How to Develop a Successful *in vitro* Screening Strategy

Developing a drug can take years and comes with significant costs. During the early phases of this journey, it is vital to have a robust process in place to allow rapid and informed decision making. A fit-for-purpose drug screening cascade is core to this and this system must have the capability to test your hypotheses in biological systems to ensure you stay on the right course.

As you embark on designing an appropriate screening cascade, it is imperative to define the fundamental properties you seek in your prospective drug candidates. These attributes will significantly influence the selection of assays to be incorporated into your screening cascade. Questions such as the importance of selectivity for your target, whether you are targeting orthosteric or allosteric binding sites, the need for blood-brain barrier permeability, the specificity of target expression in certain cell types, and concerns about on-target toxicity must all be addressed early in the discovery process. Early discussions among your team are vital to ensure the right assays can be strategically implemented as your project progresses.

The primary objective of a screening cascade, or the design-make-test cycle, is to enable swift decision-making. Screening cascades evolve and adapt as molecules advance, and a cascade developed during the hit-to-lead phase may differ significantly from one in the lead optimisation phase. Nonetheless, at each stage, the cascade should swiftly provide answers to the most pressing questions facing the project team at that particular juncture. This rapid decision-making can accelerate compound progression, facilitate timely intellectual property filings for novel chemical matter, and, importantly, lead to cost savings in the long run. Recognising when to halt a program and reconsider the strategy is as crucial as striving to achieve key milestones.

Screening cascades can vary widely depending on the stage of drug discovery. In the following sections, we will outline key considerations for constructing an effective and robust screening cascade, with a focus on the hit-to-lead phase, followed by insights into how screening cascades evolve during the lead optimisation phase.

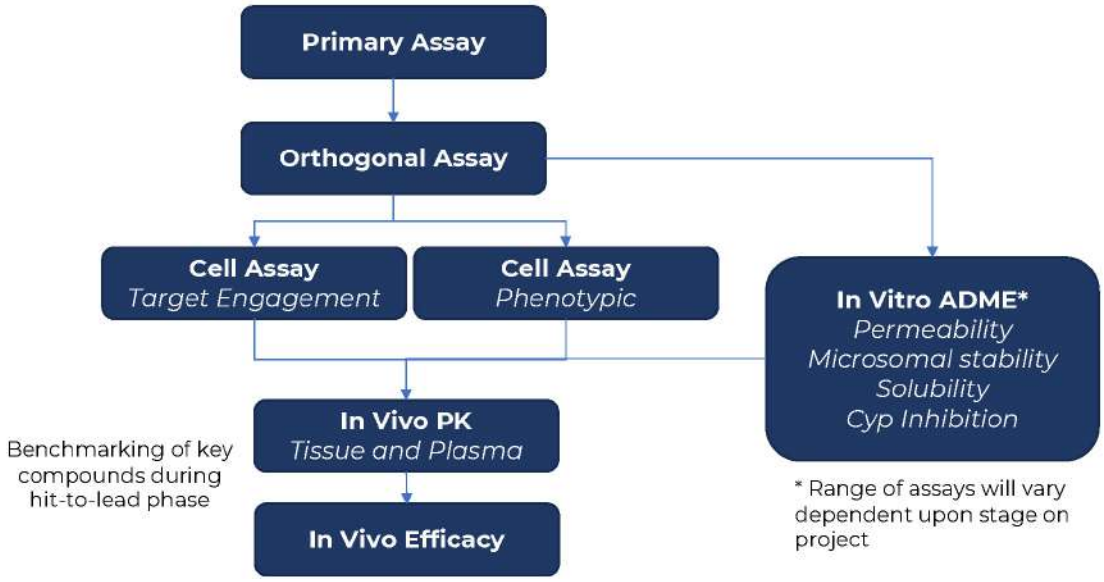
Hit-to-Lead Phase

During the early stages of drug discovery, the primary objective is to identify one or ideally several chemical series that effectively engage the target in the desired manner. While these series may still have a long journey ahead before becoming drug candidates, they serve as the foundational starting points. Consequently, the screening cascade at this stage should be simple and facilitate rapid data generation. This enables the chemistry team to develop a strong structure-activity relationship (SAR) and a grasp of the drug-like characteristics of the initial chemical compounds. This understanding is vital for instilling confidence in the selected chemical series.

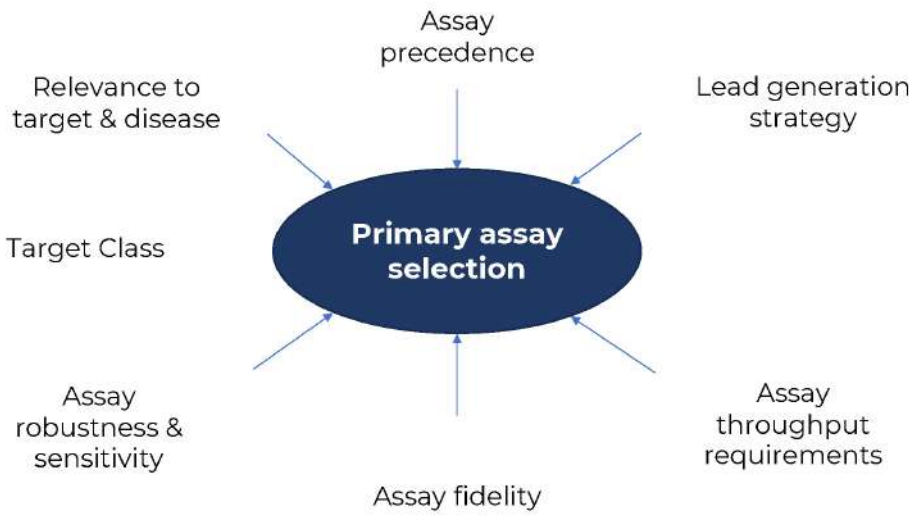
A typical cascade for this phase may include a primary assay, an orthogonal assay, a cell-based assay, and a selection of in vitro DMPK (drug metabolism and pharmacokinetics) assays. It's important to emphasize that these are guiding principles, and customising the cascade often becomes necessary to meet project-specific requirements.

Primary Assay

The selection of an appropriate primary screen is pivotal to the success of a drug discovery project, as it serves as the initial



Standard hit-to-lead screening cascade



Primary assay selection considerations

filter to identify compounds with potential therapeutic activity. Several considerations come into play when determining the optimal primary screen for a project. These considerations include the nature of the biological target, the degree of existing knowledge about target modulation, and the target's suitability for recombinant protein-based biochemical assays. Enzyme targets, for example, are often amenable to such assays. However, the choice of primary screen should align with the chosen hit-finding strategy. For instance, fragment-based lead generation typically requires biochemical or biophysical primary screens due to the need to test compounds at high concentrations.

Primary screen quality assessment is essential to ensure it serves as an effective initial filter. This involves evaluating assay sensitivity and specificity. A sensitive assay can detect subtle changes in compound activity, enhancing the likelihood of identifying hits. On the other hand, high assay specificity minimises the proportion of false-positive hits. Biochemical and biophysical screens are generally less prone to off-target effects compared to cell assays, but both types can be susceptible to compound interference mechanisms, necessitating a tailored triage strategy downstream of the primary screen.

To support high-volume screening, primary assays should be designed to be as simple as possible. The use of homogeneous assay detection technologies is advantageous in these cases, minimising the number of steps required and allowing for efficient miniaturisation.

In many instances, assays for the target of interest may already be available from previous screening campaigns or literature sources. However, these assays often require development and optimisation to suit the needs of a large-scale drug screening campaign. Parameters such as assay signal-to-background, inter- and intra-plate signal uniformity, and overall sensitivity and specificity of the screen should be fine-tuned. While IC50 values are commonly used to assess compound potency, it's crucial to evaluate concentration-response curves comprehensively. Parameters such as top and bottom values of the curve, hill slopes, and compound solubility must also be considered. Ignoring these aspects can lead to misleading results and impact project timelines and costs.

Orthogonal Assays

Orthogonal assays serve two vital roles within a screening cascade: confirmation of primary data and generation of additional data that cannot be obtained in the primary assay format. These assays complement the data from the primary assay and build confidence in the results while providing further insights into the binding interaction. Orthogonal assays are instrumental throughout the drug development process, from initial hit identification to optimisation and characterisation of therapeutic candidates.

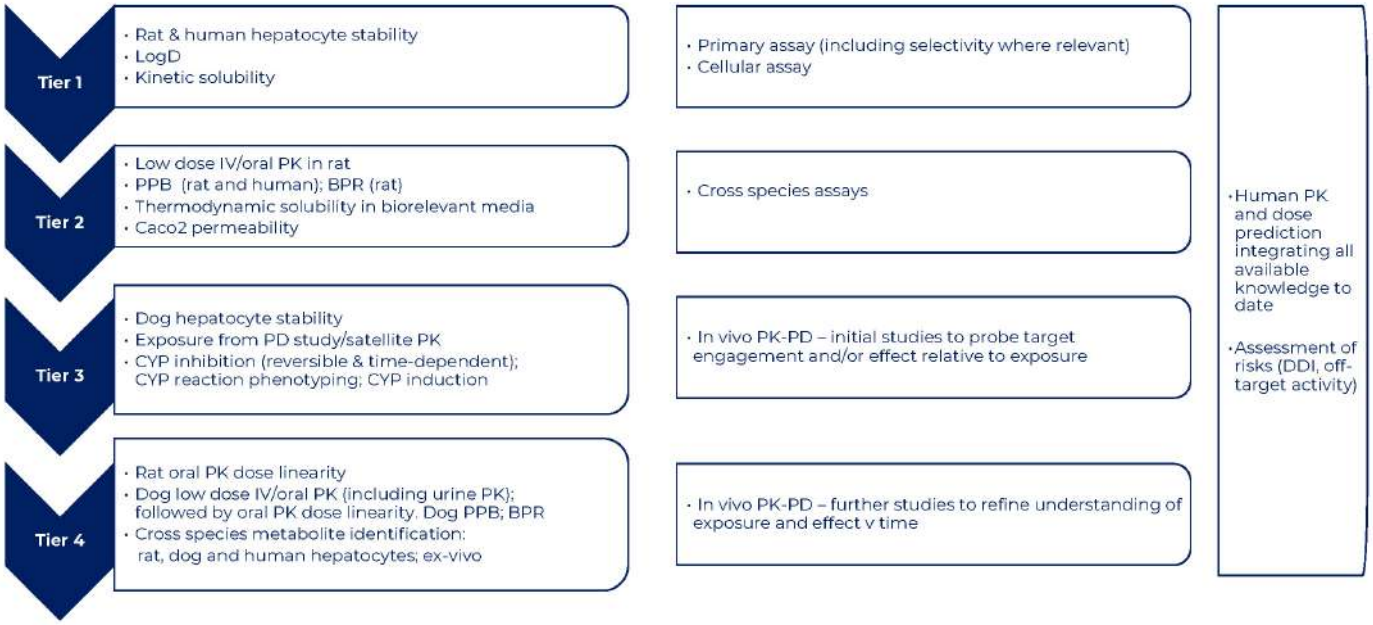
Orthogonal assays can take various forms, often being biophysical in nature to directly observe the binding of interacting molecules. While they generally have lower throughput than primary assays, they offer a deeper understanding of the structure-activity relationship (SAR) and behaviour of potential therapeutics. These assays also possess the ability to detect binding over a broader affinity range, from millimolar binders to picomolar interactions.

Confirming target engagement and generating affinity data using orthogonal techniques are essential to support IC50 data derived from primary assays. Some technologies, such as surface plasmon resonance (SPR), enable the determination of kinetic parameters and stoichiometry, providing crucial insights into binding modes and behaviour.

It's important to note that no assay is flawless, including orthogonal assays. While they offer valuable data, they still represent a departure from natural biological environments. This is where cellular assays come into play, armed with primary and orthogonal data, to further our understanding of how candidate molecules influence specific biology.

Cell-Based Assay

Gaining insight into cellular activity during the hit-to-lead phase is desirable. Developing a cell model that allows the evaluation of compounds in a relevant context is a pivotal component of the early drug discovery cascade. The choice of cell model and the endpoint to be measured should align with the target biology. For example, anti-cancer programs may seek a cell-killing phenotype, while those targeting inflammatory diseases may focus on cytokine profile modulation.



Typical LO screening cascade

The complexity of the cell assay deployed at this stage should be considered in light of the need for rapid decision-making. Simplifying the system to provide information on cellular activity at weekly or bi-weekly intervals allows for timely decisions.

Cell activity can be measured as a phenotypic endpoint (e.g., cell proliferation) or through mechanistic assays confirming target engagement within the cell. Ideally, both types of assays should be included in the screening cascade, as they provide complementary insights.

Drug Metabolism and Pharmacokinetics (DMPK)

A successful drug candidate must balance biological activity and pharmacological effects with human pharmacokinetics to ensure adequate drug delivery to the target site for clinical efficacy. During the hit-to-lead phase, compounds from different series are assessed across a range of in vitro assays to understand the properties that need to be optimized in parallel with pharmacological profile evaluation.

Interpreting data from various assays during the hit-to-lead phase is crucial for making informed decisions. Assessing properties like LogD7.4, metabolic stability, permeability, P450 inhibition, and solubility helps identify potential issues and prioritize series for progression.

The information gathered during this initial profiling phase informs the screening cascade in the lead optimization phase, focusing on properties with significant associated risks. Screening for permeability, for example, may be unnecessary if initial hits already exhibit high permeability, and the chemistry plan aligns with similar physicochemical characteristics. Flexibility is key, and adjustments should be made based on the project's specific needs.

Lead Optimisation (LO) Phase

The transition to the LO phase occurs when one or more chemical series are considered suitable for progression towards drug candidate status. At this stage, there's a deeper understanding of the liabilities associated with each series,

allowing for modifications to the screening cascade to prioritise these challenges.

While the primary assays developed during the hit-to-lead phase remain essential, additional assays may be introduced. These could include assessments of selectivity against closely related targets, cross-species evaluation, and more complex cell-based assays. The focus on the lead chemical series may evolve, and each series presents unique challenges, necessitating continuous evaluation and adaptation of the screening cascade.

In the LO phase, there's an increased emphasis on assessing the overall profile of molecules, beyond potency alone.

Data Analysis

With the substantial volume of data generated during the hit-to-lead phase, robust mechanisms for data analysis are essential. Correlation analysis plays a pivotal role in ensuring data consistency across different assay platforms. Correlations should be tracked between primary assays, orthogonal assays, and cell-based readouts to confirm that target engagement is driving the desired biological effect. Deviations from linear correlations warrant further investigation, as they may indicate issues with compound physicochemical properties or target inhibition strategies.

Bringing it All Together

Developing a dynamic, purpose-built, and robust *in vitro* screening cascade, encompassing both biology and DMPK, enhances the likelihood of discovering new drugs efficiently or making timely project terminations. Efficient assays, co-located within the design-make-test-analyse process, provide scientists with the necessary data for informed decision-making. Drug discovery thrives on collaboration, and isolated efforts are more likely to falter. his is where expert input in DMPK becomes indispensable.

Stuart Thomson

Stuart is Director of Bioscience at Sygnature Discovery, a translatlantic integrated drug discovery CRO. He has 20+ years experience working in the UK and the USA for both pharmaceutical companies and biotechs focused on oncology drug discovery and translational research. Stuart holds a PhD in cell and molecular biology and has carried out post-doctoral research at the University of Oxford and King's College London.

Graham Trevitt

Graham is the Director of DMPK Projects at Sygnature Discovery, with over 20 years of experience in DMPK. His professional background includes eight years at UCB, five years at Almac Discovery, with academic studies at Imperial College London and a PhD in Organic Chemistry from the University of Nottingham.