SYGNATURE DISCOVERY

Lead optimization: Delivering molecules fit for preclinical development

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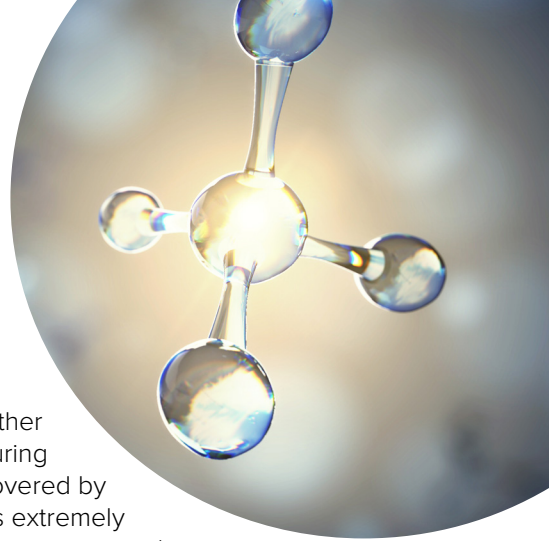

A careful lead optimization program is critical if a molecule is to progress successfully through preclinical studies and into clinical development. The initial leads that emerge from a hit-to-lead program, while they may show promising early signs of efficacy, may have features or properties that make them less likely to make a successful medicine.

A lead compound might not be particularly selective, making off-target effects or toxicities more likely to occur. The required dose might be too high, again increasing the likelihood of side-effects. It might need to be dosed several times a day, reducing patient compliance. Or perhaps there are structural features within it that might be best avoided, as they have shown problems in the past that may only emerge after long-term safety studies running alongside expensive early clinical studies.

The aim of lead optimization is to reduce or remove all such complications. Better selectivity, higher potency, an improved half-life and an absence of structural alerts would all make the molecule more likely to progress smoothly through preclinical development and into clinical trials, rather than failing on safety grounds before it makes it into a first-in-human study.

In a lead optimization program, there is no substitute for experience. Medicinal chemists and other drug discovery specialists who have been working in the field for years are well placed to know what to look for – and what to avoid. Making the best use of the modern tools at their disposal, allied to skills and expertise developed across multiple lead optimization programs in the past, will make success more likely.

Where do leads come from?

The leads that are the starting points for any lead optimization program can come from a number of sources. They may, for example, result from hits found in a screening program – either a high-throughput screen using library compounds, or from a virtual library. In each case, hit-to-lead chemistry will establish a structure–activity relationship, and there is the potential to create an optimized lead that could have good potency and selectivity against the target.

If the idea for the lead comes from a newly published program elsewhere, then speed is of the essence. Early discovery programs on novel targets are generally in stealth mode until such time as patent data is released, and the race is then on for competitors to find better alternative molecules that act at the same target. These 'fast follower', or knowledge-based, projects, add another requirement to a lead optimization program: ensuring that it explore novel chemical space that is not covered by existing IP. If the new target is promising, then it is extremely likely that several other companies will be looking at it, too, and insight into what else is going on in that space will be essential.

Success with a fast follower project will also be more likely if there is a good understanding of where the flaws in that competitor's molecule might lie so they can be avoided. It might involve making something with better selectivity, or which is safer, requires a lower dose, or perhaps something with fewer drug–drug interactions. Here, the key is chemical properties and off-target pharmacology, as the target biology has already been validated elsewhere. It's about finding something that might perform better in the clinic, and have all the requisite features to progress all the way to commercialization.

Target product profile

It is important to start out with the final goal in mind and develop a clear picture of what the eventual medicine would, ideally, look like. Design processes can then be implemented that take into account the molecular properties that are most likely to deliver that profile. If, for example, the aim is that the drug will cross the blood–brain barrier, it is unlikely to succeed if it is large and polar. Design efforts should be focused in appropriate molecular space.

Early absorption, distribution, metabolism and excretion (ADME) profiling capabilities are critical in ensuring the lead optimization program is going in the

right direction. Are the molecules behaving as predicted? And will this translate into an appropriate drug metabolism and pharmacokinetics (DMPK) profile and *in vivo* results? For example, its clearance properties will have an impact on the size of dose that will be required. A lower dose is likely to offer additional benefits such as reduced off-target toxicity, meaning that the best molecule is not necessarily the most potent, but one that gives good exposure over a prolonged period. Equally, for anti-infectives it will be important that a minimum concentration is sustained throughout the day to reduce the likelihood of resistant strains emerging.

Design–make–test–evaluate

The design–make–test cycle is at the heart of lead optimization. The medicinal chemistry team take the initial leads from the hit-to-lead process and use them as the starting point for designing new molecules they think might better engage the target without having off-target effects. Synthetic chemists will make these new molecules, and pass them on to the pharmacology team, who will run assays that determine their activity in the screen. *In vitro* pharmacology assays are key to assessing on-target interactions, but can also give an insight into off-target issues that might affect safety. The data these assays generate will be evaluated and passed back to the medicinal chemists to inform the next cycle. The cycle will repeat until a lead with acceptable activity in the screen is found.

Multiparameter optimization is another key concept for speeding up the cycle time. Instead of looking at different parameters separately, it involves working on multiple key parameters simultaneously. These include potency, selectivity and ADMET (ADME plus toxicity) properties.

Clearly, it should be faster if different parameters are considered together, rather than sequentially. But there are other advantages, too, as making changes in one area may affect others. And clues to optimization can often arise when outliers are spotted in the data.

There are many advantages in working in multidisciplinary teams for lead optimization. Rather than being siloed experts, all the scientists involved should have a good understanding of each other's areas, and the implications of results generated in their own areas for the others. And, importantly, it is a real advantage if they are co-located, rather than distributed across multiple labs that are hundreds, if not thousands, of miles apart.

This should, ideally, be allied to a collaborative relationship with clients, feeding data back to them and making joint decisions about the next steps for a program. Yet a less experienced client may need more support, and the CRO should be capable of leading the program alone if needs be. Either way, a governance structure should be in place to ensure all lead optimization decisions are made quickly.

Turnaround time on the design–make–test cycle is important, as this is what informs the next steps, indicating whether the design changes are taking the molecule in the right direction, or heading towards a dead end. If the latter, the sooner the chemistry can be refocused in a more productive direction the better.

Integrated services are key to shortening the design–make–test cycle. A good-sized team of experienced chemists who can solve synthetic route problems and make the molecules is the essential interstitial step between design and test. But if this synthesis is done a lab on one site and the testing elsewhere, that necessarily slows things down; if those facilities are on different continents, that can easily add at least a week, if not longer. In contrast, if the synthetic and analytical labs are in the same place, the biology results should be fed back to the medicinal chemistry team within a few days, ready to start the next cycle.

Drug metabolism and pharmacokinetics

The majority of the assays used in DMPK are industry standard, and it is the team's ability to interpret the data that provides the edge. Extrapolation from *in vitro* results generated in cell-based models to the likely performance *in vivo* is a key skill that relies heavily on experience. There are, of course, occasions where bespoke assays may be required, but either way, it's experience that drives the program forward, making decisions quickly and accurately based on the data.

There can be a temptation to carry out experiments incrementally, as this may appear cheaper and more efficient, particularly with a view to the amount of data generated. But in reality it can be more efficient to run multiple activities in parallel, and in a high-throughput manner, allowing molecules to be removed from consideration early on in the process if they are less likely to succeed.

With effective DMPK, absorption issues should be identified early on, potentially reducing the effort required later on for formulation. DMPK also links into toxicology, for example with drug–drug interactions, and off-target effects. Allometric scaling can also be used in combination with *in vitro* to *in vivo* extrapolation, as it can give an insight into how the drug distribution might occur in the tissues.

If there is an indicator that a reactive metabolite might be formed *in vivo*, perhaps an acyl glucuronide that might lead to hypersensitivity reactions, then an assessment of the potential risks going forward can be made. Metabolites may also have similar activity to the drug compound itself, thus could extend the effect of the drug in terms of its pharmacodynamics.

If a lead optimization program is able to give an insight into the likely dose that will be required, this can also be extremely useful. A drug that is likely to require multiple grams to be dosed to a patient every day is unlikely to be successful, for example. While exact dosing for clinical development will be determined in the preclinical stages, computer simulations are extremely helpful in giving a rough estimate of the order of magnitude of a likely dose, and whether this is feasible and practical.

Discover more:
Integrating human PK predictions and DMPK into lead optimization

The role of biology

Biology also plays an important role, taking the compound from *in vitro* assays to animal models and then into the clinic. Early on, it will show that the compound is engaging with its intended molecular target in situ in the right way, works in a cell system, and works in early *in vivo* studies. These animal models act as a platform to start thinking about moving into Phase 1 clinical studies.

Identifying and validating biomarkers, carrying them through from *in vitro* into *in vivo*, can be a huge contributor to success rates. Regulators are increasingly expecting some form of easily measurable clinical biomarker to be included as part of the regulatory package. Biomarker discovery may well include looking at more disease-relevant cell systems, such as those from patients, to show how the compound works in human disease cells.

Safety is, of course, also important. As well as a range of *in vitro* safety assays that will check for a whether a compound is likely to prove mutagenic or cytotoxic, which will be required to prove that as well as having efficacy, there are no safety red flags.

Once a promising compound has been identified, it is advisable to check it in different cellular systems related to the target, not just one. While a key cell type may underpin the project hypothesis, drug targets are generally linked to multiple cell functions.

Moving on to *in vivo* studies, in order to test a compound in an animal, it clearly needs to engage with the enzyme or receptor in that species, and in the correct way. Proving this in advance is critical if an *in vivo* model is going to be viable. This is important: while a compound may work well in a human cell model, it may not work at all in the mouse or rat equivalent, or it may do something else.

Phenotypic screens are experiencing something of a renaissance in terms of lead identification: running a target-agnostic screen to look for a compound that blocks a specific cell function. The assay will involve disease-relevant

cellular systems, or even more complex cell systems such as 3D models, organoids or even systems derived from induced pluripotent stem cells. While deconvoluting the compound's cellular mechanism to identify the molecular target is not required for regulatory approval, it can make screening simpler later on, and may even open up new opportunities.

When running phenotypic cell assays for lead optimization, it is important to ensure the cell line and assay is robust, which can be an issue if human cells from different donors are required, as it adds an extra level of complexity in terms of variation, with potential differences in the way the cells respond to compounds.

Discover more: *In vitro* to *In vivo* Translation in Lead Optimization: Bridging the Biology Gap

The role of computers and machine learning

Significant advances in computational methodology have already contributed to lead optimization programs across the board. In the design phase, ligand-based pharmacophore matching is a useful tool, while structure-based drug design using X-ray crystallography and cryo-electron microscopy can give insights into target binding, such as revealing hitherto unknown cryptic pockets. This opens up new opportunities for both potency and novelty.

In recent years, there has been a huge amount of hype about the potential of artificial intelligence and machine learning tools to revolutionise the drug discovery business. While AI programs are extremely good at generating large numbers of potential molecules, it takes an experienced chemist to triage them carefully. They will be able to pick out molecules that are sensible, whether from a synthetic standpoint or by avoiding moieties that might be expected to cause off-target effects, affect stability, or cause other issues. It takes human experience to reduce the number generated by the computer down to just a handful to focus on.

AI and machine learning can be a very useful way of generating chemical ideas, but in reality the real power lies in the combination of algorithms and experience. Technology cannot replace the human brain, but it can be a powerful tool in helping scientists get to the right molecule faster.

One area where AI/ML can be extremely valuable in lead optimization is in helping medicinal chemists identify new structures and help select druggable molecules, and even design potential synthetic routes to make them. A short, efficient route with the potential to be scaled up is a significant advantage in speeding up later stage development, if redesigns can be kept to a minimum.

Informatics is another essential tool. Lead optimization generates a huge amount of data, and an effective data handling and analysis capability is key to speed, and also to identifying outliers that, while they might not have the desired activity, might give an insight into, say, ADME properties. It might tell you which parts of a molecule to focus on next.

Beyond the Rule of Five

A dogma has appeared in recent years that suggests that a molecule is unlikely to be successful as an oral drug if it breaks two or more of the following guidelines: molecular weight below 500; no more than five hydrogen bond donors, no more than 10 hydrogen acceptors; and its ClogP (a measure of lipophilicity) below 5. In practice, while these represent a useful rule of thumb for creating a molecule with good drug-like properties, there are occasions when breaking the rules is advisable, if not essential. An experienced medicinal chemistry team will be able to navigate in "beyond rule of 5 space" by careful design of molecular properties that impact absorption.

Examples include macrocyclic drugs, such as some of the routine antibiotics. Erythromycin, which has a molecular mass of 733, is a good example. If the target is a protein–protein interaction, then it may be difficult to find a Rule of 5 compliant molecule that interacts effectively. Similarly, interactions that hide polarity for a membrane transition may need to break the rules.

Another field where non-compliant molecules are inevitable is protein degradation. This is a fairly new modality, where the drug is designed to hijack the body's own protein destruction mechanism to eliminate a disease-causing protein. These degrader molecules have both a high molecular weight, and a large exposed surface area.

Form and formulation

On-target pharmacology, off-target pharmacology and pharmaceutical sciences all have a role to play in giving a candidate molecule the greatest chances of making it all the way to the market, and early ADMET profiling allows toxicity issues to be identified. Yet there is a growing trend for the molecules emerging from medicinal chemistry programs to have issues with solubility: about two-thirds of small molecule drugs in clinical development have sub-optimal solubility.

This is a key property for a drug, as without aqueous solubility, bioavailability will be poor, however effectively it binds in lab studies when it is dissolved in polar solvents. For most indications, it is not enough simply to get sufficient exposure to show efficacy in a pharmacodynamic model – 10x the efficacious exposure will be needed to demonstrate a good safety margin in most indications.

Cutting corners adds time to the later stages, so it is well worth spending the time earlier on to optimise these properties, too. Once a candidate has been nominated, there is little that can be done to improve bioavailability other than these solubilization methods and other formulation tricks. But until that point, it is still possible to amend the structure to improve these properties. It is therefore important to consider the practicalities of delivery.

Solubility concerns will need to be addressed early on, as it may delay a molecule's progression into the clinic, or even cause it to fail completely. While this can be solved in the design process, in practice, if everything else ticks the boxes – good binding, good potency, and no obvious red flags in terms of toxicity –it can frequently be fixed at the formulation stage instead.

It can be as simple as finding another crystal structure: a different polymorph might dissolve better. If it contains at least one ionisable group, then the answer may lie in creating a salt form; if it does not, a co-crystal could work. If all else fails, an amorphous dispersion, or milling it into much smaller particles, could prove successful.

Later on, it is important to remember that the molecule will also have to succeed in *in vitro* safety screens if it is to progress into preclinical evaluation. Everything from Ames tests, Comet assays, Cerep screens and even QT elongation studies will have to be successfully navigated. Are there aspects of a molecule's design and performance in DMPK studies that might affect the success of these later assays? And what about when it moves into *in vivo* evaluation? For the drug, but also for potential metabolites, it is important to assess the likelihood of toxicity and other adverse effects.

The overall aim is to de-risk the molecule as early as possible, and not progress something that might prove to have toxicological or other liabilities that will cause it to fail later on, wasting time and precious resources. While there may be a temptation to delay some of the studies that might lead a molecule to fail, this simply leads to increased risk later on in the process. De-risking earlier might add more up-front costs but make great savings overall.

Discover more: How early access to drug formulation expertise prevents costly, foreseeable delays

And in the end...

At the end of the process, a nominated candidate should have a good chemistry profile, a good formulation profile, and there should be a good idea of what is likely happen when it is dosed to humans. The transition from lead optimization into preclinical, regulatory toxicology and safety assessment and then clinical development should be as smooth as possible if the lead has been optimized effectively.

As advanced leads are identified a scale up synthesis will be required to provide multi- gram quantities for *in-vivo* profiling. At this point if the chemistry route developed is process friendly and able to produce 100s of grams, rather than just a few milligrams it will smooth transition into development. This route would be suitable to take the program into non-GLP toxicity studies and early de-risking PK/PD studies. In an ideal world, it would also prove appropriate for further scale up, with just a few minor tweaks to make it process-ready.

There are real advantages in killing a compound that may be problematic early on. Attrition in early safety studies is a perennial problem, and so if potential problems can be identified early on, then a molecule can either be modified to fix them, or discontinued to focus on something with greater chances of success.

Once a molecule reaches the lead optimization stage, then potency and selectivity are being sought, but also some indication that exposure will be acceptable. It's all about balancing the molecule's properties and its ADME properties with potency and selectivity. Again, success is far more likely with an experienced team working on it. There should always be a rational hypothesis behind any changes made to a lead molecule, rather than simply making random changes in the hopes that they might work.

Experience within the chemistry, biology and DMPK teams combine to look at the molecule in the light of assay results and predict the liabilities it might have going forward. Market awareness of issues that have occurred with similar molecules and the same or similar targets elsewhere can also be extremely helpful.

Ultimately, the aim of lead optimization is to deliver a molecule that is ready for preclinical work, with biochemical and physical properties optimized to maximise its chances of success in the clinic later on. Drug discovery is a difficult, challenging process. A collaborative team of experienced scientists, all focused on finding ways to solve problems, is the key to creating strong molecules that move a project forwards. They will help each other – and the molecule – succeed.

To discuss your lead opimization challenges and to learn how a strategic, holistic approach contributes to successful project outcomes, 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.