plot, the well-stirred model can be applied, correcting everything to unbound CLint and for plasma protein binding. If this reveals any outliers with significant under or over prediction, these can then be further investigated.

A standard hepatocyte assay which measures loss of compound due to metabolism can be utilised. However, total CLint occurring *in vivo* should account for the different elimination parameters– hepatic metabolism, hepatic uptake, and renal clearance.

A standard hepatic uptake assay and an assay which measures loss from the media alone for drugs such as statins, can be much more predictive of elimination.

Assays at XenoGesis are optimised to give the maximal CLint and more accurate correlations with *in vivo* data than data given in literature.

Plasma protein binding – the importance of understanding the unbound drug concentration

The free drug hypothesis states that only the free drug (unbound) concentration at a receptor is responsible for efficacy. In the absence of active transport, at steady-state, a permeable compound will have the same unbound concentration on both sides of a cellular membrane. Under these conditions the free compound concentration at the receptor in the target tissue will be expected to be equivalent to the unbound concentration in blood.

In order to measure the free drug concentration, you need to be able to measure the amount of drug bound non-specifically to plasma. Plasma protein binding cannot be optimised, it just needs to be known.

PPB measurements are important to translate the *in vitro* to *in vivo* data for the IVIVE and should be compared across species. Measurements of PPB should be taken in the same species of animals as PK/efficacy and tox. data as differences can be significant.

Bioavailability – the key to the developability of a drug

Bioavailability measurements are used to determine the extent and rate at which the compound, when delivered orally is absorbed and the fraction which enters the systemic circulation. Metabolism and absorption contribute to bioavailabilty, so ideally you are looking to develop a compound which is readily absorbed with low hepatic clearance. Formulation strategies focus on increasing the fraction that is absorbed. Formulations are not able to modulate the amount of drug which is metabolised by the liver. As this is an intrinsic characteristic of the drug, it is essential that hepatic clearance is measured allowing compounds that have a high intrinsic clearance rate, which results in an inadequate drug exposure level, to be removed prior to the selection of a candidate drug.

Influence of DMPK on drug discovery

Drug metabolism and pharmacokinetics (DMPK) is an integrated process and issues should be identified and addressed early in the drug discovery process. Assessment should include an assessment of the structure, bioavailability, prediction of human PK and dose, and assessment of the drug-drug interaction risk. With a robust dataset, the influence of DMPK should extend through to Phase IIa clinical trials. This way, the asset is prepared for due diligence by large pharmaceutical /partnering companies and divestment opportunities.

About the author

Richard Weaver, *XenoGesis*

Richard is the founder and CEO of XenoGesis. His clear scientific rationale, business direction, vision and entrepreneurial approach have seen XenoGesis consistently expand and grow to become the UK's largest independent DMPK provider.

Richard set up the business in 2011, when he spotted a gap and opportunity in the market. XenoGesis now works with over 200 companies and Universities across the globe and has provided experimental data, advice and PK predictions on eight compounds that are now in the clinic.

The business has grown from three to nearly 40 employees in over nine years.

In 2018, Richard led the move into state-of-the-art laboratories and offices at BioCity's Discovery Building to provide the platform for future growth.

Richard gained a first-class honours degree in chemistry with awards for the best performance in every year, followed by a medicinal chemistry PhD with a Wellcome Trust scholarship at the University of Leicester and two subsequent postdoctoral positions at the Welsh School of Pharmacy. In 1997, Richard joined Astra Charnwood within Discovery DMPK, and progressed to Group and Project Leader at AstraZeneca.

XenoGesis

XenoGesis can identify the potential 'winners' and 'losers' in a selection of compounds synthesised in drug discovery campaigns. They provide data-driven iterative feedback to the client, and recommending next steps is a key focus. They combine state-of-the-art *in vitro*, *in vivo* and bioanalytical capabilities with expert pharmacokinetic/pharmacodynamic (PK/PD) data interpretation services.

In-vivo imaging to understand the biodistribution of a candidate compound

Using imaging in drug discovery

Multimodal and multiscale imaging solutions enable quantification and understanding of organ accumulation and biodistribution, target expression and engagement, optimal biological dose, toxicities/drug interactions and preclinical studies to predict what is seen in the clinic.

Overcoming the challenge of quantitatively determining organ accumulation and distribution at specific time points

In vivo and *ex vivo* preclinical imaging offers a compelling approach and solution to non-invasively characterise and validate compound distribution and accumulation.

Medicines Discovery Catapult (MDC) has imaging expertise in multi parametric imaging modalities:

- Functional radiological imaging techniques such as positron emission tomography (PET)
- Computed tomography (CT)
- High-frequency ultrasound (HFUS)
- Bioluminescence/near-infrared imaging (NIR)
- *Ex vivo* imaging techniques:
  - Mass spectrometry imaging (DESI and MALDI)
  - Digital spatial profiler (nanostring DSP)
  - Advanced microscopy imaging suite (super resolution/confocal/multiphoton)

How can imaging be used to understand distribution of a therapeutic?

The rate and degree of drug distribution reflects the extent it is present in extravascular tissues and depends on blood flow, capillary permeability, and protein binding. The administered substance goes through a series of cascades to be effective and imaging can track the progress from the systemic level to the cellular level to understand and characterise it.

The 5 steps are:

1. Circulation
2. Accumulation
3. Penetration
4. Internalisation
5. Release

Examples of imaging techniques that can be used include:

- Fluorescence near infrared imaging device for longitudinal Cy-7 biodistribution. Cy-7 imaging allows deep biological penetration within the body and the ability to image the whole-body distribution with good signal-to-noise. There are many ways to label a molecule with a dye enabling it to be tracked.
- ^{89}Zr PET can also be used to look at whole body distribution and also off target toxicity and nonspecific binding. 89Zr PET is an ideal tracer due to its half-life, allowing repeated images to longitudinally assess distribution of the compound. This technique can also be used to assess tumour penetration longitudinally.
- MDC's collaboration with the University of Leeds and the therapeutic microbubble consortium in cancer nanoparticles uses an enhanced tumour delivery of microbubbles as a drug carrier in combination with a HFUS platform. Using imaging it can characterise the delivery system and demonstrate that accumulation and distribution of the microbubbles are predominantly through the reticulo endothelial system (RES).
- Three imaging techniques can be used to assess distribution and penetration across the blood-brain barrier (BBB) including PET, near-infrared imaging and bioluminescence probes offer a non-invasive method, and mass spectrometry imaging which has the potential to define drug distribution in very small brain structures with a non-labelled approach.
- Non-invasive mass spectrometry imaging has broad potential in the drug distribution space. Two main mass spectrometry imaging techniques used include desorption electrospray ionisation (DESI) and matrix assisted laser desorption ionisation (MALDI).

Summary

Imaging at MDC offers huge potential as a platform to non-invasively measure the pharmacokinetics (PK) and characterisation of small molecules, biologics and, non-biological complex medicines to facilitate faster translation into clinical development.

About the author

Dr Juliana Maynard, *Medicines Discovery Catapult*

Juliana is Lead Scientist in Pre-clinical Imaging at MDC. She works with a wide range of molecular imaging modalities including PET, SPECT, CT and ultrasound. Previously she was Head of Imaging Services at Alderley Imaging and worked at AstraZeneca for 9 years. Juliana has a PHD in neuroendocrinology from the University of Edinburgh.

5 | Chemistry and Formulation

The chemistry discipline is instrumental at all stages of drug discovery, from the creation of diverse or targeted libraries for screening, through design, synthesis, formulation and ultimately the scale up and manufacture of the final drug product.

In this webinar we heard from three chemists. Trevor Perrior described how fragment-based drug design can be applied, taking us through a case study which illustrates how low molecular weight fragment hits can be turned into drug candidates. James Hitchin focussed on synthetic chemistry and the different skills required from early Drug Discovery to Development phases, with particular reference to the way in which transformation maps can be applied to guide decision making. Alison Foster described how the formulation of a compound can be optimised to maximise dose and absorption in preclinical studies and enhance the properties of the API to meet the Target Product Profile in the clinic.

View the recording and slides for the fifth webinar
Chemistry and Formulation

IKKε/TBK1: A case study of approaches to turning fragment hits into drug candidates

Domainex is a leading provider of high quality innovative and efficient scientific solutions that enable successful drug discovery programmes against a wide range of drug targets. Amongst its clients are pharmaceutical, biotechnology, academic institutions, and patient foundations from all around the world.

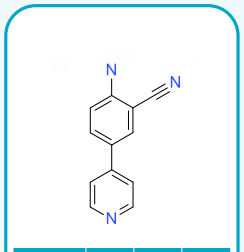
Fragment-Based Drug Design (FBDD) is an approach where libraries of low molecular weight compounds are screened to identify highly-efficient chemical starting points. Skilful elaboration of these very efficient fragment hits with the addition of molecular weight only where this is of optimal benefit affords potent and bioavailable drug candidates.

This case study illustrates the use of FBDD to invent an efficient and selective drug candidate against IKKε and TBK1. Both of these kinases play an important role in regulating the immune response in a group of inflammatory diseases known as interferonopathies e.g. systemic lupus erythematosus, Sjogren's syndrome, and scleroderma. This class of autoimmune diseases are caused by upregulation of interferon signalling leading to inappropriate activation of interferon-stimulated genes, many of which lead to the expression of inflammatory mediators. Inhibition of IKKε and TBK1 will prevent this upregulation and reduce the pro-inflammatory signals. The development of small-molecule inhibitors has been challenging, and the biologic treatments that are available for interferonopathies are often ineffective and expensive. Therefore, there is a very high unmet medical need for specific and effective treatments for these diseases.

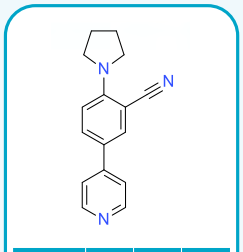
Key results

By screening a fragment library, a hit was selected based on its potency against the target, molecular weight, and a ligand-efficiency metric. Enzyme crystal structure studies revealed opportunities to increase potency without affecting efficiency. By manipulating the initial hit fragment by the substitution of an amine, followed by aryl or heteroaryl amino substitution on the pyridyl ring, the potency of the compound was increased with no loss in ligand efficiency. A final replacement of a pyridine with a pyrimidine ring enhanced potency further to give a 50 nM compound with a distinctive cyano group.

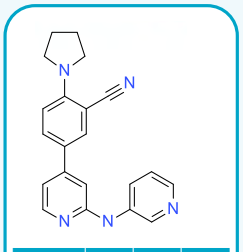
Project Progression (4)



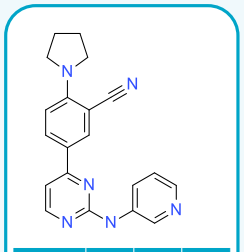
IKKε (nM)	MW	LE	LLE
13700	195	0.45	3.5



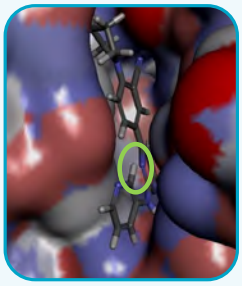
IKKε (nM)	MW	LE	LLE
1600	249	0.43	3.2



IKKε (nM)	MW	LE	LLE
166	341	0.37	3.1



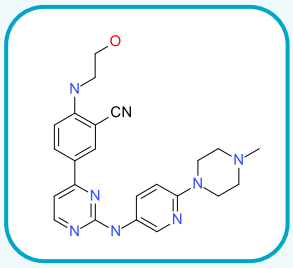
IKKε (nM)	MW	LE	LLE
48	342	0.39	3.9



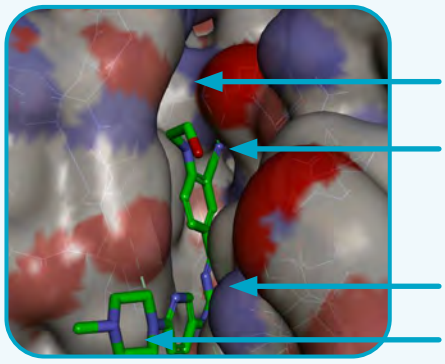
Changing pyridyl to pyrimidinyl enhanced both potency and LE

X-ray crystallography provided a high-resolution crystal structure of an exemplar protein-ligand complex, where the CN group fitted into the narrow pocket in the kinase, forming a hydrogen bond to lysine 38.

X-Ray Crystallography (DMX2302)



X-ray resolved crystal structure bound to IKKε protein



Space for larger amine substituents

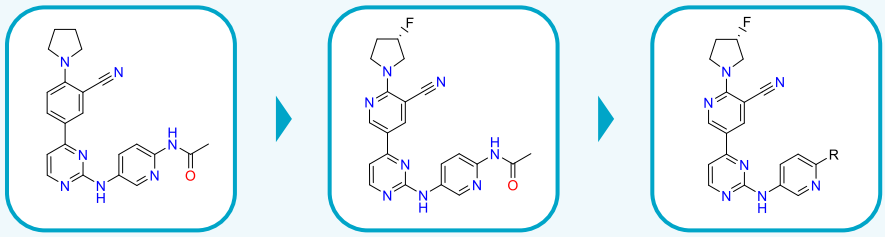
Selectivity pocket occupied by CN group H-bond to Lys-38

Hinge-binding by aminopyrimidine moiety

Solubility-enhancing group into water

Structure-based drug design and manipulation of molecular and physical properties allowed the solubility, metabolic stability and permeability to be further enhanced. By lowering the logD with the introduction of polar atoms and substituents, improved metabolic stability was achieved whilst maintaining the high selectivity and potency and DMXD-011 was identified.

Evolution of DMXD-011

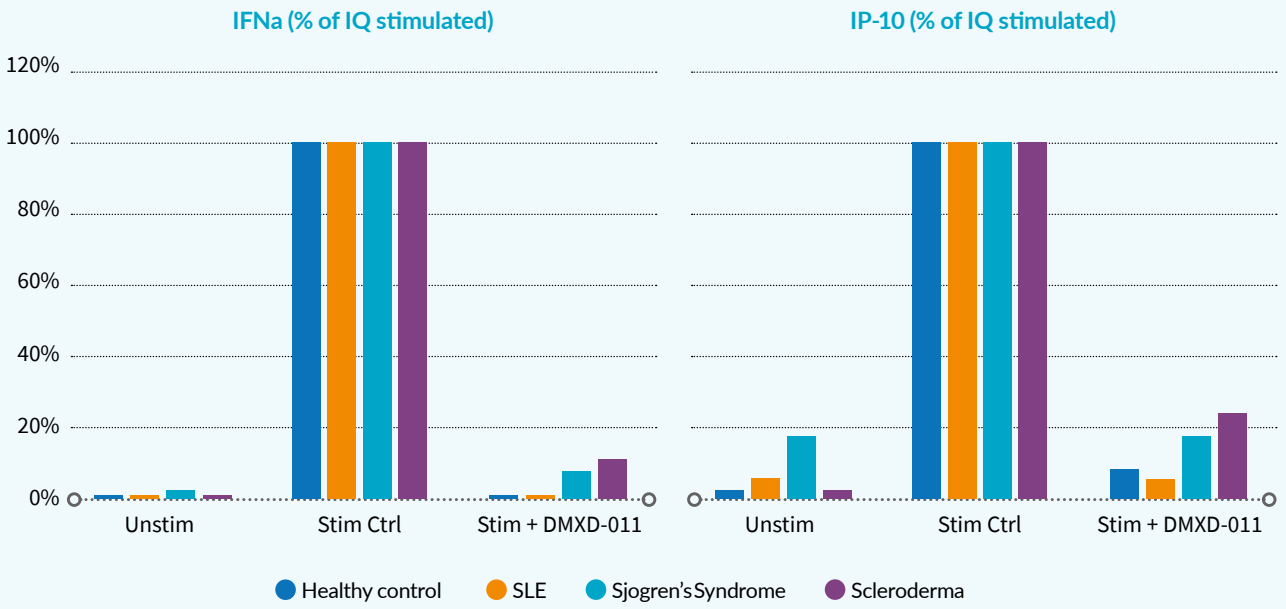


DMX ID	1338	3179	DMXD-011
IKKε (IC_{50}, nM)	2	22	16
TBK1 (IC_{50}, nM)	4	26	9
$LogD_{7.4}$	4.7	3.1	2.0
Aq $sol_{7.4}$	-	1µM	>1mM
MLM (µl/min/mg protein)	343	18	21

Will this work in patients?

Ex vivo studies using inflammatory cells from healthy donors and patients with interferonopathies showed that the compound almost completely blocked the release of interferon-alpha and other inflammatory mediators, leading to reduced expression of interferon-stimulated genes demonstrating excellent proof of concept.

DMXD-011 dramatically reduced the effect of IQ stimulation on cytokine levels



ISG profiles also reduced in all patient groups (data not shown)

How we did it

These results were achieved using a FBDD approach based on identifying small chemical fragments which bind to the biological target. Here Domainex fragment libraries were screened using a high concentration biochemical assay against the IKKε enzyme, and a unique in-house crystal structure of IKKε was obtained using a domain approach. Through structure-based drug design and profiling with a cascade of enzyme and cellular assays, we optimised the compound. By using the FBDD strategy we efficiently designed DMXD-011, a unique low clearance, metabolically-stable IKKε/TBK1 inhibitor, that was validated in proof-of-concept studies in animal models and an ex vivo human assay. DMXD-011 is now in pre-clinical development.

About the author

Trevor Perrior, *Domainex*

Trevor is now a technical consultant to Domainex. Following his education at the University of Cambridge Trevor undertook academic research at Cambridge, Oxford and in the USA. He then held a number of senior R&D leadership roles at ICI, Zeneca, AstraZeneca and Celltech, where he worked in the UK, USA, and Switzerland. In 2005 Trevor joined NCE Discovery as Chief Scientific Officer, and when NCE Discovery merged with Domainex he became Director of Research for the enlarged company. In 2016 he was appointed Chief Scientific Officer of Domainex, and then in 2018 Chief Executive Officer. In April 2020, Trevor retired from this role, but continues to support Domainex as a scientific advisor. Trevor is also a scientific consultant to a number of venture capital and charitable funds.

Domainex

Domainex is a high quality, fully integrated drug discovery service company based near Cambridge, UK serving pharmaceutical, biotechnology, academic and patient foundations globally. They offer a tailored range of biology and chemistry services from a single location, taking our clients from target nomination to delivering pre-clinical candidates. Approximately 80% of their scientists have PhDs and have over 10 years of average industrial experience across a number of therapeutic areas.

Designing formulations for pre-clinical and early stage clinical studies

Quay Pharma are a contract development and clinical manufacturing organisation who provides expert services in the formulation of molecules for systemic and localised delivery.

The development pathway

- The active pharmaceutical ingredient (API) is supplied by the contract manufacturing organisation
- Quay Pharma formulate the API for preclinical studies to maximise dose and absorption in the animal models
- DMPK and toxicity evaluation are carried out by a CRO
- DMPK and toxicity study results guide the Quay Pharma clinical formulation development starting with pre-formulation studies
- Formulation development is based on data generated from the pre-formulation studies, to generate early prototypes
- A feasibility batch of the lead formulation is produced, followed by ICH stability prior to the actual GMP manufacture.

General considerations in pre-clinical formulation

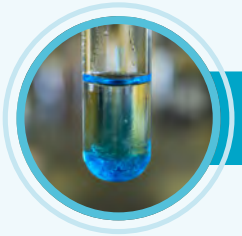
The aim is to maximise dose and absorption, typically using a liquid dose and often with poorly water-soluble molecules with little API available. Excipients should be carefully considered due to potential side effects which can differ between animal species. Useful data to aid this process include chemical structure, solubility data, logP, pKa, melting point, Caco-2 data, amount of API available, maximum target dose required in the animal species and intended pre-clinical species.

Quay Pharma utilise 3 main platforms for pre-clinical formulation, initially aiming for a simple solution formulation before investigating more complex formulations (solid dispersion or nanoparticles) if a simple solution is not possible.

A comparison of a typical 'macro' equilibrium solubility screening method (UPLC) with Quay Pharma's own screening method revealed similar trends for both methods and analytical techniques, however, the Quay Pharma method used 50% less API and took a quarter of the time.

Formulation For Pre-Clinical Studies

Quay Platforms



Solubility

Solid Dispersion

Nanoparticles

Three platforms

- Solubility screening
- Simplest option and starting point
- Includes range of solvent and surfactant solutions, lipids etc

- Solid dispersion and nanoparticles
- If solution or lipid formulation not viable
- More complex route aimed at poorly soluble BCS Class II molecules
- Can bridge to Phase 1 formulation development

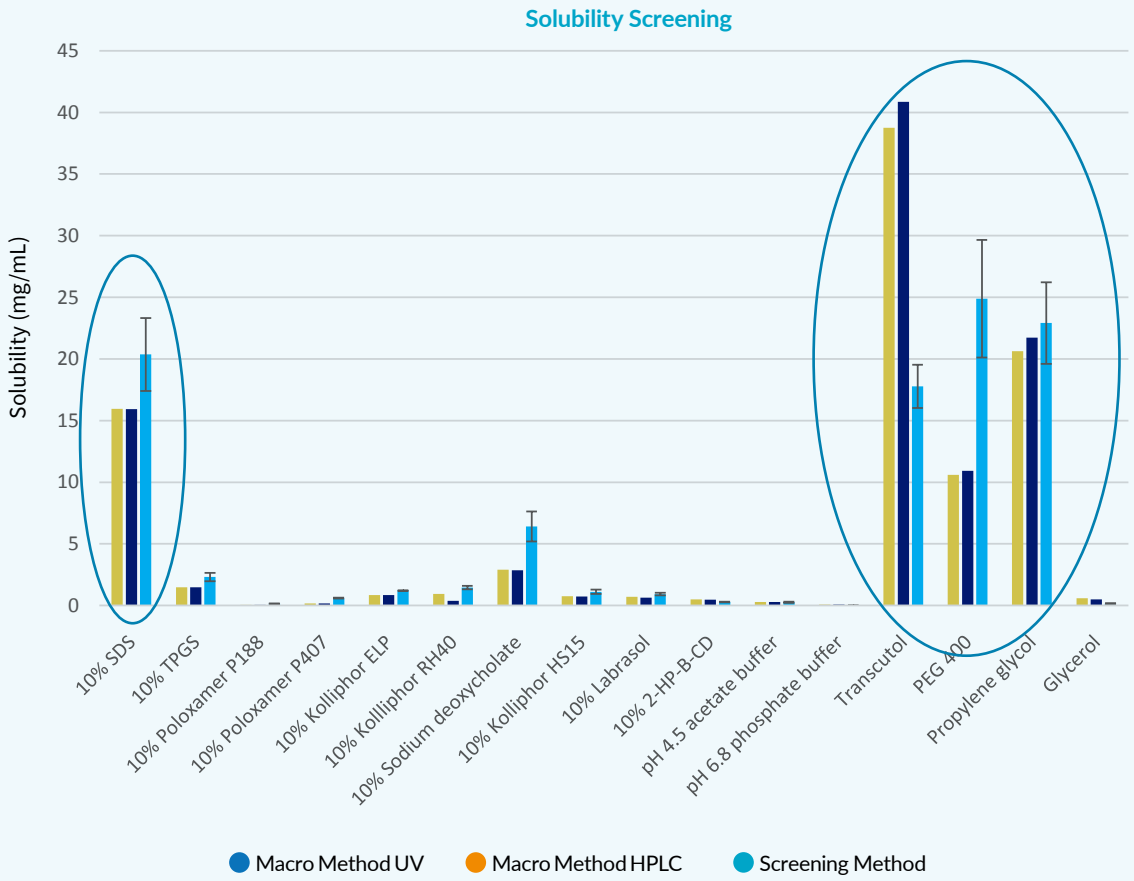
Bespoke - excipient selection based on API characteristics, animal species and downstream requirements

Minimal API

Rapid turnaround

Formulation For Pre-Clinical Studies

Solubility Screening – Case Study



- Comparison between typical solubility 'macro' method and HT screening method
- Maximum 20 mg/mL target in screening method
- Same trends observed for both methods and analytical techniques

	Macro Method (UPLC)	Screening Method (UV)
Amount of API	430 mg	200 mg
Time required	6 days	1.5 days
Lead solvents	Transcutol Propylene Glycol PEG 400 10 % SDS	Transcutol Propylene Glycol PEG 400 10 % SDS

General considerations in early clinical studies

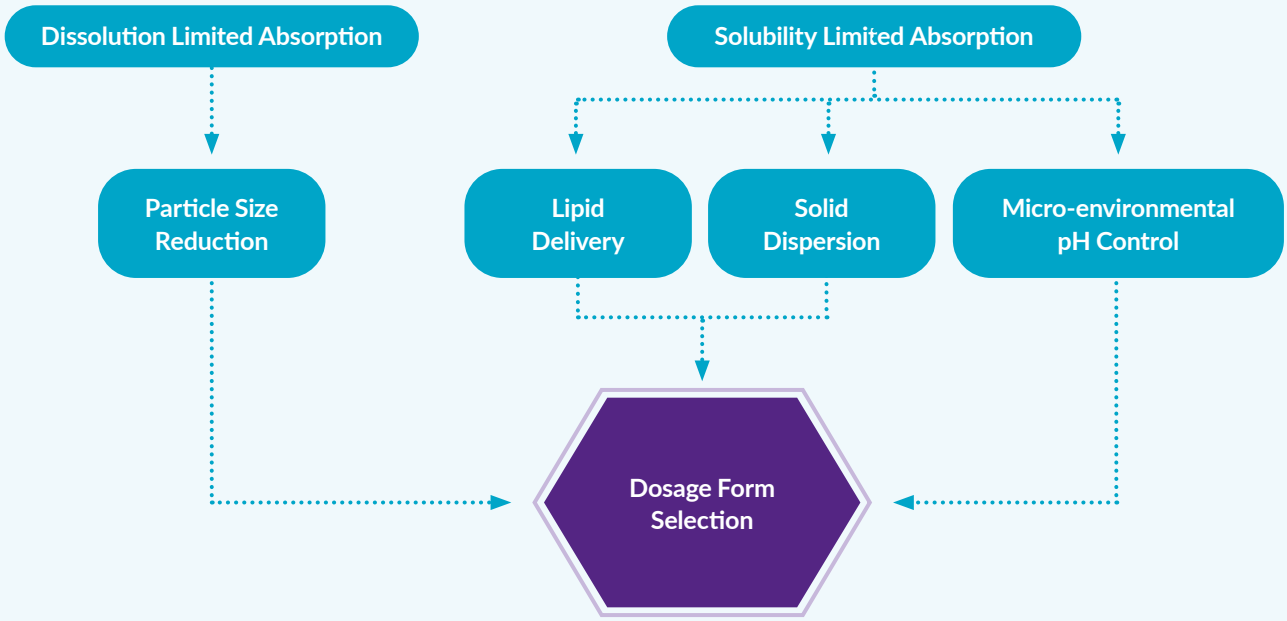
Considerations include patient health benefits and requirements, the phase I study goal, preferred route of administration, target product profile, target release profile and target absorption site. The balance between the chance of success against the degree of acceptable risk and the balance of the costs and time to reach a phase I study is critical. Further consideration is a submission strategy, commercial considerations, API availability and finally the regulatory timeframe.

Prior to formulation, data required include the physicochemical characteristics of the API, particle size, distribution, shape or density, flow characteristics of the API, polymorphic form, and stability data. Biological characteristics include permeability, Caco-2 cell data, any potential efflux mechanisms, and the existing pharmacokinetic data.

Data obtained from pre-clinical studies can help define the developability classification system (DCS) together with the target dose from the target product profile. Molecules fall into one of 4 categories–Class 1 which show good solubility and good permeability, class 2 and 3 which have poor solubility or permeability, respectively, and class 4 molecules which are difficult to formulate based on both poor solubility and permeability. Most compounds fall into the class 2 category.

Formulation For Early Clinical Studies

Low Solubility, Good Permeability (Class II)



- Majority of new candidates fall into this category
- Absorption rate drives the selection of approach
- Higher risk
- Rationale must be based upon available data (complex decision tree!)
- Case by case evaluation
- Rapid screening approach available in pre-clinical platform can help select formulation options

Essential to the entire process is the balance of risk, time, and cost. An advanced formulation at phase I leads to an easier transition to subsequent phases but is likely to take longer to develop to meet the Phase I study timeline. Conversely, moving to a more complex formulation at a later stage (Phase II/III) may be a riskier approach as the complex formulation may lead to a difference in the bioavailability in man compared to the results obtained from the formulation used in the Phase I study.

About the author

Alison Foster, *Quay Pharma*

Alison moved to Quay Pharma 7 years ago and is currently the Head of Technical for Pre-Clinical Services. Prior to this she was one of the founders and Pharmaceutical Programme Director of a small nanotechnology start-up company focussed on improving bioavailable of poorly soluble drugs. She has a wide breadth of experience having worked previously for Unilever in their cross-category research unit focused on Oral Care and Hair projects. Alison gained Post-Doctoral experience in medicinal chemistry from the School of Pharmacy, University of Manchester following a Ph.D. in synthetic chemistry.

QuayPharma

Established in 2002, Quay Pharma has become one of the world's leading CDMOs offering a complete service through all stages of drug development and clinical supply as well as pre-qualification batches to support Marketing Authorisation applications and initial manufacture of commercial product.

In recent years, the company has pioneered work in live biotherapeutics for oral and topical delivery, being the first UK clinical contracting company to be licensed for live biotherapeutic manufacture for finished products. Quay is also one of the few CDMOs developing many types of biologics and microbial therapies for oral drug delivery and manufacture.

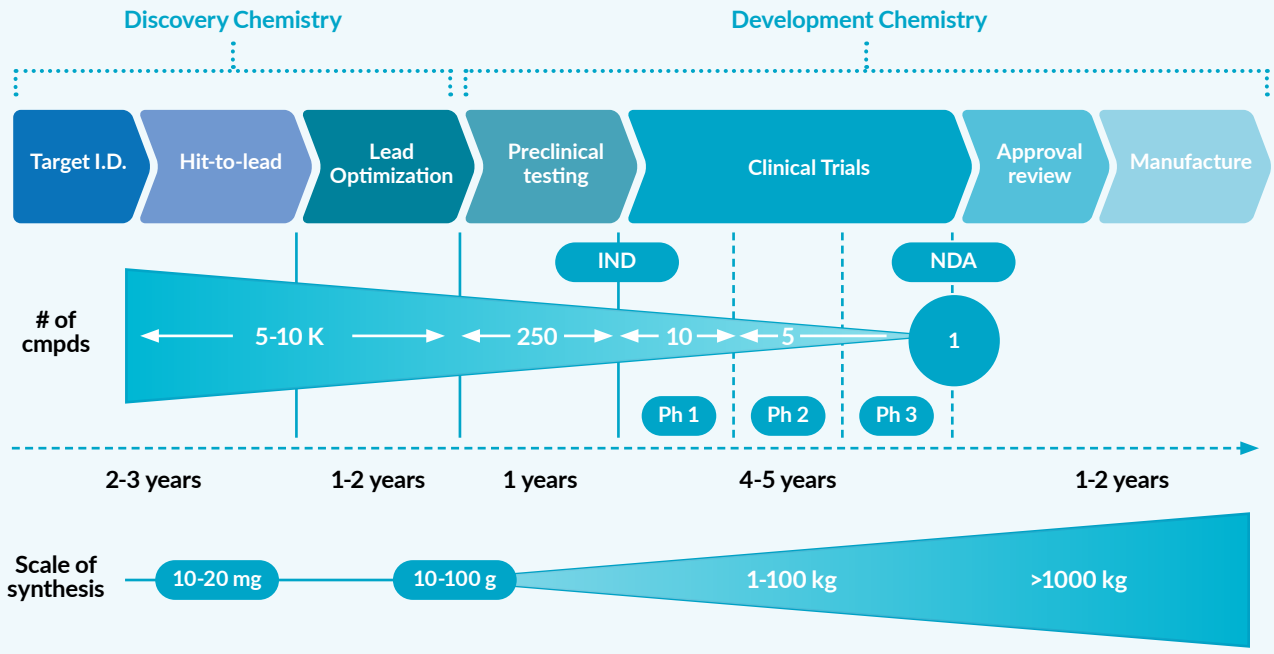
Synthetic chemistry aspects of Drug Discovery and early Development Projects

Charnwood Molecular is a chemistry CRO, providing medicinal and synthetic chemistry services to the pharmaceutical, biotechnology and chemical industries. Charnwood Molecular support client projects from hit identification through the preclinical development and non GMP toxicity studies.

Synthetic chemistry in drug discovery

The drug discovery process is complex and scientists collaborating across a variety of different disciplines and areas of expertise to go from identification and validation of a new target, to an approved and marketed small molecule therapeutic agent. Chemistry is fundamental to the drug discovery process with discovery chemists at the front end of the process and development chemists in the latter stages.

Specific skills are required of the chemist to successfully navigate the earlier and latter stages of the drug discovery process, and the two disciplines have apparent differences



Discovery/medicinal chemists

- Design, synthesise and purify small libraries of compounds
- Use screening cascades to establish structure activity or property relationship
- Understand target biology to ensure desired therapeutic effects are achieved and DMPK liabilities are considered
- Uses computational modelling to generate new ideas for synthesis

Development chemists

- Focus specifically on the synthesis of a small number of compounds nominated as candidates at the end of a lead optimisation campaign
- Design robust, highly efficient, and cost-effective routes to the candidate compounds
- Require mechanistic understanding of each stage in this synthetic scheme
- Can identify impurities formed during the reaction and to design them out of the process
- Understand the regulatory framework and chemical engineering process to better facilitate the transfer to the pilot plants and beyond

Use of transformation maps

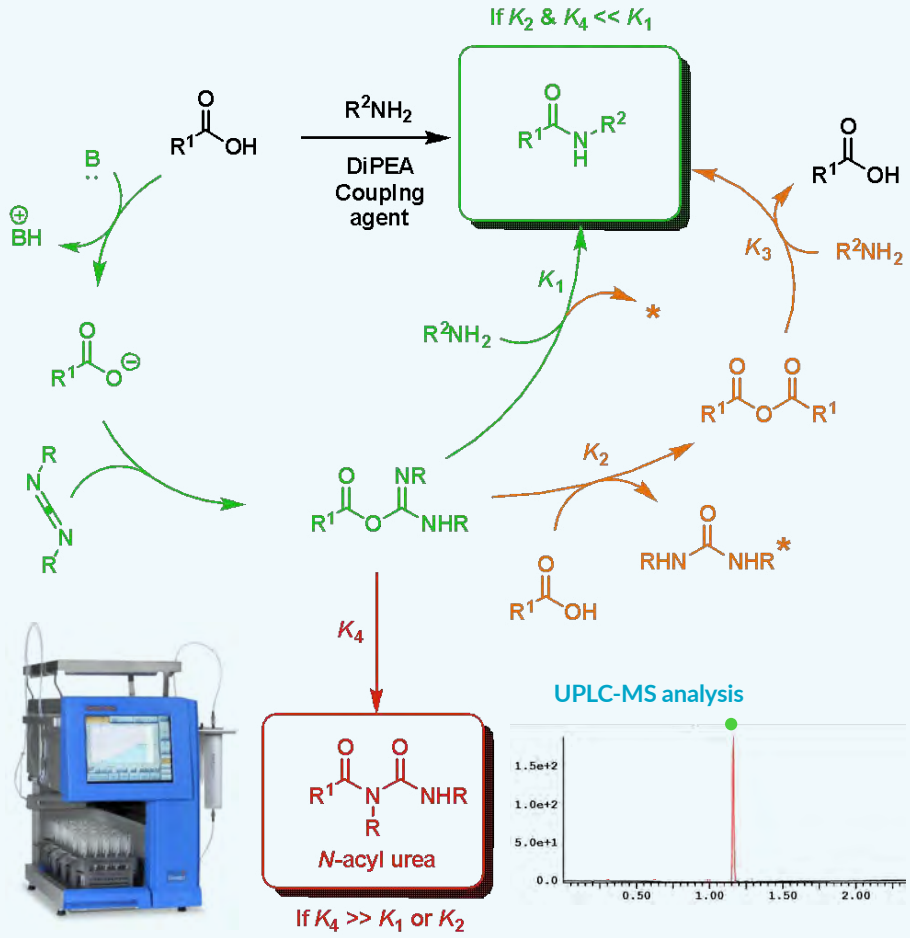
Transformation maps can be applied at any stage in the drug discovery process to provide an in depth understanding of the different pathways available to the many components of the reaction beyond the desired transformation. Understanding and minimising the rate factors driving the different diversionary pathways ultimately facilitates the optimisation of the transformation.

A transformation map can be used to highlight processes that represent the desired transformations (green), semi desirable pathways that have the potential to undermine yield (amber) and deleterious pathways (red) that are of no benefit to the reaction.

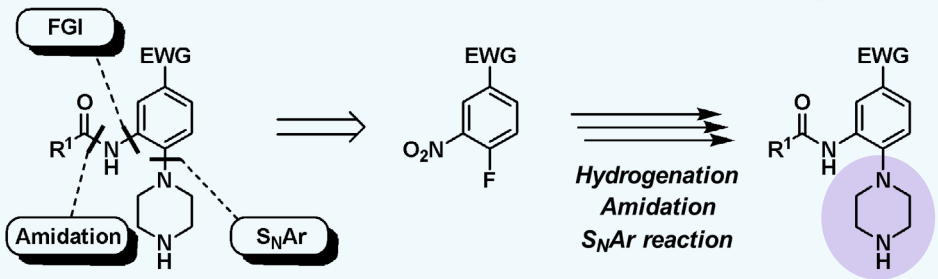
For example, unwanted products may form via the rearrangement of an intermediate or its reaction with the starting material or another by-product that may otherwise be considered benign. Understanding these processes can provide a rationale for a low yield, allowing for a solution to be devised, rather than simply purifying the material by column chromatography. In some cases, however, diversionary pathways can also generate useful materials that could appear problematic at first sight. Understanding the nature of these materials can present opportunities, avoiding the need for purification and allowing crude mixture to be advanced into subsequent steps, such as the construction of a small library of analogues, without undermining the efficiency of the process.

Transformation maps provide a detailed understanding of the potential challenges and opportunities in any reaction and provide the potential to deliver considerable savings in time and money.

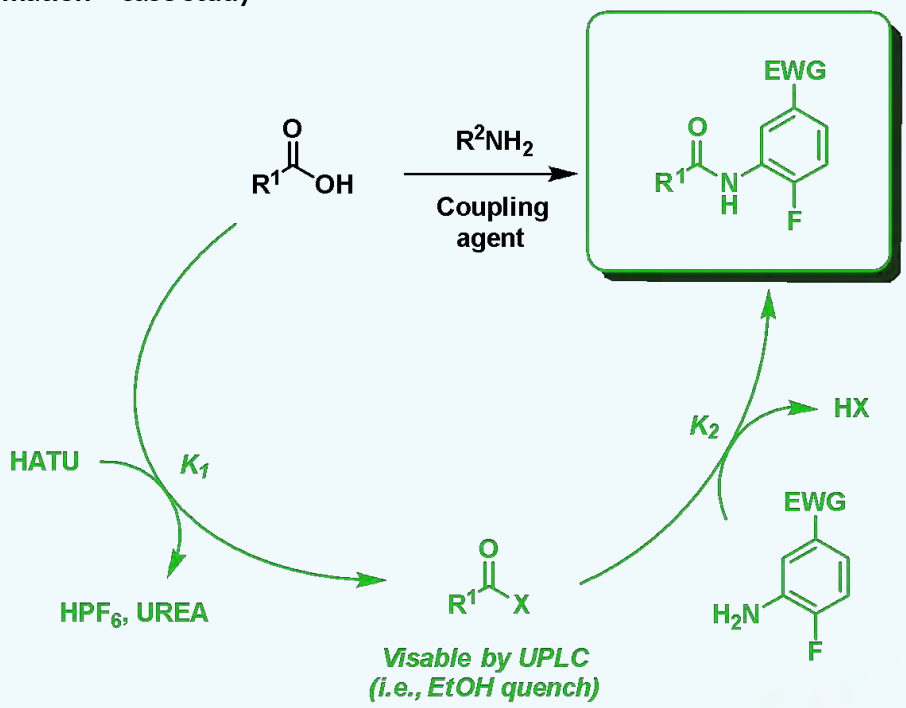
Amide bond formation - transformation map



Recent discovery project



Amide bond formation – case study



Further reading

For a more in-depth understanding of synthetic chemistry in the drug discovery process, the following references are recommended:

1. Roughley SD and Jordan AM. The medicinal chemist's toolbox: an analysis of reactions used in the pursuit of drug candidates. *J Med Chem* 2011;54(10):3451-79
2. Brown DG and Boström J. Analysis of past and present synthetic methodologies on medicinal chemistry: Where have all the new reactions gone? *J Med Chem* 2016;59(10):4443-5
3. Brown DG and Boström J. Where do recent small molecule clinical development candidates come from? *J Med Chem* 2018;61:9442-68
4. Beutner GL, *et al.* TCFH-NMI: Direct access to *N*-acyl imidazoliums for challenging amide bond formations. *Org Lett* 2018;20(14):4218-222

About the author

James Hitchin, *Charnwood Molecular*

James joined Charnwood Molecular in December 2016 as Head of Medicinal Chemistry. James brings extensive experience from across multiple therapeutic areas in drug discovery, having previously held senior positions at SAFC Pharma, Pfizer Pharmaceuticals and KemFine Oy. Prior to joining Charnwood, James was Senior Medicinal Chemist at the Cancer Research UK Manchester Institute, where he worked on various target classes, including kinases and epigenetic targets. James has led numerous projects from hit identification right through to lead optimization and beyond and has an in-depth knowledge of modern drug discovery, as exemplified by his impressive publication record.

Charnwood Molecular
Excellence in Chemistry

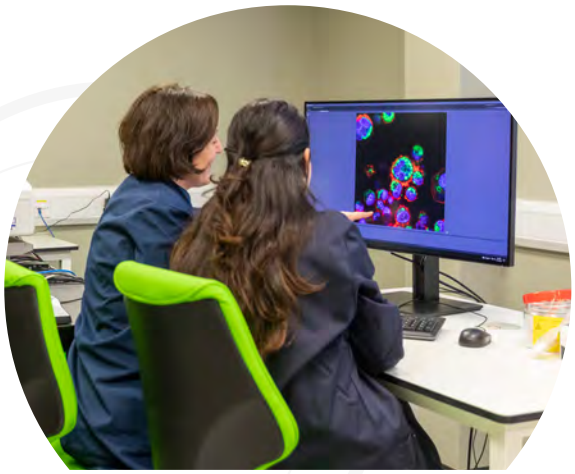
Operating from state-of-the-art facilities in Loughborough and BioCity, Nottingham, UK, Charnwood Molecular is an award-winning Contract Research Organisation providing synthetic chemistry services to the global pharmaceutical, biotechnology and chemical industries.

6 | Understanding the PK-PD relationship

Understanding the pharmacokinetic-pharmacodynamic (PK-PD) relationship in preclinical models is crucial to predicting an efficacious dose regime in man. Preclinical PK-PD analysis investigates the dose-response relationship of exposure and biological effect. The exposure levels of a drug following dosing is quantified using PK analysis in the plasma or target tissue and the measurement of a PD marker show how the drug is acting at the biological target.

In this webinar, Graham Trevitt described and explained the principles of PK-PD modelling, how it can be applied in early stage drug discovery and how it supports decision making throughout the lifetime of the project, increasing in complexity and accuracy with time. Jenny Worthington outlined what a successful PK-PD study should include and draws on case studies to demonstrate the benefit of PK-PD models prior to efficacy studies in preclinical animal models; and finally Neill Gingles described the approaches for evaluation of PD endpoints, using non-invasive imaging modalities to visualise and quantify clinical response to a drug.

View the recording and slides for the sixth webinar
Understanding the PK/PD Relationship



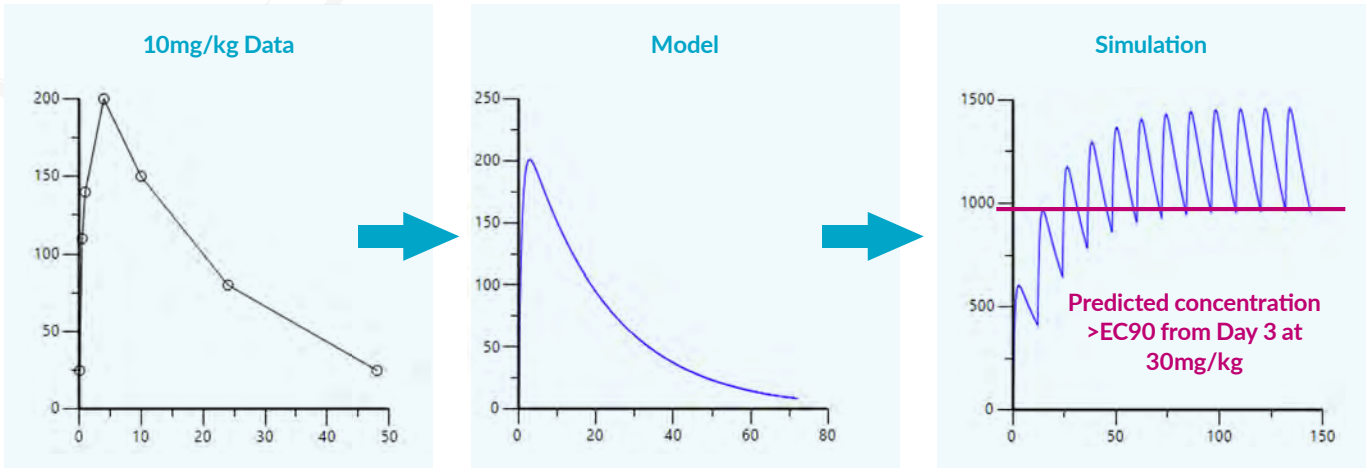
Principles and modelling of pharmacokinetic and pharmacodynamic relationships

Mathematical modelling is currently hard to avoid due to headlines regarding COVID-19. Models are being used to predict situations such as the worlds response to COVID-19, how many will die globally from the pandemic, etc., and not all the headlines are complimentary. At the start of COVID-19, these models were simple models based mainly on assumptions with little data. As more data become available, the assumptions can be replaced by data and the models become more robust, however this incurs both time and financial costs.

XenoGesis aims to offer simple, but useful modelling of pharmacokinetic (PK), pharmacodynamic (PD) and integrated pharmacokinetic-pharmacodynamic (PK-PD) modelling requiring minimal data and therefore cost. This can allow for efficient design of pre-clinical PK-PD studies and, through the integration of predicted human PK, predictions of efficacious dose regimes in the clinic.

How does XenoGesis do this?

PK modelling can be useful in early stage drug discovery. If the plasma exposure is known from a single 10 mg/kg dose in a mouse, modelling could be used to predict what could happen to trough concentrations with a twice daily 30 mg/kg dose, which could then help define a hypothesis for the pharmacology stage.



PK, PD and PK-PD – what's the difference?

PK measurements (concentration vs time) can be often be described using a single compartment IV model with 2 parameters: clearance, and volume. These are then plotted with observed data and a linear line is drawn. When the data do not fit this linear line, and show over and/or under prediction, a more complex model is required with additional compartments e.g. volume (V2) and clearance. In addition, an oral compartment can be included adding further complexity to the model. To this a PD effect can be added (effect versus concentration) and a direct PK-PD relationship can be observed i.e. the integrated relationship between the plasma exposure time (PK) and effect versus concentration (PD), for a given dose, and route of administration.

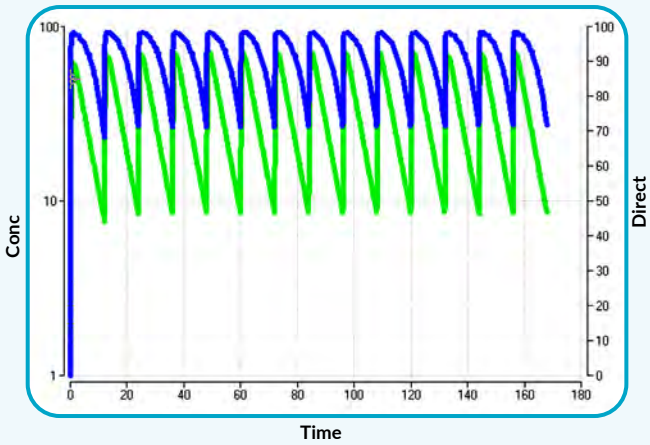
Direct PK-PD models are the simplest, meaning at all time points the concentration in plasma is directly related to the effect. Indirect PK-PD occurs when there is a delayed response *in vivo* and the maximum effect occurs later. The plots show the same underlying PK and EC50. The direct PK-PD needs twice daily dosing for a >70% effect, whereas an indirect PK-PD achieves >80% effect from once daily dosing. A hysteresis loop indicates 2 different response levels for one drug concentration.

XenoGesis believe studies should be designed to integrate all available knowledge to test a hypothesis using dose levels and samples that investigate concentration effect whilst also investigating time dependence allowing indirect PK-PD to be seen. Models integrate knowledge of drugs and help support decision making throughout the life time of the project increasing in complexity and accuracy with time.

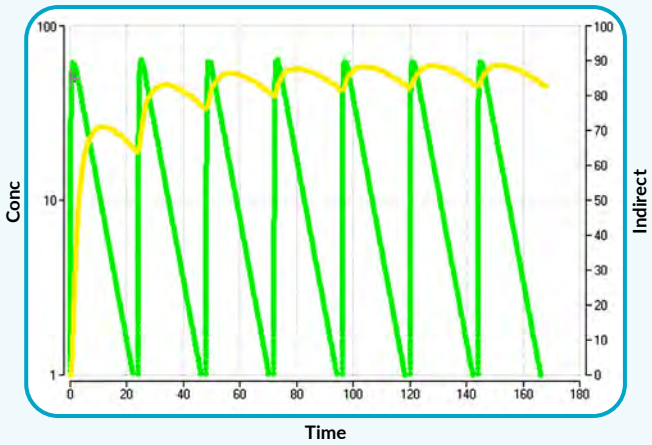
To find out more about PK-PD, the DMDG run a 2-3 day residential PK-PD course:

info@dmdg.org

Direct PK-PD: BID Dose schedule



Indirect PD-PD: QD dose schedule



About the author

Graham Trevittt, *XenoGesis*

Graham is CSO and heads up the scientific team at XenoGesis. He joined XenoGesis in 2015, bringing over 14 years' industrial drug discovery experience, as well as a track record of delivering pre-clinical drug candidates in oncology and inflammation through integration of DMPK properties into compound design to increase the probability of creating successful drugs. Prior to joining XenoGesis, Graham worked at UCB for 8 years before joining Almac Discovery in 2009. Graham graduated from the University of Nottingham with a PhD in Synthetic Organic Chemistry and went on to complete his Postdoctoral Research at the University of Geneva.

XenoGesis

XenoGesis can identify the potential 'winners' and 'losers' in a selection of compounds synthesised in drug discovery campaigns. They provide data-driven iterative feedback to the client, and recommending next steps is a key focus. They combine state-of-the-art *in vitro*, *in vivo* and bioanalytical capabilities with expert pharmacokinetic/pharmacodynamic (PK/PD) data interpretation services.

Understanding PK/PD using pre-clinical models: Lessons for efficacy studies at Axis Bio

Axis Bioservices is a preclinical contract research organisation based in Northern Ireland, providing services in oncology, inflammation, and respiratory disease. The company specialise *in vitro* efficacy and mechanistic studies through to *in vivo* target engagement.

Value of PK-PD studies to maximise pre-clinical efficacy

Preclinical pharmacokinetic-pharmacodynamic (PK-PD) analysis looking at dose-response relationships of exposure, and biological effect both in the plasma and target tissue can enhance the drug discovery process. Benefits include predicting time course effects of drug doses (single or multiple doses) and pharmacological effects and provide an understanding of target modulation and the dosing schedules required to lead to statistically significant efficacy whilst minimising toxicity.

The PK-PD relationship

The exposure levels of a drug following dosing in both plasma and target tissue can be quantified using PK analysis. PD analysis studies how the drug is acting at the site of action and helps answer questions such as does it modulate the target? What happens downstream of the target? Are there biological effects/efficacy?

Analysing a range of doses is key, alongside looking at effectiveness over time. In addition, it may be possible to correlate the PK-PD parameters with any adverse effects seen.

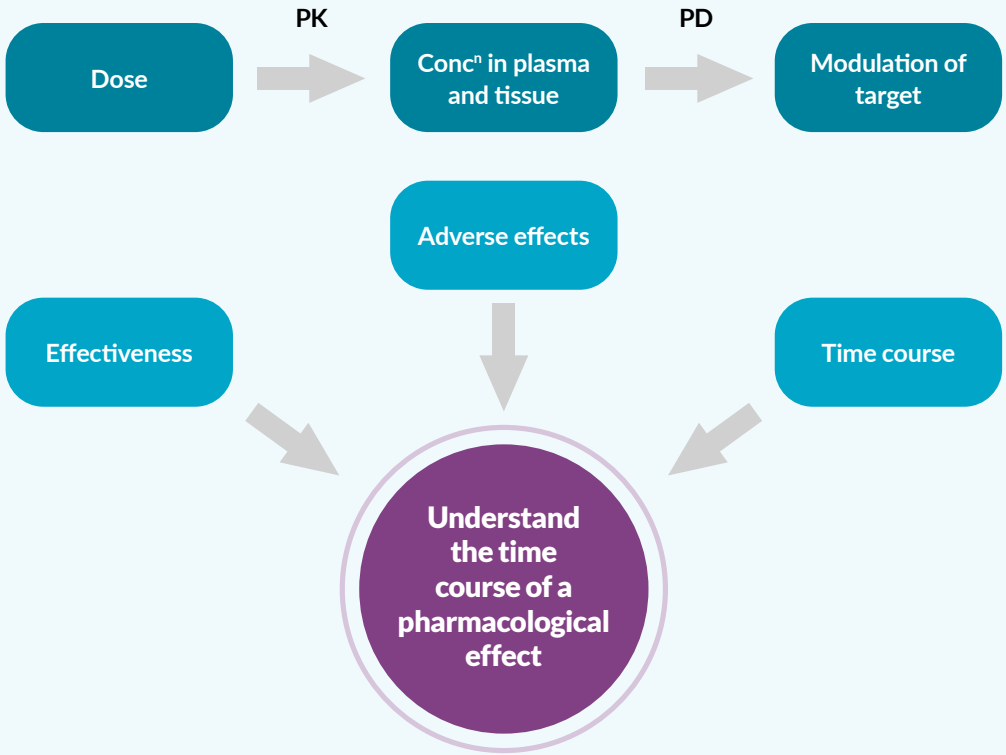
Integration of knowledge

Knowledge integration is critical to design a successful PK-PD study and should combine the knowledge of chemists, pharmacologists, and biologists.

A successful PK-PD study should include:

- A range of doses e.g. 3
- A range of time points e.g. 5–6 around Tmax
- A washout phase to assess direct or indirect effects on the target
- Measurement of plasma levels, and target tissue
- Multiple samples from individual animals

PKPD Relationship



Demonstrating the benefit of PK-PD models prior to efficacy studies

CASE STUDY 1 – NO PK-PD ANALYSIS

Problem:

Following plasma PK analysis, the drug moved directly to an MTD efficacy study with a 30 mg/kg dose. Whilst efficacy was apparent, tolerability was an issue leading to early termination of the study.

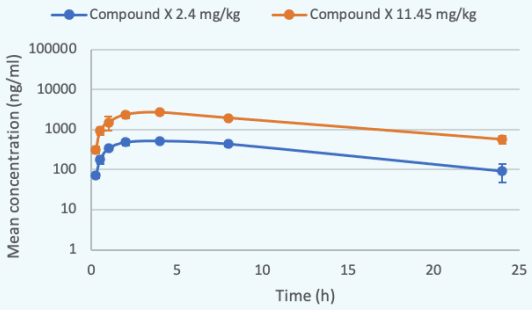
Solution:

Axis Bioservices designed a single dose PK-PD study at three dose levels (3, 10 and 30 mg/kg) with longer time points compared to the original PK study. Serum exposure levels, tumour exposure levels and both tumour target protein levels by Western blot and the associated downstream processes were analysed.

Outcome:

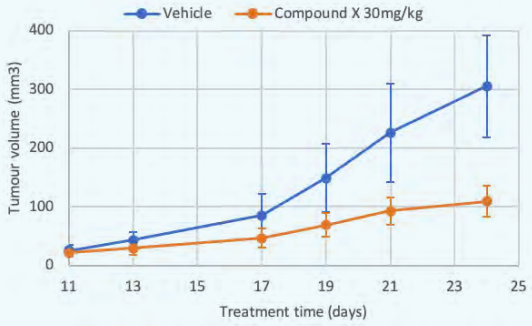
Exposure levels were very similar at 10 and 30 mg/kgs, as was target protein modulation. However, indirect effects were apparent downstream. The results led to a change in the dosing schedule from 30 mg/kg once daily to 10 mgs/kg 3 times per week to give good efficacy and acceptable toxicity. Conducting these studies prior to animal models would have saved time and budget.

PK data



MTD DATA (14 days dosing) 30 mg/kg is MTD

Efficacy data



CASE STUDY 2 – PK-PD ANALYSIS

Respiratory studies can take 6 months, so problems encountered can impact on timelines.

PK-PD study design

Previously obtained PK data from naïve and diseased animals, *in vitro* data, and modelling data, were used to design a PK-PD study with a 14-day optimised dosing schedule.

Results

The study designed led to a successful efficacy study with full scale biomarker analysis. This has allowed the client to proceed to the clinic with confidence in the dosing schedule.

Planning a PK-PD study provides the opportunity to maximise efficacy studies in the short term and plan for long-term success.

About the author

Dr Jenny Worthington, *Axis Bio*

Dr Jenny Worthington is Co-Founder and Director of Science at Axis Bio. She 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 preclinical research. Jenny has used her background in cancer research and drug discovery to move into a commercial setting. Using her knowledge and experience she has nurtured excellence in a team of scientists who provide clients with an excellent preclinical service portfolio.

AXISBIO DISCOVERY SERVICES

Axis Bio is an independent, privately owned preclinical CRO located in purpose-built facilities on the North Coast of Northern Ireland. Founded in late 2013 by Dr Jenny Worthington and Catherine Maguire, the company has grown organically with a strong and diverse client base across the UK, Europe, Middle East and North America.

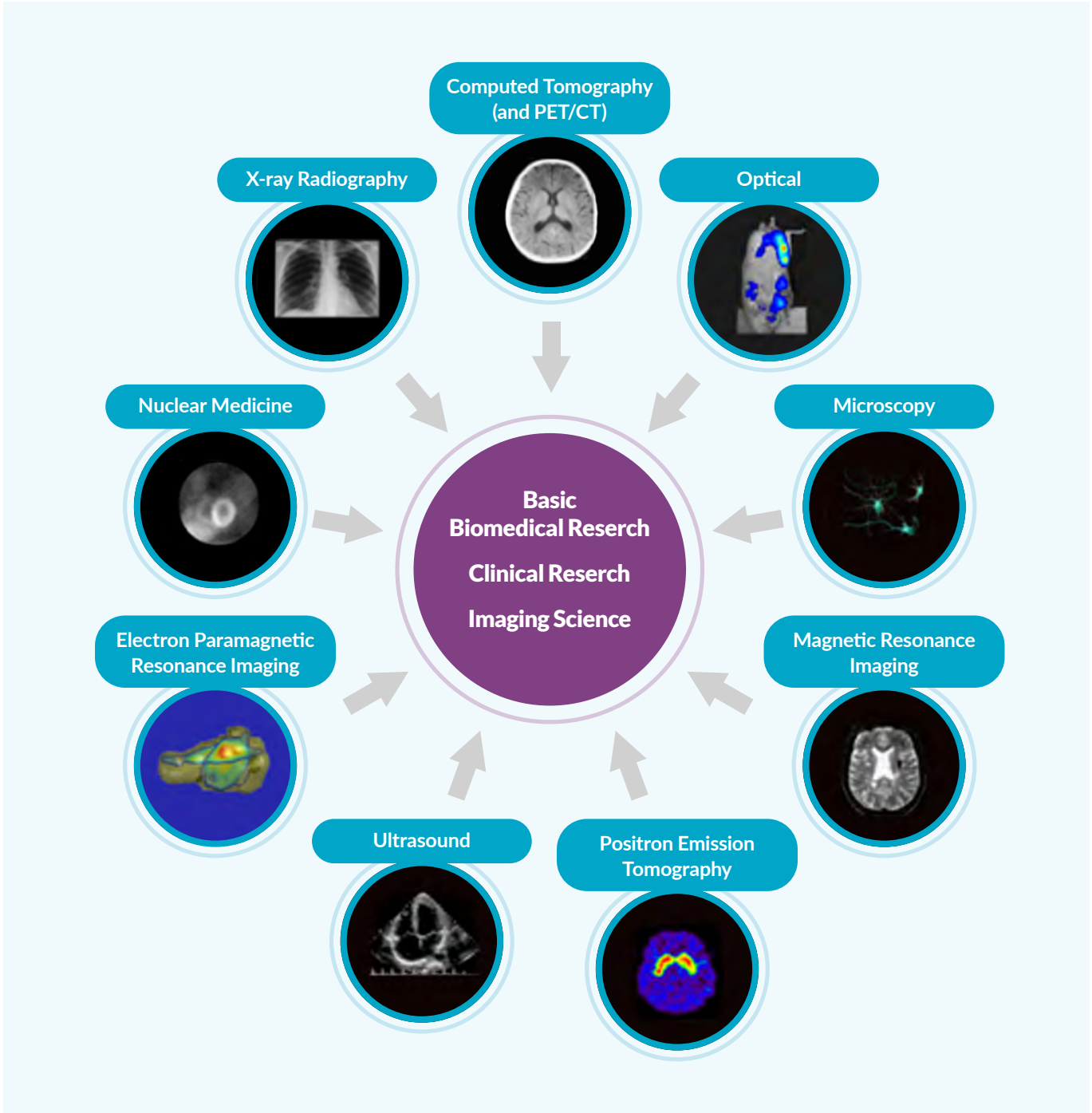
Evaluation of clinical and pre-clinical pharmacodynamic endpoints using non-invasive imaging modalities at Medicines Discovery Catapult

Preclinical and clinical imaging endpoints can be used for key decision making throughout drug development through to regulatory approval and are essential to the drug development process.

Pharmacodynamics and imaging

In pharmacodynamics, imaging offers a non-invasive opportunity to visualise and quantify clinical response to a drug. In oncology trials, PD endpoints can be used as surrogate biomarkers of a clinical response.

There are a variety of different imaging modalities in preclinical and clinical use, as shown below. Each modality has different ranges, spatial resolution, and depth of penetration, alongside advantages and disadvantages, for example, the need for complex technology or bolus contrast.



CASE STUDY OF PRECLINICAL IMAGING PD USING FDG-PET

Hypothesis to test:

Tumours with a PTEN deficiency will benefit from the novel oncology drug (AZD8186) and ^{18}F-FDG could be a suitable endpoint biomarker.

Results:

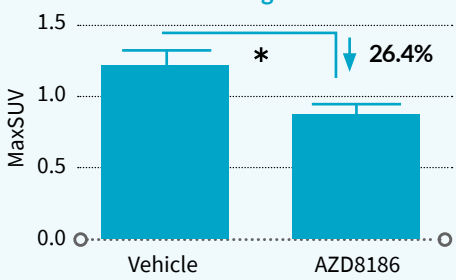
Preclinical FDG-PET imaging in xenograft mouse models of cancer showed a significant reduction in FDG uptake in tumour cell lines 786 and U87 (both PTEN nulls) versus the PTEN avid cell line BT474C. The FDG-PET data correlated with other PD endpoints such as the tumour size and protein biomarkers.

Conclusion:

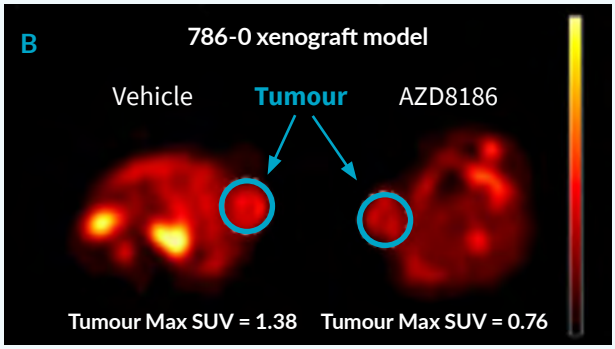
18F-FDG PET imaging can be used as a non-invasive pharmacodynamic biomarker for clinical studies with the drug.

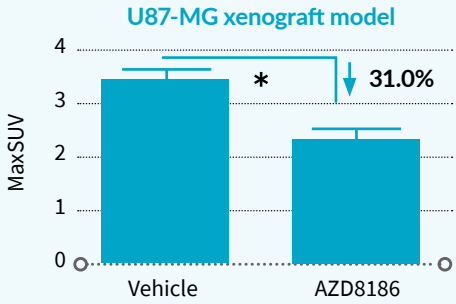
A

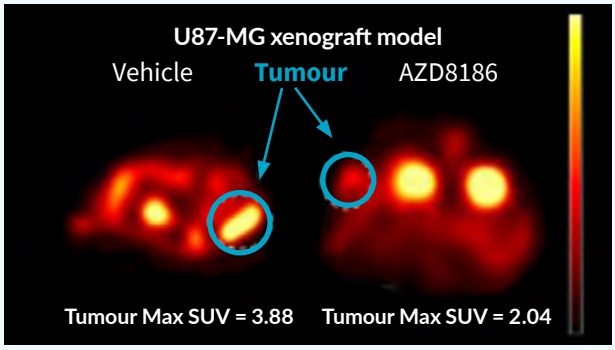
PTEN null cell line



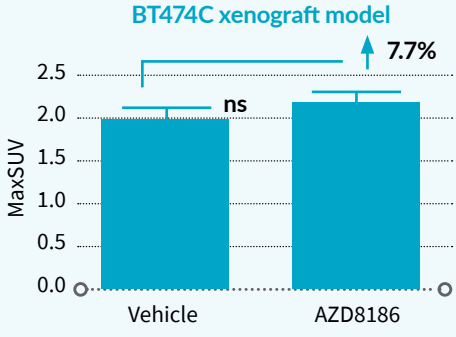
B

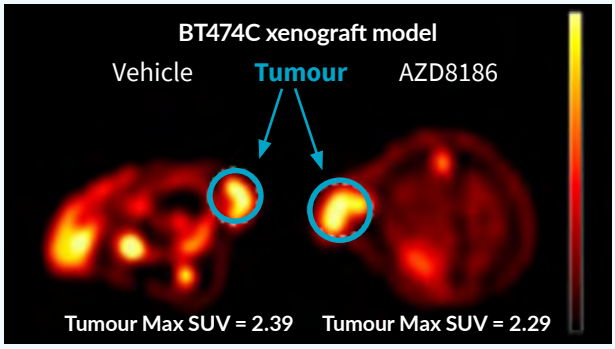






PI3Ka cell line





CASE STUDY OF PRECLINICAL IMAGING USING HIGH FREQUENCY ULTRASOUND AND PET

Aim and Methods:

Using a $FeCl_3$ rodent model to induce thrombus formation on exposed femoral veins in mice, the hind limb functional flow with velocity 3D vasculature and time to occlusion will be measured. High frequency ultrasound can generate a 3D representation of the vasculature at baseline after injury and with drug treatment. PET whole body fibrin binding using a novel PET tracer (fibrin binding peptide label with fluorine-18) will be studied.

Results:

Evaluation following different treatments are underway.

Imaging in clinical trials

Imaging is routinely used in clinical trials e.g. PET, CT, and MRI. Objective tumour response using the RESIST criteria is an example of an oncology biomarker.

Computed tomography (CT) and magnetic resonance imaging (MRI) are used to measure lesion size, detect appearance of new lesions, and provide anatomical information. Advanced imaging techniques such as PET, SPECT, dynamic contrast enhanced MRI, and perfusion CT can provide further information on anatomical structure of tumour sizes and functional information such as metabolic activity, expression of molecular targets, and cell proliferation.

CASE STUDY OF IMAGING PD MARKERS IN METASTATIC COLORECTAL CANCER

Methods:

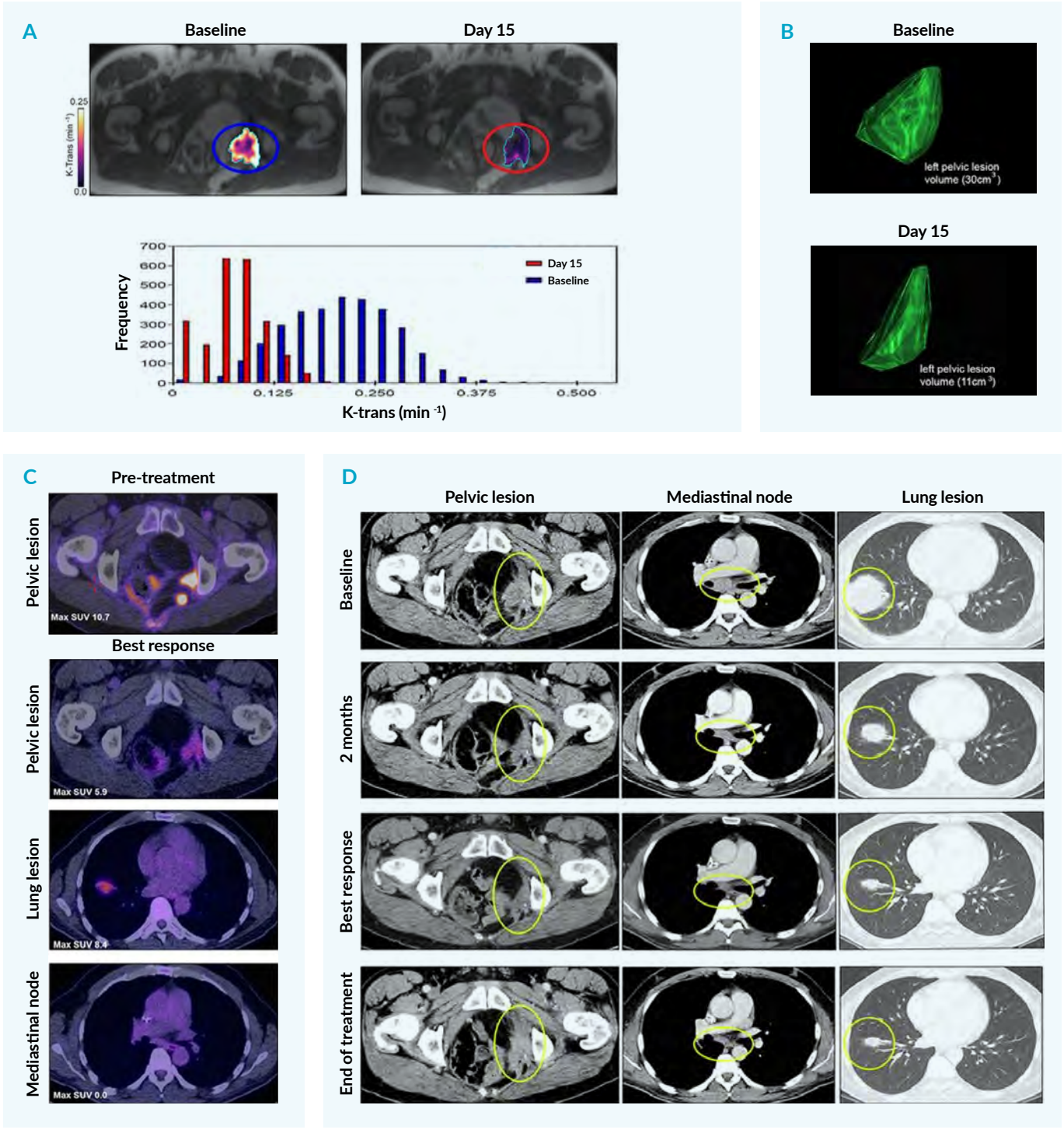
Imaging was performed at baseline and followed up at various time points. CT was used to assess volume change in tumour size, using the RESIST criteria and FDG-PET to assess metabolic activity of the tumour. Permeability and flow in the tumour and angiogenesis were assessed using dynamic contrast enhanced MRI.

Results:

Reduction in permeability and flow in the tumour were observed (A) and tumour volume (B). FDG PET showed a decrease in metabolic activity (C) and CT images measured by the RESIST criteria showed a partial response to the drug for 31 weeks (D).

Conclusion:

The imaging endpoints correlate with improved progression-free survival and overall survival in patients with metastatic disease in these trials.



Khurum Khan *et al.* Gut 2018;67:1484-1492

About the author

Neill Gingles, *Medicines Discovery Catapult*

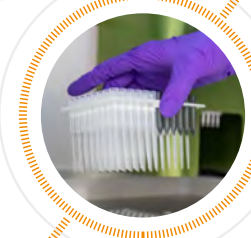
Neill has spent over 25 years working in academia, pharmaceutical and biotech SME industries. He has experience working in various therapy areas such as infectious disease, cardiovascular disease and oncology from preclinical early development through to late stage and marketed products.

7 | Target Validation and Efficacy

Project failure due to lack of clinical efficacy during development remains an issue and can in part be attributed to inadequate pre-clinical target validation or clinical translation data. Target validation is required to build confidence in the biological hypothesis, and the strength of the hypothesis is increased as the complexity of the system increases; from validation in cell lines, to primary cells, to complex cell systems and animal disease models before ultimately efficacy is tested in human clinical studies.

In this webinar, Matt Burnham described the importance of confirming target engagement, the ligand-target interaction and its mechanism, and provides examples of methods to measure this. He explained the importance of understanding target engagement in the event of a lack of efficacy to establish whether this is due to the compound not engaging with the target or a failure of hypothesis. Amanda Woodrooffe discussed the advantages and disadvantages of *in vitro* cell models using primary cells and the importance and relevance of these approaches in modelling native biology in human systems prior to the clinic; and finally Lorraine Mooney outlined the considerations for *in vivo* proof of concept studies with reference to experimental design and model choice based on the molecule's mode of action.

View the recording and slides for the seventh webinar
Target Validation and Efficacy



Strategies for target and pathway engagement in cellular assays

The importance of target engagement studies

Key to the target validation process and improving clinical attrition is testing the biological hypothesis. It is important to establish if a lack of efficacy is due to the compound not engaging with the intended target or alternatively, engaging with the target but the target not modifying the disease pathway. Achieving this in a cellular context is an important step in mechanistic validation.

Three main steps are involved in target engagement:

1. Does the lead compound reach the intended site of action?
2. Does the ligand interact with the target with the intended mechanism?
3. What are the downstream consequences of the drug-target interaction?

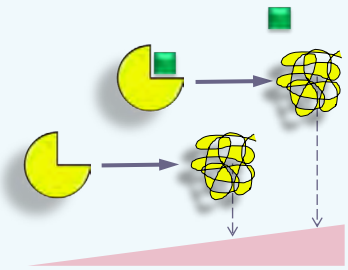
Cellular target engagement

Cellular target engagement models the complexity of the cell environment compared to studies in isolated protein or protein domain approaches. Achieving this for intracellular targets can be more challenging compared to targets located on the cell surface and can be exemplified by three broad categories:

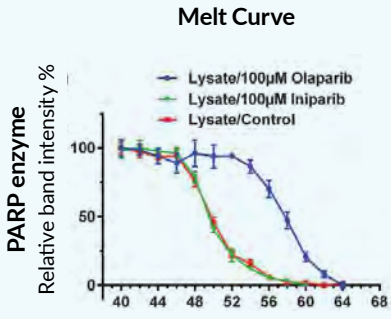
1. Label free approaches (e.g. Cellular Thermal Shift Assay (CETSA)) which do not require modification of ligand or target.
2. Approaches using both a modified ligand and a modified target (e.g. Bioluminescence Resonance Energy Transfer (BRET)) which require an engineered target as well as a tracer ligand molecule.
3. A diverse collection of modified ligand approaches such as fluorescence-based ligand tracking (e.g. Fluorescence Polarisation or Fluorescence Correlation Spectroscopy) or alternative chemical biology approaches such as the conversion of a ligand into a PROTAC or affinity-based proteomics (which may be difficult approaches to achieve in practice).

CETSA relies on the stabilising effect that small molecule binding can confer on the structural confirmation of its target. When the target protein is subjected to a heat pulse, the ligand-bound form will unfold at a higher temperature compared to the unbound form, generating a shift in what is known as the melt curve. A variety of detection methods can be applied to readout this difference caused by ligand-induced stabilisation, ranging from Western blot and antibody-based detection through to mass spectrometry-based proteomics. It should be noted that CETSA can give false negative results for compounds that genuinely bind the target but do not lead to a thermal stabilisation, however, it is a flexible approach that may have minimal reagent requirements.

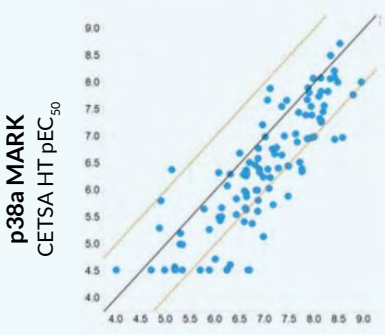
BRET is a cell-based assay that utilises a luciferase enzyme tag and a fluorescent tracer ligand. While similar to fluorescence resonance energy transfer (FRET), BRET has the advantage of improved signal due to the absence of widefield illumination with the luciferase tag producing the donor emission. Compound binding is detected by displacement of the tracer and decrease of the BRET signal, allowing the binding of the compound to be followed in real time. It is one of the few techniques allowing intracellular residence times to be measured, which may provide an additional avenue to improving drug efficacy and safety profiles.



Temperature

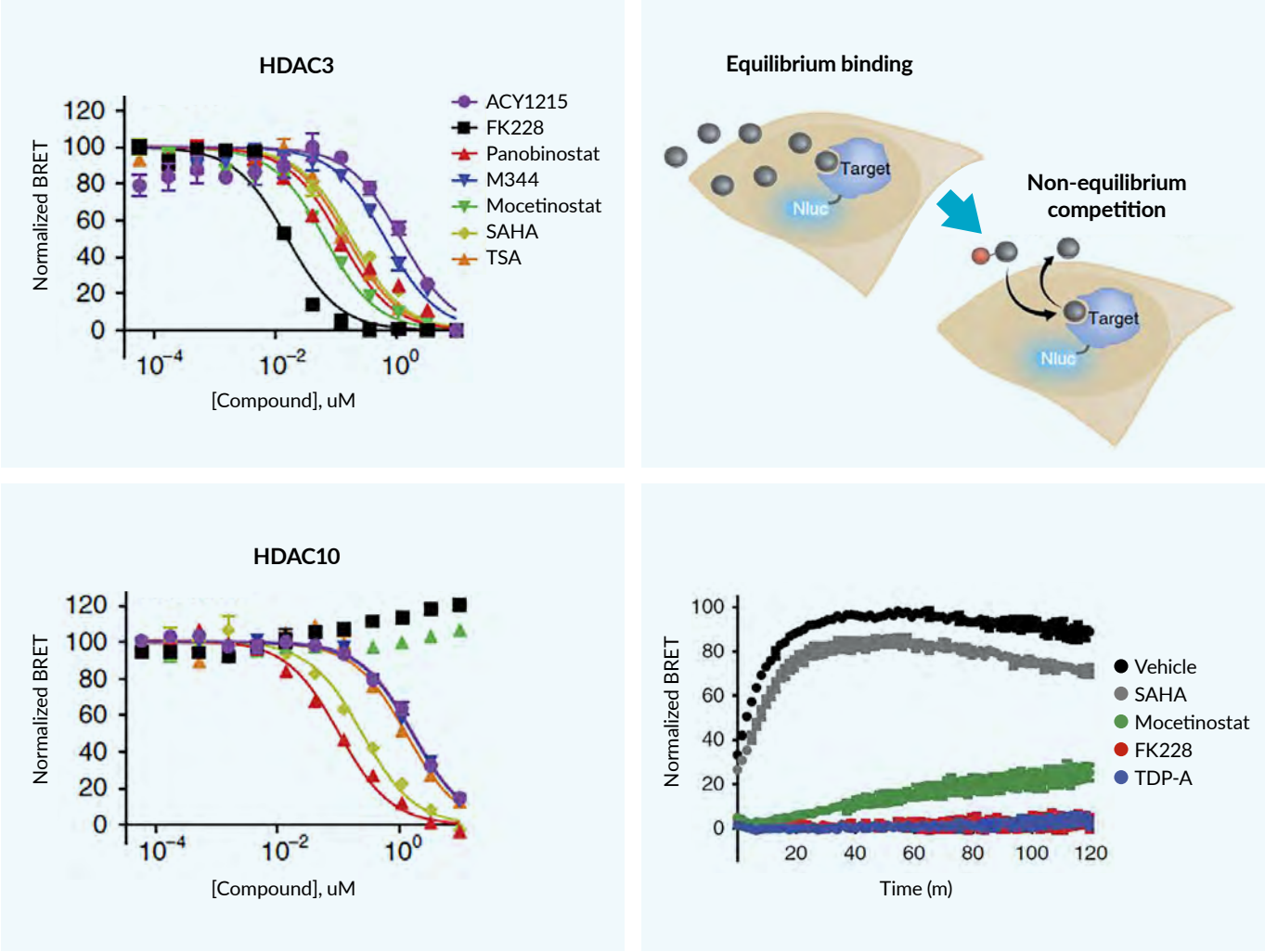


Temperature °C
Western Bolt



Biochemical FP assay PIC_{50}
HT (AlphaLISA)

Molina *et al* 2013; Shaw *et al* 2019



Robers *et al* 2015; Target engagement and drug residence time can be observed in living cells with BRET

Pathway engagement

Demonstrating modulation of the disease-relevant pathway often involves validating proximal markers downstream of the target. Ideally, these markers are specific to the desired interaction between ligand and intended target, and well validated. At MDC, we offer highly-sensitive detection of peptide and proteins using platforms such as Quanterix™ single molecule array Simoa® platform and advanced microscopy. In addition, global tissue analysis such as Nanostring GeoMx™ digital spatial profiler and mass spectrometry imaging.

Conclusion

Target engagement should demonstrate the ligand reaches its site of action, confirm the ligand-target interaction and its mechanism, and identify and measure proximal markers that can report on the modulation of the disease pathway. This concept of target engagement carries through all the way from *in vitro* cascades in early discovery to the use of proof of mechanism biomarkers to inform outcome in clinical trials.

About the author

Matthew Burnham, *Medicines Discovery Catapult*

Matthew is a Lead Scientist at MDC. He has expertise in drug discovery with nine years previous experience at AstraZeneca in both the Mechanistic Biology and Profiling Department and the Safety Screening Centre, developing and validating cellular assays for drug efficacy studies and building understanding for predictive *in vitro* toxicology. Previously in academia, he specialised in elucidation of complex molecular pathways and electrophysiology of vascular biology as a BHF investigator.

Go Native… Characterising therapeutic effect in primary cellular models

Clinical attrition due to lack of clinical efficacy during development remains an issue and can in part be attributed to inadequate pre-clinical target validation and/or suitable clinical translation data. Using relevant preclinical cell-based models can help ensure the clinical validity of the target & therapeutic effects prior to clinical efficacy studies and ensure a better understanding of complex biology and complex diseases.

Prior to assay development, it is important to consider the type of model required and whether it can adequately reflect the complexity of the disease. There should be a good understanding of the actionable data required, how to characterise the relevant biology in the model and therefore decide which is the most suitable model approach for the study. Sometimes a simple model that faithfully recapitulates a key functional requirement may be suitably fit-for-purpose. Sometimes a more complex model may be needed. The main consideration is that the model best reflects the biology required.

Advantages and disadvantages of primary cell models

The use of primary cells models has advantages, disadvantages and some opportunities. Primary cells have the advantage that they are native cells, the use of which enable a better understanding of the native physiological, morphological and molecular processes in human cells and are therefore provide the most *in vivo*-like cellular biology possible.

The disadvantages of using primary cells include the routine availability and sourcing of cells, the need for regular access, the scale/numbers required for higher throughput screening models and therefore the need to often source cells from individual donors resulting in interindividual variation within the assay and the challenge of maintaining the cellular phenotypes in culture. Whilst interindividual variation can be a disadvantage for certain applications (e.g. screening), it can also provide an opportunity, as it ultimately better represents what variation may be observed in the clinic.

The key opportunities with primary cells is the increasing ability to perform/access high volume isolations for certain cell types e.g. PBMCs which can undergo cryopreservation for future studies and provide consistency across multiple assays. Also, the ongoing evolution of more sophisticated, multicellular culture platforms offering longevity of relevant function.

Primary cell-based assays are typically positioned at the tertiary screening or candidate selection stage. However, appropriately validated primary cell-based assays can also be used to generate bioequivalence data to support marketing authorisation applications. Ultimately the choice of model and the positioning of the assay and relevant clinical translation of biomarkers carried through to clinical development need to be considered within the preclinical development cascade.

Primary cell assays can be useful to explain the pharmacology and show differences between primary cells and other cell lines. The use of primary cellular models can allow not only support validation of the target and therapeutic efficacy but also demonstrate the potential for any safety issues. It's equally important to appropriately model both the efficacy and disposition/safety characteristics of a novel drug.

Summary

Clinical translation of the model is key to its success. Modelling of native biology, at native receptors in simple or complex systems, is possible. For it to be a success the positioning needs to be considered – the format, complexity, different cell types, the throughput required, and the source materials. The model must then characterise as fit for purpose and to ensure it can generate the data required.

About the author

Amanda Woodrooffe, *Precision for Medicine*

Amanda has 25 years' experience in drug discovery and *in vitro* ADME gained from her roles in the biopharma and CRO industries. She has been responsible for operations management and scientific leadership of the UK-based discovery research services business for Asterand/BioIVT since 2010, This business has very recently been acquired by Precision for Medicine, becoming their 2nd European laboratory operation. Prior to this, Amanda held various positions responsible for developing business to business partnerships, pharmaceutical licensing, patent portfolio management and supporting corporate development. Amanda received her PhD from the University of Cambridge.

PRECISION for medicine

Precision for Medicine is the first global, precision-medicine, clinical research organization. Purpose-built to shift the development curve for life sciences clients, they incorporate laboratory expertise, clinical trial excellence, and advanced data sciences at every stage. Known as Precision Convergence, this integrated approach enables them to deliver critical insights into patient biology from early development through approval. The result: More predictable trial outcomes. Accelerated clinical development. New life-changing treatments for the patients who need them everywhere around the globe.

Use of preclinical models to deliver proof of concept efficacy at Sygnature Discovery

Recently, Sygnature Discovery extended its *in vivo* pharmacology capabilities by acquiring a translational oncology team which offer bespoke, high-quality oncology *in vivo* pharmacology services in support of drug discovery projects.

The Translational Oncology team provide in depth oncology and drug discovery knowledge and expertise to their clients. They offer an extensive range of disease-relevant subcutaneous and orthotopic mouse xenograft models and have experience of working with a wide range of therapeutic modalities.

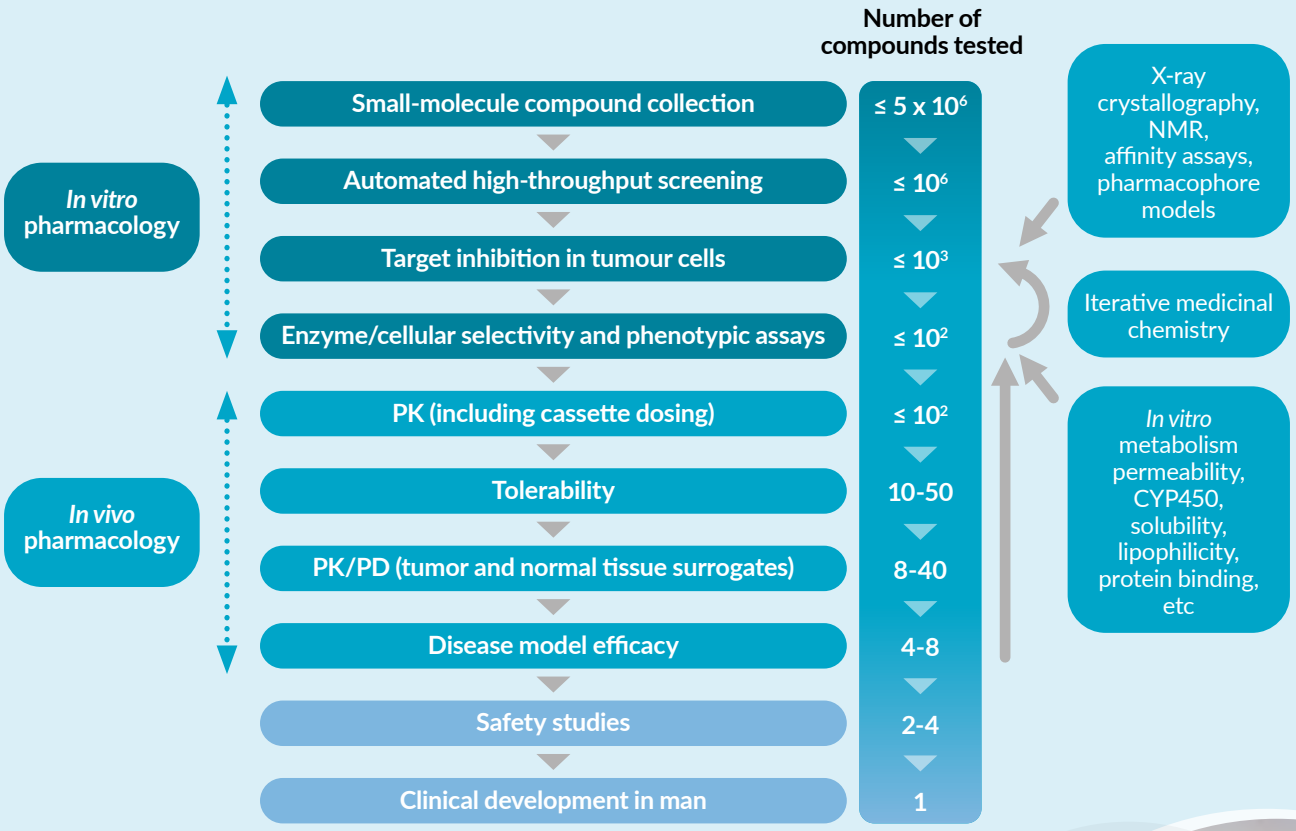
In addition to the team's expertise in therapeutic PK and tolerability, pharmacodynamic, target engagement and efficacy-based studies, the team are also skilled at model development. They are able to undertake model development with commercially available models or work with clients to establish models bespoke to their needs.

Key to success

Target validation is the key to a project's success. As the biological complexity of the system increases i.e. validation in cell lines, to primary cells, to organoids, through to complex *in vivo* systems and finally human clinical studies, validation becomes more challenging, however, at each stage, positive data validates the target and decreases the risk of the project failing.

There are several steps required for testing a molecule prior to *in vivo*, as shown in the drug discovery cascade below. Initial *in vitro* high throughput screening to identify 'hits' involve large numbers of compounds, whereas in *in vivo* pharmacology, the number of target compounds tested include only compounds with the desired *in vitro* profile and properties.

There are a number of preclinical murine models available which serve as surrogates for patients including syngeneic models, human cell line derived models, genetically modified models, patient derived explant models and humanised mouse models. The model choice is dictated by the target, the *in vitro* cell line data and the hypothesis being tested.



Considerations for oncology *in vivo* proof of concept studies

To ensure a successful *in vivo* study it is important to consider three key elements:

Experimental design:

- Suitable tool molecule for testing (confidence in potency, selectivity, PK)
- Understand PK – dose and schedule (acute and chronic dosing)
- PKPD relationship – not always known as at the start of the *in vivo* cascades
- Suitable Formulation
- Appropriate controls – vehicles and positive controls
- Monotherapy/combination therapy
- Samples – ensure samples are taken for PK, tumour biomarker assessment and other tissue biomarkers.

Model choice – based on the molecule's mode of action

- Model should be well characterised – Gene expression, Mutational analysis, Immunophenotype,
- Targeted therapy – cell line derived or patient-derived xenograft models expressing target of interest
- Immune therapy – requires models with a functional immune system e.g. syngeneic models or humanised models

Understand the model of choice

- Understand the utility
- Know how to interpret and use the data

CASE STUDIES

Dysregulation of the fibroblast growth factor (FGF)/FGFR pathway is frequently found in many cancer types making this an attractive therapeutic target. AZD4547 is a potent and selective FGFR inhibitor which suppressed FGFR signalling and growth in tumour cell lines with deregulated FGFR. Selection of sensitive and resistant cell line derived xenograft models allowed proof of concept to be tested *in vivo*. These successful proof of concept pre-clinical studies for AZD4547 have led to Phase 2 clinical trials in lung cancer with clinical proof of concept ongoing.

Syngeneic preclinical models can be used to explore proof of concept for immune therapy agents. AZD8835 a dual PI3Kα/δ inhibitor was being tested clinically to target tumour epithelial cells in solid tumours. However, the impact of AZD8835 on the tumour microenvironment and anti-tumour immunity had not been explored. Syngeneic models were utilised to investigate the mode of action of AZD8835, these studies revealed a novel immunomodulatory mechanism to deliver anti-tumour activity independent of its effect on tumour cells.

Selecting the right *in vivo* model to answer the scientific question, to better understand PK, PD, potential biomarkers and establish PK/PD and efficacy relationships is key to validating your target and increasing the probability of success of your project.

About the author

Lorraine Mooney, *Sygnature Discovery*

An experienced Bioscientist with *in vivo* pharmacology expertise and 15 years' experience in Oncology Drug Discovery both at AstraZeneca and in contract research. Lorraine has delivered pre-clinical *in vivo* strategies for large and small molecule drug discovery projects from identifying leads through to supporting early clinical development. Lorraine has expansive knowledge of tumour xenograft models as well as significant experience of utilising syngeneic models to support immune oncology targets. Lorraine received her PhD in Cancer Biology from the University of Sheffield and completed two Postdoctoral Research Positions, studying Stress Kinase Cell Signalling Pathways at the University of Manchester and then at AstraZeneca exploring *in vivo* optical imaging techniques.

SYGNATURE DISCOVERY

Sygnature Discovery is a leading independent provider of integrated drug discovery and pre-clinical resource and expertise.

Sygnature offer fully integrated discovery project support, as well as discipline-specific support in medicinal and computational chemistry, bioscience, DMPK, *in vitro* pharmacology, and *in vivo* pharmacology, as needed by their collaborating clients.

Sygnature's primary focus is value creation for clients – providing advanced scientific knowledge and intellectual input to accelerate customers' drug discovery projects from target validation and lead optimisation through to pre-clinical candidate.

8 | Is my Compound Safe?

Safety plays a huge part during preclinical development, and any drug must be extensively tested to regulatory standards before approval to test in humans. Safety-related hazards can arise from target-mediated risk, off-target activity, pharmacodynamic effects on the major body systems such as cardiovascular, central nervous and respiratory systems, as well as gross behaviour and pathological changes in animals. Comprehensive testing strategies, combining in vitro and in vivo safety studies enable liabilities of a new molecule to be identified and assessed in line with the clinical patient setting and can significantly influence compound selection, ensure the safety of clinical trial candidates and increase the chances of success.

In this webinar, Richard Knight highlighted the importance of thinking about safety early in the drug discovery programme; considering target, mechanistic and compound related safety. Pauline Garner provided a practical guide to the design of safety studies and considerations of formulation, toxicokinetics, species selection and regulatory requirements. Finally, Malcolm Haddrick discussed the challenges and opportunities of using complex cell models in early de-risking strategies and toxicity testing.

View the recording and slides for the eighth webinar
Is my Compound Safe?

Making safety part of drug design

The importance of safety in the drug discovery process

The aims of safety in the drug discovery process are to develop an effective new medicine which can be given to patients quickly and safely. Studies have shown that clinical development attrition rates are high. A study analysing over 7,000 development programs with nearly 10,000 clinical and regulatory phase transitions, reported <10% of them reach the market (all indications).

A key challenge in the drug development process is reducing this high attrition rate. Data have shown clinical failure at different stages of the project can be attributed to safety issues. Safety issues may stop the project, or dramatically slow the project down and increase the costs associated with it.

The ideal time to optimise the safety characteristics of novel compounds is in the early discovery phase which should be integrated with chemistry, biology, and DMPK. There are three key areas to consider; the safety risks associated with the drug target itself, the risks associated with the chemical space and patient safety.

Safety risks

Safety issues related to the primary target remain a major reason for drug project failure. Data from AstraZeneca has shown that up to 25% of discovery projects and up to 50% of early clinical phase studies were stopped for safety concerns with the primary target. Understanding of the target in normal physiology means it may be possible to anticipate potential toxicities, recognise the difference between 'on' and 'off' target toxicity, and may influence dose scheduling, route of administration or combination opportunities. It may also drive choices around selectivity and be instrumental in the decision on whether to pursue the drug lead.

Chemical toxicity

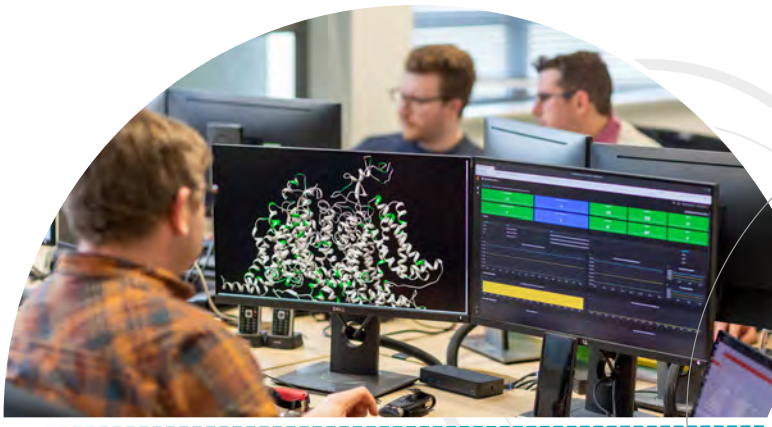
Various in silico approaches can investigate chemical related toxicity or risks relating to the liabilities other than those of the target, that might limit an efficacious dose range. Key liabilities can be assessed with *in vitro* safety screens, secondary pharmacology screens, or investigation on the genotoxicity of the primary compound, for example. Bespoke investigational studies can identify potential safety issues early on and potentially eliminated through the molecule design. The key message for safety endpoints is they are implemented to help increase the quality of the compound that enters the clinic.

Patient safety

The final area of interest is patient safety where an informed risk assessment can be made. This should be based on patient demographics, risk/benefit profile, patient age, and comorbidities, type of illness i.e. a severe life-threatening illness or short-term illness such as an infection and the dosing schedule i.e. short-term dosing or lifelong dosing. Understanding the patient need in the early phase of development allows the qualities of the compound to be refined.

Summary

Safety in the drug discovery process is more about defining the right question and using this to guide the right experiment to derive data that allows a compound to be developed, with the greatest chance of long-term success.



About the author

Richard Knight, *ApconiX*

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

APCONIX
a better decision

ApconiX was formed by three AstraZeneca colleagues with the drive and ambition to create a world-renowned company known for its expertise in nonclinical safety toxicology and ion channel electrophysiology. The company is founded on the skills and experience of a growing team with a wide range of expertise in preclinical drug safety.

ApconiX is continually forming collaborative relationships, helping customers large and small. The changing face of drug discovery and development means a safe pair of hands is needed to help companies navigate the difficult pathway towards safe, effective and profitable drugs.

How to get your molecule into humans: A practical guide for the present and a look to the future at Sequani

Understanding the main reasons for delay and maximising the opportunities available to improve the efficiency of the transition to first-in-man clinical trials are paramount to the success of a drug development programme.

The ultimate aim for a non-clinical program is to ensure the safety of the clinical trial subjects but, to improve the efficiency of drug development, we should seek opportunities to achieve this better, cheaper and faster, whilst utilising as little active pharmaceutical ingredient as possible. Elements that should be considered are a full risk-benefit assessment focusing on what is required and when to progress the compound to clinical trials but de-risking will take longer and cost more. Appropriately designed lead optimisation studies combined with an early focus on the non-clinical strategy can save time and unnecessary expense.

When developing the non-clinical strategy, there are a number of elements to consider – the duration of dosing, daily or cyclical dosing, recovery periods, the choice of relevant rodent and non-rodent species. Strategy should be based on science and regulatory understanding. Regulatory advice, or advice from experienced CROs will help support the strategy. The extent of testing required will depend on the nature of the pharmaceutical development and design of the proposed clinical trial.

To enhance efficiency, the common reasons for delay should be considered and avoided in the strategy. The most common reasons for delay are shown in the graphic below.

Steps to enhance non-clinical efficiency opportunities include a mutual agreement CDA with the CRO, a good relationship/ partnership with the CRO, integrating the CRO as part of the project team, and using the CRO for advice and guidance on the programme design. The key to a successful programme is good communication.

To maximise the value of toxicology studies, integrating additional measurements can help e.g. genotoxicology endpoints can be added to pivotal repeat dose rodent toxicity studies using flow cytometry analysis of micronuclei, which can reduce animal usage and cost. Safety pharmacology can be included in repeat dose toxicity studies: non-invasive telemetry, and Irwin style observations or combining a male fertility element in the sub-chronic or chronic rodent toxicity studies.

Histopathology, the pivotal endpoint of the toxicity studies can itself be rate limiting. By processing all tissues from all animals to slides and making the slides available for evaluation when required will save time. Development of clinically relevant biomarkers alongside non-clinical studies enables a direct comparison of non-clinical data to clinical data, helping to inform on clinical design and dose level selection in the clinical trials. Micro sampling allows for more efficient science with fewer animals by reducing or removing satellite animals for toxicokinetic sampling.

Availability of API	Formulation Issues	Insufficient Capacity	Unexpected TK
Single most common reason for delay Quantity and quality of API Clinical formulation Contingency	Also very common Higher doses than previous studies Appropriate vehicles for test species Check solubility at appropriate concentrations Suspensions more appropriate?	Repeat dose tox are rate limiting CROs have finite capacity Select CRO early Know strategy Avoid last minute changes	May invalidate studies Include additional dose groups Animals, API, money, and time wasted Conduct lead optimisation with TK

Unanticipated Toxicity	Analytical Method Development	Species Selection	Late Reports
Can result in significant delay Can result in unnecessary use of TI Conduct appropriately designed lead optimisation	Bioanalysis and formulation analysis methods required Formulation analysis a GLP requirement Fully validated (in each matrix) Start as early as possible	Fundamental basis of all non-clinical programmes Traditional 'rat and dog' approach no longer acceptable Species selection impacts on API Biologicals – relevant species	Can delay regulatory submission Avoid by careful CRO selection Track record of quality reports delivered on time Experience in FIM programmes Communication is key

What does the future hold?

Concentrating specifically on animal models there is an increased uptake of mini pigs which have a number of advantages including similar omnivore digestion to humans, having an oestrogen cycle similar to humans, being sexually mature by six months of age, and having similar skin to humans. The mini pig offers a viable, non-rodent species as an alternative to the commonly used dog and non-human primate (NHP) models and, may provide an improved prediction of clinical efficacy.

Disease models (usually mice) can be of value in providing a safety assessment in the context of the disease and, in some cases, may demonstrate toxicological responses more representative of the response in patients. Humanised transgenic animal models can be used for toxicology studies to produce better read-outs, but these humanised models are very expensive. Uptake of different models could result in a concomitant reduction in the use of NHPs and dogs, which has ethical and economic benefits.

In summary there are significant opportunities to save time and money during the implementation of the non-clinical programme but retaining quality is paramount. The non-clinical strategy must be defined and a good partnership with an experienced and flexible CRO is essential to improve efficiency.

About the author

Pauline Garner, *Sequani*

Pauline started her career in non-clinical 23 years ago working in the Genetic Toxicology department at Sequani as a Study Director and latterly managing the team. Then in 2012, she made the transition to the Business Development department at Sequani. In her current role as a Programme Manager, Pauline manages the programmes of work that are placed at Sequani, encompassing General, Reproductive, Juvenile and Genetic Toxicology, as well as all supporting scientific disciplines such as Analytical Chemistry, Bioanalysis, Clinical Pathology and Pathology. Sequani Limited is a Contract Research Organisation in Ledbury offering non-clinical expertise and support throughout the development of a pharmaceutical, crop protection product or chemical

sequani NON CLINICAL
great people, great work, real results

Sequani's heritage spans more than 40 years when a company called Toxicol Laboratories opened for business in London before moving to the company's current site in Bromyard Road, Ledbury. A little while later they went on to join IQVIA (Quintiles); a venture destined to grow into one of the largest contract research organisations in the world. Sequani's customer feedback consistently shows that the service and commitment their clients enjoy is the most personal and direct in the business. But they take none of this for granted. Their focus is continuous improvement so that they carry on growing through the repeat business of loyal customers as well as attracting business from new clients.

Challenges and Opportunities of Complex Cell Models for Toxicity Testing

Availability of safety data is critical in early studies and in later pre-clinical development. A key focus is to improve the safety related attrition in clinical studies which could be achieved using model systems closer to humans and therefore able to generate more reliable, translatable data. These data can then be used to reliably identify toxicity flags throughout discovery.

The current cell models used are often too simple and inadequate to provide these data. Cells are immortalised and have aberrant cellular metabolism, models are often single cell, cultured in a simple 2D setup, used with a glass or plastic substrate, lacking in relevant biological stimuli, with no communication with other cells or cellular models and there is an absence of relevant physical stimuli.

Next generation models accessing stem cells, primary cells with the ability to modify using CRISPR represent more biologically relevant models. Multiple different cell types should be cultured together to mimic *in vivo* situations, newer models could be 3D spheroids unsupported structures or with matrix gels, or even patient-derived organoids. Available technologies to help better mimic biological environments should be utilised eg. the presence of flow.

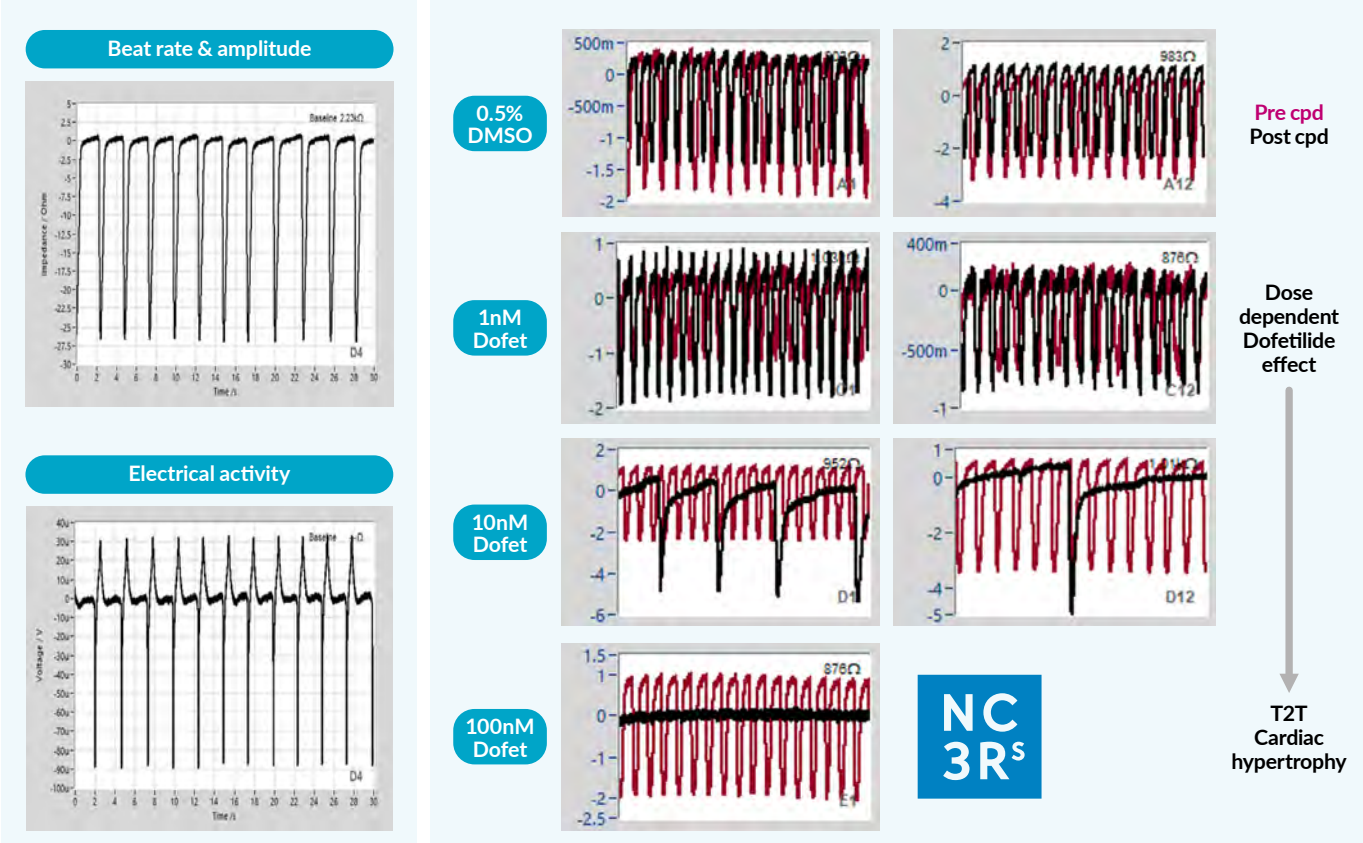
To exploit more complex cell models, it will be necessary to validate and use humanised isolated organ on-chip models (OOAC) and connected on chip models which better mimic a normal biological environment, for use in both efficacy and safety determination.

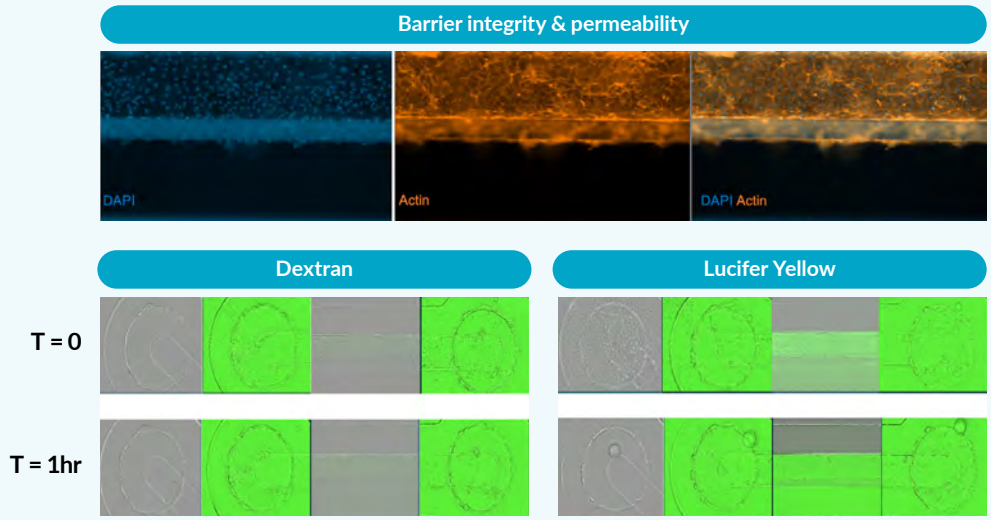
A key step is to develop isolated models and multiple organ models using new technology and identifying promising models that can be used. Industrial experience can provide the expertise to characterise the model, elucidate how good it is, how robust it is, and identify limitations.

Medicines Discovery Catapult (MDC) is developing and evaluating next generation cell models. Examples include the CMEF Cardiac Model, a 3D model for assessment of cardiotoxicity. It uses stem cell derived cardiomyocytes joined with primary cardiac fibroblasts and cardiac endothelial cells (CMEF). These cells, placed in ultra-low attachment wells of a plate, will form spheroids and will then spontaneously beat. By positioning the cells on a recording electrode of a cardioexcyte instrument, it is possible to determine the beat rate and amplitude and produce a trace.

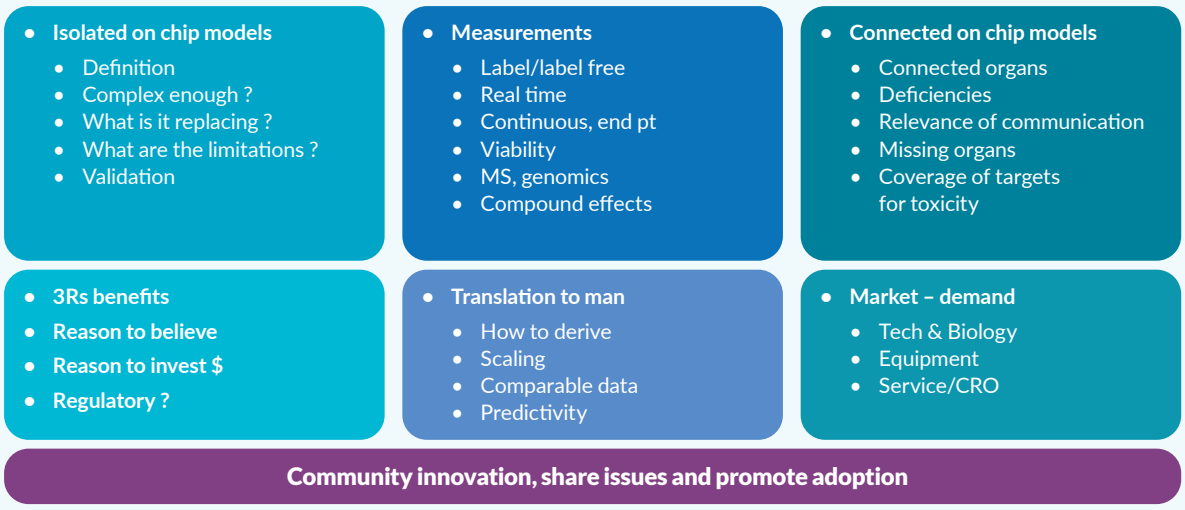
The model has been characterised using known clinically relevant human cardiotoxins and results demonstrated improved predictability, with stepwise increases in predictivie power with the ccuulation of each cell type. The model is amenable to screening at discovery scale, exploits label free, continuous and real-time measurements. There are current ongoing collaborations for CRISPr introduced cardiac mutations and endothelial cell optimisation to further enhance translation to patient physiology.

Neurotoxicity is also a significant problem and the blood-brain barrier (BBB) represents an opportunity to produce a model to give more accurate and predictive results. Current standard *in vitro* BBB models use Caco-2 or MDCK-MDR1 cell lines in transwell plates alongside *in vivo* rodent models for compound permeability and neurotoxicity, however they have limited physiological relevance. MDC are evaluating a simple BBB model in a Mimetas OOAC -384 well SBS plate format. It has a 96-chip or 40-chip format, and the ability to form a tubule from for example, primary human endothelial brain cells, with a simple flow. It has compartmentalised channels and allows cross-talk between cells. Images below, using a fluorescent dye, show the barrier integrity and permeability - there has been some leakage across the barrier so further optimisation is required of the model.





Highlighted here, are just two examples of models being evaluated at MDC, however other models include a liver model using a Physiomimix OOAC platform and primary hepatocytes. The ultimate aim in next generation model development is to connect together robust and translatable relevant human models. There is currently a lot of activity in the cell nanochip space looking to do this. However, there are major challenges to overcome as shown below.



In order for these next generation models to replace or augment animal studies it is important to understand where they can be positioned in drug discovery for safety testing and the value of the data that they will generate. Progression of these models will involve a collaborative approach across UK academia and drug discovery communities.

About the author

Dr Malcolm Haddrick, *Medicines Discovery Catapult*

Malcolm is a Lead Scientist at Medicines Discovery Catapult with specific responsibility for complex cell model development and organ-on-a-chip applications. He has worked across efficacy and toxicity projects at AstraZeneca and Pfizer for the last 20 years, developing and running screening assays for small molecule drug discovery.

9 | Developing a Biomarker Strategy

A common theme throughout our MDC Connects series is that of building confidence in a hypothesis and improving the chances of success in the clinic. To achieve success, the project team needs to understand the key clinical questions and build testable and scientific evidence throughout the preclinical phase employing a clear biomarker strategy. The biomarkers used to transition from the preclinical into the clinical setting will ensure development of robust clinical studies, enabling the monitoring of effects and an understanding of the outcomes.

In this webinar, Gayle Marshall sets out the importance of thinking about biomarkers early in your drug discovery project and defining a clear biomarker strategy to address a range of clinical questions. Helen Hind outlined the importance of clinical samples in R&D and how to access them, and finally Russell Garland discussed biomarkers in the context of immunology. He provided examples of how biomarkers can be identified and measured using different platforms and what validation of a biomarker looks like.

View the recording and slides for the ninth webinar
Developing a Biomarker Strategy

Designing a biomarker strategy at Medicines Discovery Catapult

What is a biomarker strategy and why is it important?

Within the drug discovery pipeline there are several steps a compound must progress through prior to the clinical stage – initial target identification and validation, lead identification and optimisation and pre-clinical stage. Even so, drugs that enter the clinic still have a high attrition rate due to lack of efficacy, PK/PD, safety issues or the wrong strategy i.e. incorrect patient population.

The key to greater success is to understand the key clinical questions and build testable and scientific evidence to transition between the preclinical and clinical setting.

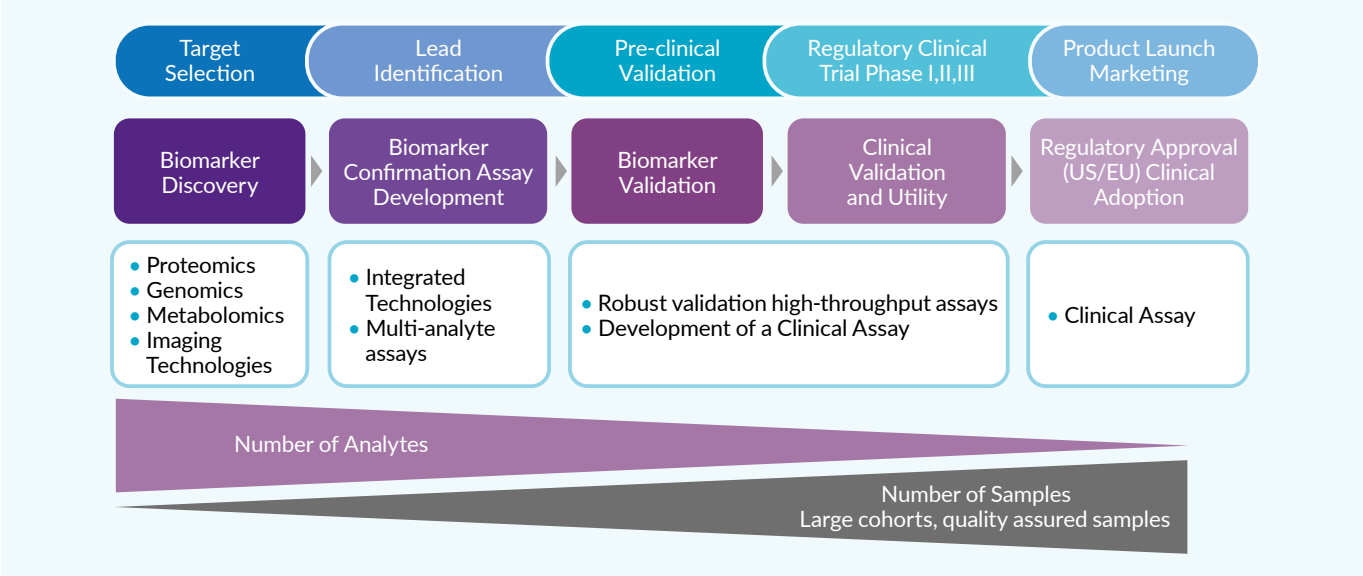
A biomarker strategy is developed to answer a range of key clinical questions and to help develop a robust clinical study. For example,

- What disease will the drug treat?
- Who are the target patients within that disease?
- What dose will be used and how often i.e. daily, weekly etc.?
- How does the drug perform compared to current treatments/ standard of care? A drug that is comparable to an established standard of care will not succeed
- Will a combination therapy with the standard of care further improve treatment outcomes?
- Could acquired resistance develop following long-term treatment?

An understanding of these key questions allows them to be tested in a preclinical setting to help mitigate some risks

Optimal time to introduce biomarkers in drug discovery

Biomarkers should be introduced at the start of the drug discovery pipeline at target selection stage. It is important to understand the mechanism of action with the target and all the markers involved. This can be established using different multi analyte assays and techniques shown below to identify some key markers that can be monitored once the compound reaches the clinic, using a robust assay that has been developed during the biomarker identification.

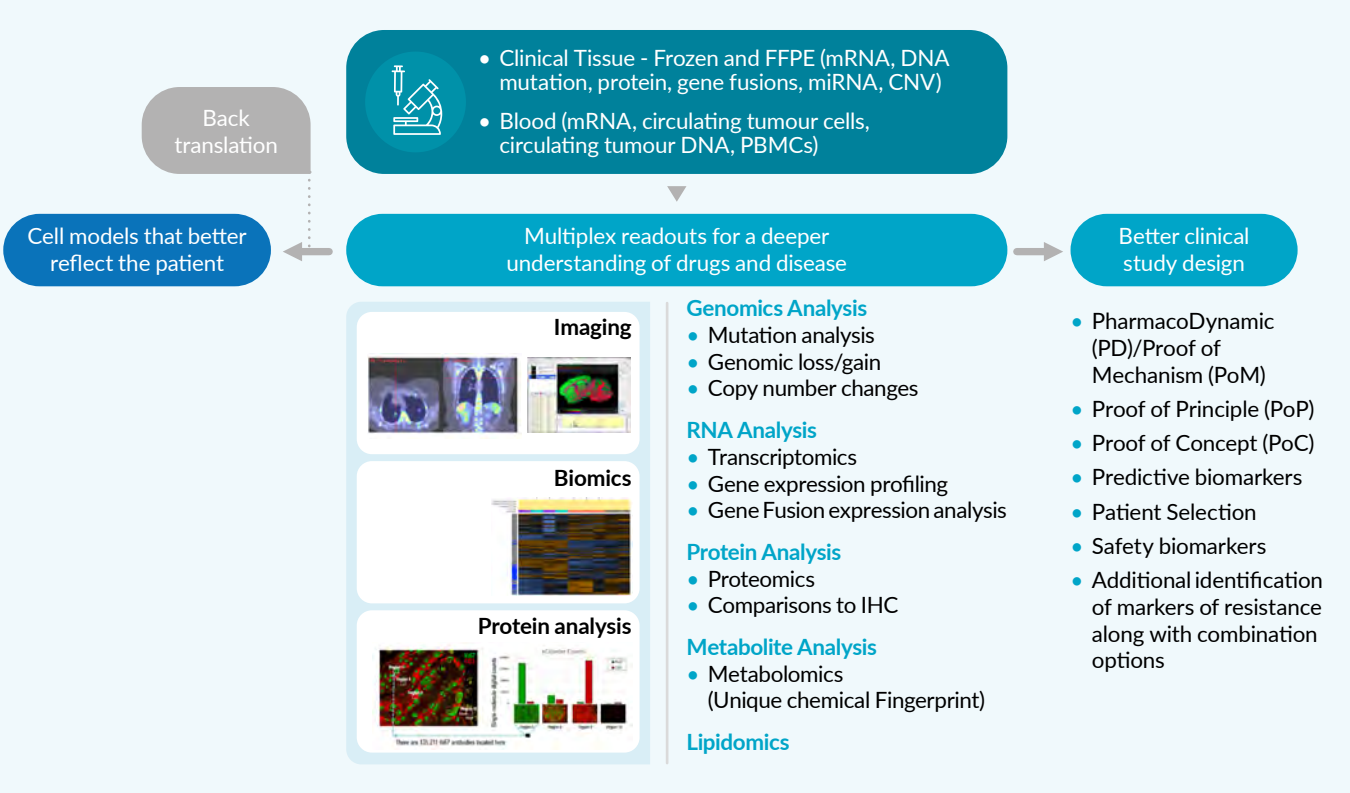


Important considerations to build into a clinical trial design include:

- Pharmacodynamic markers i.e. is the compound hitting the target in treated patients. This in turn allows the dose schedule to be determined, proof of mechanism and dose range
- Proof of principle i.e. proliferation markers or cell death markers, does morphology change due to modulation of the target?
- Proof of concept i.e. what clinical effects occur?
- Predictive biomarkers – is it possible to predict an effect on the target?
- Patient selection –the most common biomarker to be measured is which patients will respond?
- Safety biomarkers are key to your biomarker strategy
- Are there any markers of resistance?
- How does it compare to that current standard of care?

Whilst there are many potential applications for the use of clinical biomarkers, very few have entered the clinic as a diagnostic. Lack of biomarker uptake may be due to lack of clinical utility, complex and underestimated biomarkers, lack of understanding of the pathology and the heterogeneity of the disease, use of inappropriate samples for discovery and validation and methodology limitations.

Medicines Discovery Catapult have several technologies for biomarker discovery and development as shown below which can be utilised by companies to incorporate relevant biomarkers into their drug discovery programmes, develop robust methods to analyse large numbers of analytes, and provide integrated data sets across different technologies to support the biomarker studies.



About the author

Gayle Marshall, *Medicines Discovery Catapult*

Gayle is Lead Scientist for Biomarkers at MDC. She has extensive experience in clinical and pre-clinical biomarkers, previously leading a translational science laboratory team within large pharma. At MDC, she is responsible for delivering biomarker strategies and developing robust assays for clinical utility through delivering key data to support clinical development.

The challenges of accessing clinical samples at MDC

Medicines Discovery Catapult launched their sample access capability following a report that was published called 'State of the Discovery Nation'. The report was based on a survey of UK SMEs around the various challenges in R&D which revealed that whilst access to clinical samples was important, in practice it was challenging to achieve.

Importance of clinical samples for SMEs

SMEs require clinical samples as part of their core R&D activity. Clinical samples can be accessed via collaborations with clinical academics or key opinion leaders, from NHS or academic biobanks identified through the UK CRC tissue directory, from commercial sample supplier organisations or via procurement platforms. Clinical samples can be used to support method development, assay validation, regulatory submission packages, and for biomarker analysis.

Within the UK there are rich sources of clinical samples, consented for research however, these are often not used to their full potential. This could be due to lack of biobank promotion, websites may not be accessible or user-friendly, or the lack of listing in a tissue directory. Researchers may not be aware of individual biobanks especially in the small, early stage organisations with limited experience in sample acquisition. There is a lack of UK wide infrastructure which can impact access committees, governance requirements e.g. ethics approvals, and speed of access. Speed of access is critical for SMEs who need to progress quickly to the next milestone or investment decision in order to survive in a competitive market.

Making samples available to SMEs

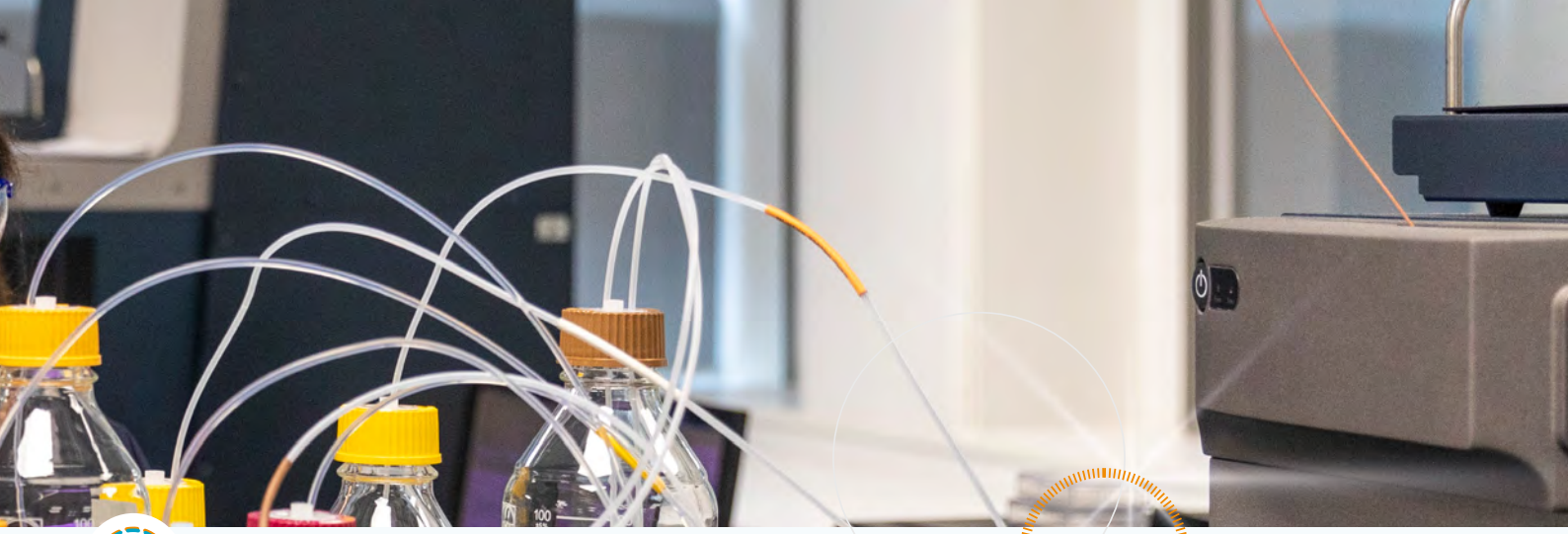
In recent months, the pressure on NHS biobanks to deliver diagnostic work has escalated due to the COVID-19 pandemic and resources are extremely stretched. To support biobanks and help them deliver the services, requests should be submitted with enough lead time as possible, requests should be researched to ensure essential criteria as opposed to the nice to have data variables are identified, and no last minute changes are made, which will impact timelines.

Within the UK the Health Research Authority (HRA) could help enforce registration of biobanks on the UK CRC tissue directory. The Human Tissue Authority (HTA) are committed to trying to encourage the use of biobanks however research only makes up a small proportion of what they oversee. In addition, the patient groups can support the promotion of biobank use. For UK biobanks to continue, they need to be used. Many UK biobanks are established using grants and need to be self-sustaining after several years otherwise they either close or merge with other bioresources.

About the author

Helen Hind, *Medicines Discovery Catapult*

Helen is Biosamples Lead at MDC. She has over 25 years' experience in the life sciences, having previously held both lab-based and clinical research roles at AstraZeneca; she has also worked within sample access for AZ Discovery scientists. Prior to her current role Helen worked in academia on clinical trials methodology and within the NHS as a Business Development Manager, supporting commercial research in the Liverpool city region. She leads Medicines Discovery Catapult's Samples and Data capability to help UK drug discovery companies – primarily SMEs – access the clinical samples they need to develop novel medicines faster.



Biomarker identification: Assessing immune function

Why is biomarker identification so important? Clinical trials are expensive with each phase becoming more expensive as the number of subjects increases, and the attrition rate is high due to a lack of clinical efficacy. Data from the AZ pipeline have shown that programmes that have a Pharmacodynamic (PD) biomarker associated with them, where target engagement or proof of mechanism has been demonstrated, have a better chance of success and proceeding to phase 3 clinical trials or launch.

PD, or Response, biomarkers included early in the preclinical stages of the drug discovery process can be used to confirm target engagement. Informative biomarker assays can be translated to the clinical phases where they can add value and provide proof of mechanism and help inform the sponsor and support their go/no go decisions.

Modulation of the immune system unpins treatments across a range of therapeutic areas, from infectious disease to autoimmunity and immuno-oncology. There are a number of powerful techniques available to measure biomarkers of immune function. For a given study, the experimental solution needs to be selected based on an understanding of the immune component which is being targeted and the logistics of the particular trial.

In the context of PD biomarkers, the aim is to establish a 'window of effect' between the biomarker level following modulation by the test compound of interest. A range of experimental platforms can be employed to identify immune biomarkers. For example, ELISA or multiplex platforms (e.g. Luminex) or can be used to identify protein products stimulated by an immune response, such as an antigen-specific antibody titres or a cytokines/chemokines produced in response to an antigen. qPCR or nanostring can be used to study the molecular 'message' (mRNA or DNA) for a particular biomarker in a biological sample or to assess the modulation by sample treatment or stimulation. Nanostring technology is a powerful technique that can measure the expression of up to 800 genes simultaneously, profile them and the results used to select a smaller panel of appropriate biomarkers, which can be validated by higher throughput methods. At the cellular level, ELISpot can be used to confirm the success of increasing the frequency of antigen-specific T cells in vaccination studies, whilst flow cytometry is a powerful technique that can be quantify the frequency of multiple subsets of immune cells simultaneously in a biological sample. The technique selected for biomarker identification, validation and quantification will be the most appropriate for the needs of the study.

Sample logistics considerations are also essential for successful biomarker identification – how will be samples be taken, how will they be stored, how will they be shipped? This is particularly important for a pre-clinical assay to be successfully translated for use in a clinical trial. A clinical assay will typically be preceded by a validation phase which will mimic the conditions which the trial samples will be exposed to – such as exposure to freezing or fixation – to stabilise the markers before testing. Finally, the assays require fit-for-purpose validation, including confirmation of parameters such as how the standard curve performs and the intra- and inter-assay precision, which will allow batch analysis of samples to be done.

Immune biomarkers can be integrated into the drug discovery process to confirm target engagement (or proof of mechanism) from pre-clinical through to clinical phases. An optimal strategy requires informed choices regarding what immune parameter to measure, how to measure it and how much validation is necessary. Ultimately, PD biomarkers can impact positively on project success rates.

Core Techniques
Depth and breadth of data acquisition

About the author

Russell Garland, *Charles River*

Russell is the Group Leader for Analytical Services at Charles River Laboratories. He is a member of the British Society of Immunology and has 20 years of experience working with immunological assays. Russell was awarded his PhD in 2000 by the University of Bristol. The activities of Russell's group spans readouts of immune modulation in both pre-clinical and early phase clinical phases of drug discovery programmes. He is based at CRL's Portishead site in the UK, which specialises in immunology and infection. Russell is Analytical Project Manager for their clinical services, with a particular focus on pharmacodynamic (response) biomarkers.

charles river

Charles River provides essential products and services to help pharmaceutical and biotechnology companies, government agencies and leading academic institutions around the globe accelerate their research and drug development efforts. Our dedicated employees are focused on providing clients with exactly what they need to improve and expedite the discovery, early-stage development and safe manufacture of new therapies for the patients who need them.

About Medicines Discovery Catapult

Medicines Discovery Catapult (MDC) is enabling the community to reshape medicines discovery in the UK. It is ambitious, and it is achievable.

We do this by championing innovative life science technology and new approaches, supporting UK innovators to succeed.

We are helping to industrialise and drive the adoption of new techniques and technologies.

We are driven by helping our community make their mark on the industry and patients.

If you're involved with medicines discovery in the UK, MDC can almost certainly assist you. Our impact is measured by your success.

Visit our website:
md.catapult.org.uk

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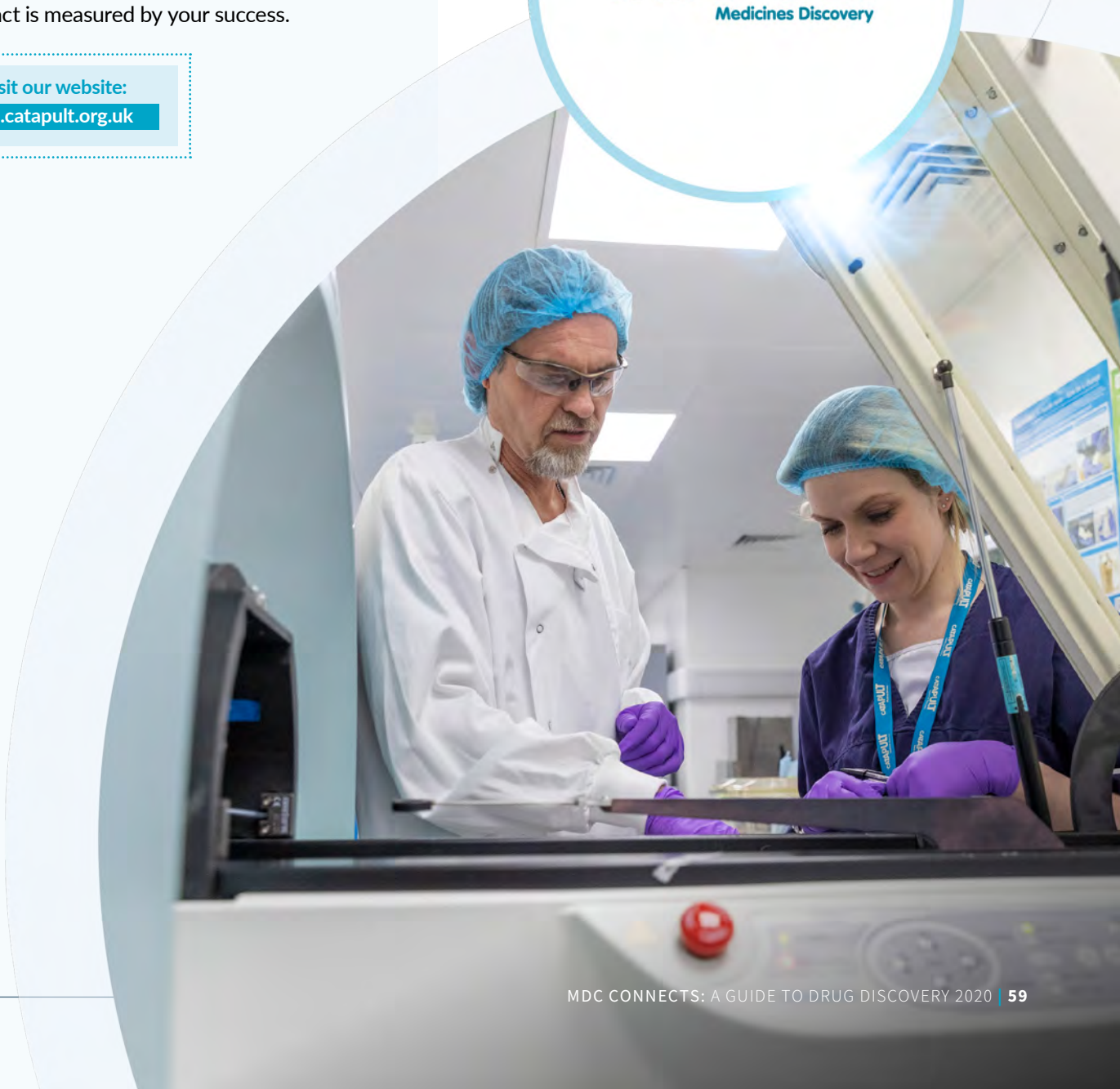
Medicines Discovery

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