SYGNATURE DISCOVERY

Developing Effective Hit-Finding Strategies

ORIGINALLY PUBLISHED BY:

FIERCE Biotech

NEW EDITION: JUNE 2023

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Challenge

Discovering and developing a new drug is a complex and time-consuming endeavour. Pharmaceutical and biotechnology companies are under tremendous pressure to be first to market. Yet, many of the best-validated targets are in challenging protein classes with little precedence for small molecule ligand binding. This creates a significant hurdle for initial hit-finding. A carefully devised strategy is, therefore, essential when looking for validated hit series with a good chance of being developed into drug-like compounds.

Best practice strategies

Consider overall program goals

Selecting the best methodologies and assays for the hit identification phase of a project requires a deep understanding of the program goals and an awareness of the most appropriate approaches in generating validated hit matter for related proteins or biomolecules.

Many different screening modalities have been successfully applied to drug discovery, but each presents its own challenges, notably translating those initial actives or binders into well-characterized hits that can be developed into qualified leads and, ultimately, drug candidates. When working with an external partner for hit-finding, it is important that they are fully aware of these challenges.

Furthermore, they should have sufficient experience to select an appropriate set of assays and filters for sifting through the initial list of hits. The key is to focus on genuine hits that engage the target and with a well-defined mechanism. (see figure 1)

Figure 1: Target classes

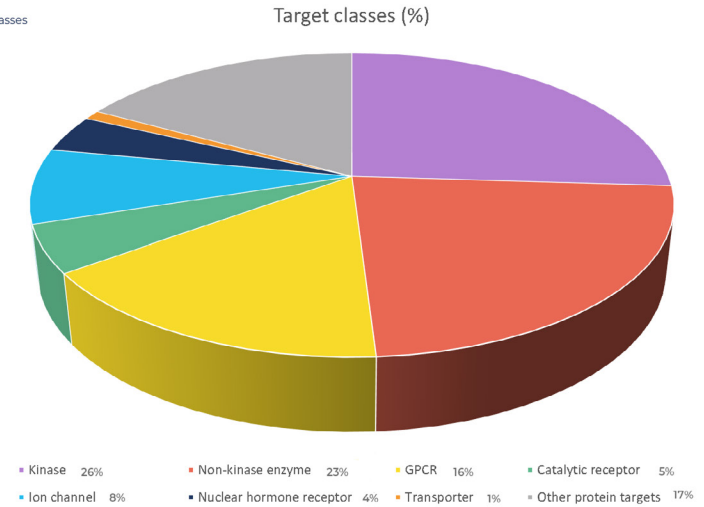


Figure 1 reflects the common target classes.

Which method of hit-finding to use?

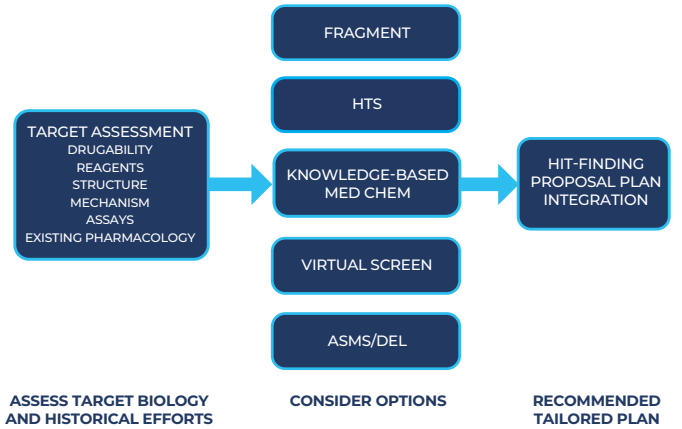
An analysis of the methods used to identify the starting points for launched drugs since 2016 was published in 2018[1] and recently updated in 2023[2]. Both analyses highlighted that the starting point for nearly half of the drugs in the reviews (222 in total) was a previously known compound, and almost a third came from high-throughput screening (HTS). Similar conclusions were also reached by Genentech/Roche for their internal projects[3] and for drugs discovered at Sygnature Discovery.

Given the time it takes to develop a drug, this almost certainly underestimates the value of more recently developed screening methods, such as affinity screening using DNA-encoded library (DEL)[4] technology or affinity selection mass spectrometry (ASMS)[5].

Nevertheless, it is clear that most new drugs came from a knowledge-based or HTS hit-generation strategy. Whether the recent focus on targeted protein degradation modalities will enhance the usefulness of affinity screening methods such as DEL or ASMS remains to be seen. However, it does seem likely that it will.

It is important to develop a strategy that uses the most appropriate hit-finding approaches. Depending on the budget, more than one technique could be used to maximize the chances of identifying a strong starting point for the subsequent optimization process. What are the benefits and challenges of the more commonly used approaches?(see figure 2)

Figure 2: General Hit-Finding Strategy



Knowledge-based design approach

Existing published ligands are required for the target of interest – or close homologs – when taking a knowledge-based design approach. A team of highly experienced medicinal and computational design chemists is key if a novel ligand structure is to be created outside of the currently published art. A significant advantage of this approach is that novelty might be found with only relatively minor changes to the published lead structures while maintaining their high potency and selectivity.

Ligand-based 3D virtual screening can sift through commercial databases of billions of molecules to find a novel scaffold that matches the relevant pharmacophore. These molecules will be commercially available or can be bought on a synthesis-to-order

basis. High-powered computational platforms enable these searches to be done quickly.

A proprietary protein structure can be created with a bound ligand. This offers a significant advantage, as it can underpin a program of structure-based drug design to improve ligand efficiency and selectivity, resulting in molecules with novel 'unexpected' properties. Novel IP can also be generated by exploring new physicochemical properties that might enable alternative routes of administration.

A project can typically start quickly, as reagents and assays will be commercially available. The translational journey to *in vivo* is likely to be fairly obvious.

There is, of course, a caveat: the original IP holders might be exploring the expansion of their initial series of leads, too, and may patent the chemistry you are working on before you file. Other competitors might also be taking a similar approach. A rapid Design–Make–Test–Evaluate cycle leading to an early IP filing will be critical to reducing the risk of being beaten.

Either way, a proactive, continuous assessment of competitive intelligence will be key if the project is to stay up to speed and respond to competing commercial activities.

Targeted Protein Degradation (TPD)

This is a variation of the knowledge-based approach. It takes a known ligand with affinity for an intracellular protein target of interest to generate a PROteolysis-TArgeting Chimera molecule (PROTAC, developed by Arvinas).

Here, the protein-targeting molecule is linked to a second molecule that recruits an E3 ligase protein, inducing a stable ternary complex. As a result, the target protein is first ubiquitinated and then destroyed by the proteasome.

TPD does not always begin with a known ligand binder of the protein target. Affinity screening methods such as DEL and ASMS can generate novel ligands as starting points. These may not necessarily directly modulate target protein activity.

There are likely to be fewer IP risks with these molecules as the field is so new. There are also potential advantages in efficacy, selectivity and, perhaps, even tissue targeting.

However, there are also potential downsides regarding the development challenges they pose, as they are likely to be beyond the Rule of Five (bRo5)[6] guidelines for the molecular properties of oral drugs. Nevertheless, eighteen PROTACs have entered the clinic and we are starting to understand their safety, efficacy and PK in patients[7].

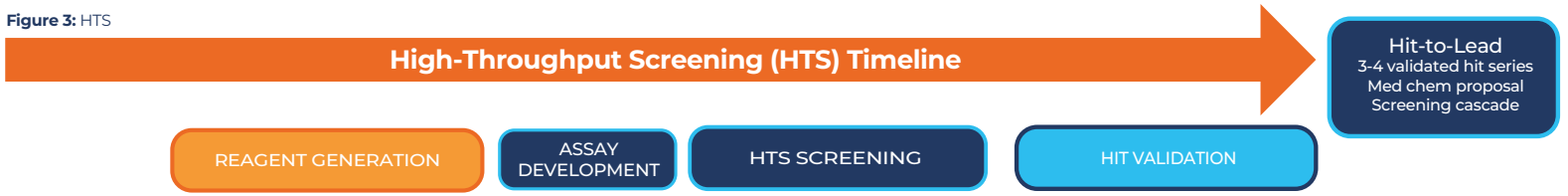
High-throughput screening (HTS)

If there is insufficient prior art for a target's pharmacology to enable a knowledge-based approach, then high-throughput screening can be employed instead. This could be applied to either an existing or a novel target modulation mechanism. (see figure 3)

Any HTS program will require very careful consideration of which assay–compound library combination that will most likely pinpoint molecules with the desired mechanism of action.

Several important factors are important for a successful outcome. An assay with a robust signal window will need to be developed and miniaturized, together with counter-screens

Figure 3: HTS



Time	3 months	1 month	3 months	3 months
Function	Protein science	HTS Group	HTS Group	Multidisciplinary integrated team
Delivery	Reagents for all assays in HTS	Primary assay Counterscreen Automation HTS screen ready	Delivery of hits with IC50 values Enrichment via computational chemistry	2-4 validated hit series Med chem HTL proposal Screening cascade
Compounds		Tool compounds	Solubilised compound libraries	New solid compound samples
Key activities	Production of project-specific proteins, cell lines, probes etc.	Existing assay transfer or de novo development	Primary & technical counter screen	Primary, orthogonal, and counter screens to remove interference compounds
	Cell, biochemical and biophysical assay reagents	Convert assay to automated mode	Computational chemistry – clustering and triaging	SAR by catalog, with some design & synthesis
	Production of reagents at scale for HTS phase	Test tool components	Order solid compound samples for hit validation	Broader profiling – chemical properties and *in vitro* DMPK

for technology artifacts. And, given the sheer number of data points such a screen will generate, a fully integrated informatics platform is essential to ensure data fidelity.

Most large pharma companies have now moved away from screening very large collections of historical research compounds, instead taking a more targeted approach. This relies on well-curated 'lead-like' diversity decks and sets of compounds targeting specific protein families, such as kinases GPCRs, or ion channels. Many HTS service providers still use historical drug-like libraries.

Hit validation and evaluation is a critical part of the HTS process[8].

It is not unusual for early hits to be artifacts resulting from unwanted properties such as fluorescent interference, compound aggregation or redox properties. A careful assessment of target engagement and mode of action is critical before an expensive hit-to-lead program is begun.

The final, validated set of hits can then be studied by experienced medicinal chemists. They will select promising

structures, hunt for close analogues in commercial databases, and carry out resynthesis and hit expansion programs to identify hit matter with a clear structure–activity relationship as the starting point for an optimization project.

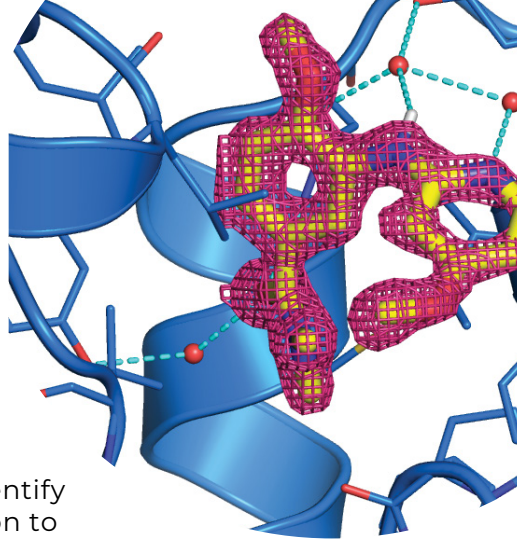
A successful HTS campaign relies heavily on generating reagents and developing automated assays. It is crucial that any external provider closely integrates these activities with both the HTS screen and the follow-on hit validation phase.

The number of protein structures in the public domain has increased exponentially over the past two decades. These structures and homology models, based on them, can provide a very cost-effective method of hit generation by high-throughput docking of commercial databases. They can also be an efficient way of augmenting or enriching an HTS campaign.

Structure-based drug design (SBDD)

Struture-based drug design has been greatly facilitated by a similarly exponential growth in computational power. While open-source software does exist for structure-based drug design, many of the most accurate, effective packages require licence payments. A good vHTS campaign may use several such tools to generate models, map binding sites, and dock or match pharmacophores. This will require buying these tools, or working with a partner that already has a broad set, along with experience in their application.

Generating a proprietary and novel protein structure is likely to present a significant competitive advantage when addressing a new, well-validated target. It also opens up the potential for structure-based drug design and fragment approaches for less druggable protein classes, including protein–protein interactions. Indeed, in a recent review of successful drugs making it to the clinic, 65% used SBDD[2].



vHTS has the potential to be a less expensive way of discovering novel ligands, particularly if there is a well-defined binding site that is known to modulate protein function. It can be particularly useful if there is limited screening capacity, as can be the case for phenotypic screens or complex, high-content assays. It is commonly used to identify compounds for an HTS screening collection to increase the chances of finding hits.

Fragment screening for biophysical, target-based approaches

Fragment-based drug discovery[9] relies on a range of techniques that detect the low-affinity binding of low molecular weight compounds to proteins or other biomolecules. These will either be immobilized or in solution.

Several techniques are commonly used:

- Surface plasmon resonance (SPR)
- Microscale thermophoresis (MST)
- X-Ray crystallography (XRC)
- Nuclear magnetic resonance (NMR)
- Differential scanning fluorimetry (DSF), also known as fluorescent thermal shift assay (FTSA)
- Chemoproteomics (protein MS) for reversible, covalent fragments

A relatively small library, of maybe 1000 to 3000 molecules, is often sufficient to identify fragment hits in screens for challenging, undruggable targets. This hit rate is much higher

than a standard HTS because of the low complexity of the fragments, which typically have a molecular weight below 300.

Their small size enables them to orient themselves to maximize productive contacts in a binding pocket. By limiting the number of atoms, a fairly broad swathe of chemical diversity can be covered with a few thousand molecules[10].

With a hit in hand, the next step is a careful structural expansion from that weak binding fragment, guided by iterative co-crystallization and taking into account lipophilic ligand efficiency. This enables an atom-efficient optimization of potency and selectivity, resulting in leads with good drug-like properties.

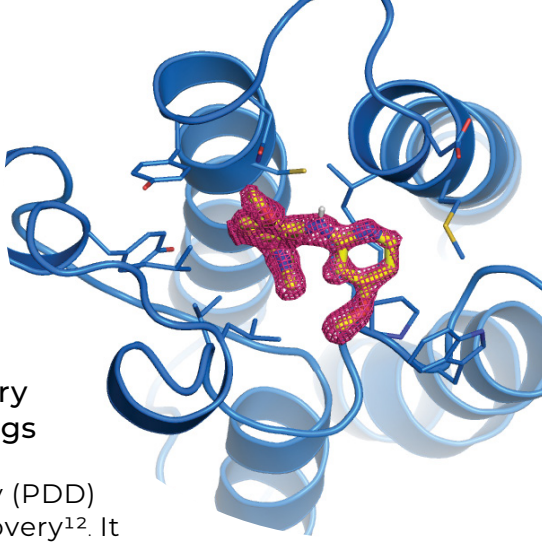
The caveat with this approach is, of course, that investment in a robust crystallographic system will be required. Also essential is patience in the fragment-to-hit phase, as early gains in potency are achieved without disturbing the rather weak initial binding affinity.

Covalent drug discovery

Over the last decade advances in covalent drug discovery have led to the launch of many successful drugs, for examples, inhibitors of EGFR, BTK and KRAS (G12C)[11]. Reversible covalent drug research is expected to grow as we continue to explore and optimise covalent ligands to obtain the right balance of target potency and selectivity across different disease settings.

A significant recent change in the reversible covalent approach has been use of covalent-first discovery strategies using covalent fragment screening with chemoproteomic analysis using protein MS – we expect this to continue grow and develop.

Traditional ligand-first strategies will still be highly relevant for making covalent drugs against proteins when existing reversible ligands are already known to bind near a nucleophilic amino acid such as cysteine.



Phenotypic cell assays for the discovery of novel targets, mechanisms and drugs

As a concept, phenotypic drug discovery (PDD) differs somewhat from target drug discovery[12]. It can be used to identify novel targets, mechanisms and drugs that would not be obvious via a classical molecular target approach. PDD is primarily used when the amount of available information about a molecular target is limited.

There are several requirements for a successful PDD project.

There must be:

- A disease-linked (patho)physiological functional HTS
- Natural product-derived high-complexity compound libraries
- Systems biology analysis tools
- Technology to deconvolute and identify the molecular target of the phenotypic hit

The attraction of PDD is that it uses empirical, target-agnostic screening processes to identify pharmacologically active molecules and novel therapeutics with unprecedented mechanisms of action.

However, this must be balanced against the under-defined scientific path forward for a novel mechanism of action. Those managing development portfolios will have to balance the potential advantages of a truly novel, and potentially game-changing approach from PDD, with the more streamlined and predictable approaches that take a mechanism-first approach. Whatever techniques are used, they can be applied in parallel.

Hit validation

Hit validation* is critical to any hit-finding approach and is a multi-discipline project specific process that aims to demonstrate that a newly discovered hit has a specific mechanism and a confirmed chemical structure[13].

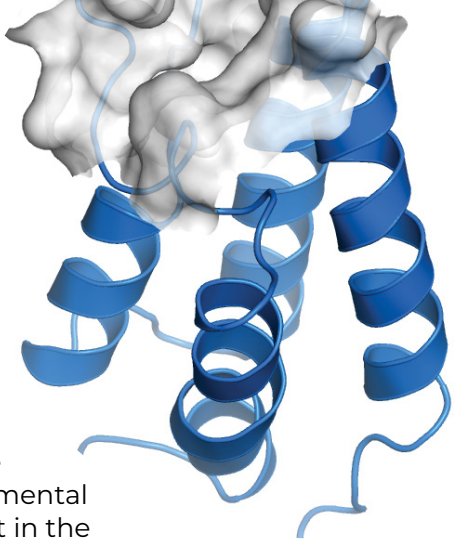
In general, demonstrating a hit modulates a target specifically involves the use of tailored screening cascades incorporating orthogonal, selectivity and counter-screens.

The structural validation of a hit, usually a member of a cluster of related compounds, can include resynthesis/repreparation, synthesis or acquisition of analogues with computational chemistry support, all to show early SAR and confidence in a structure-based target interaction.

The hit validation phase aims to deliver 2-3 different hit chemical series supported by medicinal chemistry optimization plans and a screening cascade to drive the project forward to lead optimization.

* Discover more about hit-finding in drug discovery: this blog provides insights on determining true hits, from our VP of Oncology Drug Discovery, Allan Jordan and Principal Scientists from our Assay Development team, Alex Stowell and Chris Tomlinson.

Trends in drug targets



As the past decades have made abundantly clear, scientific and technological advances have had a huge impact on the way we look for the drugs of the future. In the coming decades, further advances will, inevitably, change how they are found, too. So what advances – whether incremental or fundamentally new – might have an impact in the next few years?

Cytokine targets

While enzymes, receptors, ion channels and transporters have all been fruitful fields for novel drug targets, another is starting to have an impact: inflammatory cytokines. These cytokine targets, many of which have already been validated in the clinic with biological drugs, might prove amenable to small molecules, too[14].

Structure-based drug discovery is being used in tandem with HTS and DEL to underpin the search for novel small molecule modalities. There has already been some success with IL-17 and TNFa, for example, and this type of systematic search is likely to become more common in the coming years.

Challenging targets (undruggable targets)

Many readily accessible receptors with good binding sites have been studied extensively, so attention is increasingly drawn towards targets where a traditional drug–target interaction is difficult to achieve[15].

This is often because there is no well-defined pocket for ligand binding. Such targets include intracellular protein–protein interactions, transcription factors, RNA/DNA handling enzymes and chaperones, and even solute transporters. There have already been some notable successes for 'undruggable' targets such as RAS and Bcl-2, and many more might be expected in the future.

An interdisciplinary approach is required if the undruggable is to be successfully drugged. This will require a heavy emphasis on biology. As well as taking imaginative approaches to target interaction, assay development and protein production will certainly be more challenging. But all these new and powerful ways to address disease represent a tantalizing prospect.

RNA targets

RNA is increasingly of interest as a target for drug discovery, and many of the techniques that are established for protein-targeting therapeutics are applicable to RNA targets, too. There is no specific technology platform for RNA hit-finding per se: rather, an emphasis on handling and designing assays to detect RNA interaction and, ultimately, binding and function.

A drug that affects protein splicing, Biogen's Spinraza (nusinersen), is already available to treat spinal muscular atrophy. With numerous other RNA-targeting projects in the pipeline, many more can be expected to reach the market in the coming years.

Proximity-inducing drugs and molecular glues

As Protacs advance to and through the clinic, thoughts turn to what other approaches might be taken to protein degradation[16]. Various other heterobifunctional molecules are being investigated, designed to bring two (or more than two) molecules together so they can interact. And molecular glues[17] that stabilize protein–protein interactions in some way have huge potential.

The aim might be to induce protein degradation, such as the E3 ligases for PROTACs, but many other mechanisms could be possible[18], such as acetylation, glycosylation, or either phosphorylation or dephosphorylation or even RNA degradation. They could even be designed to induce autophagy or interact with the lysosome, for example. Many possibilities might be imagined, any of which might prove to be effective novel therapeutics in the future.

New technologies

It is difficult to predict which new technologies will power advances in drug discovery in the coming years, but perhaps the answers lie in new ways to use existing technology.

Affinity-selection mass spectrometry (ASMS)

ASMS is already proving its worth in the detection of new protein ligands[5]. It can identify via affinity all the sites at which a ligand binds, not just the active site.

The technique bridges the gap between combinatorial chemistry and molecular biology, accelerating the speed at which studies can be completed. It also provides an ideal way to identify ligands for linkage to design new PROTACs. (see figure 4)

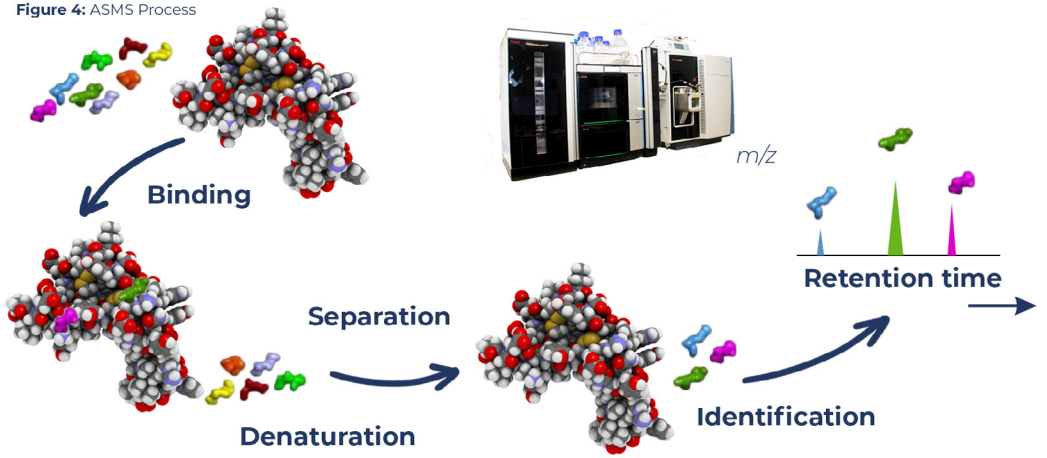
Label-free MS detection

Rather than relying on fluorescent and other tags for detection, LCMS can detect and measure metabolites and other analytes. Increasingly, enzymes are being studied that generate relatively small metabolites, particularly in the field of immunometabolism. LCMS is the only realistic way of detecting them. As well as removing the need for a labeling step, the rate of false positives is much lower[19].

Native MS

As well as measuring the binding of a compound to an individual protein, native MS also allows its interactions with associated proteins in a system to be assessed. This enables binding and function to be looked at simultaneously. It is particularly powerful for undruggable membrane protein targets and multicomponent systems that are difficult to unravel.

Figure 4: ASMS Process



AI in HTS and hit-finding

While AI is increasingly being investigated across the different phases of drug discovery, it is really coming into its own in the hit-finding arena. HTS produces vast amounts of data, so AI enables datasets to be integrated more readily and large amounts of data to be analyzed faster and more effectively.

Better computing power

The exponential increase in computing power, allied with significant developments in automation, is revolutionizing many existing techniques, ranging from HTS through to cryo-EM and high-throughput XRC. Better results can be achieved more quickly and economically and have a huge impact on hit-finding success. This is only likely to increase as computers get ever faster and cheaper.

Conclusions

The hit-finding stage of drug discovery is becoming ever more sophisticated, with multiple complementary techniques often used in parallel to improve the likelihood and speed of identifying a hit series. Longer term, the strategic integration of different approaches is likely to lead to faster, better results.

Choosing the right combination of technologies and techniques is essential to improve the likelihood of success. An experienced CRO will be able to help you consider all the screening techniques that are appropriate for each target and select those that are most likely to benefit for your target and desired project outcomes.

A positive result is far more likely if the project is run in a truly collaborative way, not least because it is an interdisciplinary endeavour. A knowledgeable CRO with a proven track record will work closely with you to find properly characterized hits in a timely way. If it has made thoughtful investments in curated libraries and automated technology, rapid delivery is more likely.

And, of course, all involved need to keep up to speed with emerging technologies for finding hits and best practice for advancing new target classes. This, underpinned by the expertise of an experienced team of drug hunters, will be key to success in finding new hits, targets, and medicines.

To discuss your hit-finding strategies, please contact the Sygnature Discovery team via our website.

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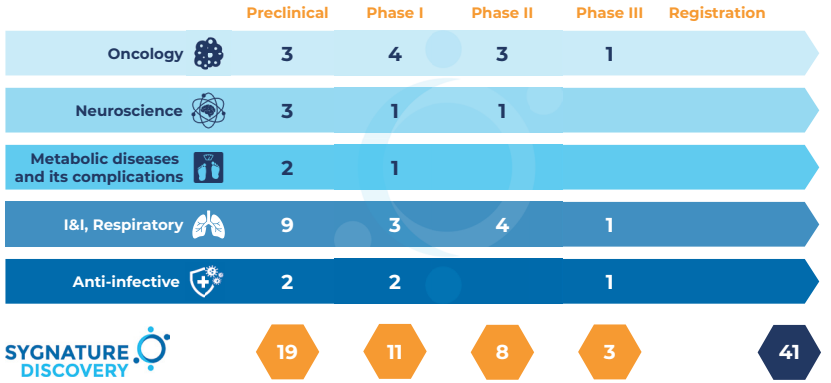
Sygnature Discovery is a world-leading integrated drug discovery and preclinical solutions provider, offering expertise across a broad range of therapeutic areas and biological target classes.

Figure 5: Sygnature Discovery's Track Record



Sygnature Discovery has delivered over 40 preclinical compounds and 22 clinical compounds. Sygnature Discovery scientists are named on over 170 patent applications.