

Safety-Directed Drug Design: Failures Are Successes

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Few, if any, products undergo more safety testing and scrutiny than a pharmaceutical. This, of course, is essential as the most critical issue with regard to pharmaceuticals is the risk benefit ratio. Whilst patient benefits can be quite clear in most cases, the risk of undergoing a treatment regimen must be as carefully and broadly quantified as possible. Often this characterisation results in findings that result in discontinuation or withdrawal. Thus as drug withdrawals and experimental therapeutics that do not fulfil safety criteria during preclinical and clinical trials are regarded as 'failures', they are actually successes for patient safety and for the science base of drug discovery. The lessons learned and the data gathered during this sometimes painful process is of critical importance in buttressing future efforts against similar problems.

The failure of a drug or clinical candidate is invariably associated with a massive amount of basic science that goes into problem-solving activities. This effort, if captured and integrated into the Discovery process, can contribute to the development of better and more sophisticated approaches to discovery safety assessment and meaningful refinements in the regulation of drug development and approval. Thus, the resources and momentum behind a problem solving effort, particularly around a late-stage clinical candidate present a unique opportunity to develop a more stable and valid science base upon which to build a more rational approach to discovery safety.

What does an increase in the sophistication of a safety science base look like and to whom should it be directed? The choice of therapeutic target is one critical area and many advances have been made in recent years in the characterisation of a protein's role in various tissues. This 'target safety' aspect is not a one-time exercise to be carried out at the beginning of a project, but, as the recent experience with Vioxx has shown us, a constant vigil to relate all aspects of the complicated life of a therapeutic target to the adverse event signals coming from our preclinical and clinical studies.

THE ROLE OF THE MEDICINAL CHEMIST IN SAFETY

It is the area of chemical design that perhaps the most value can be gained from translating safety data to real, tangible decisions. This is a long, tedious process but several successful examples have been identified in which a valid set of decision-making tools can be used to warn for chemical designs that contain inherent safety liabilities. In some cases this can lead to decisions before synthesis is even undertaken. In other cases the level of confidence is lower, but the tool can spark the decision to do further experiments in more sophisticated model systems to investigate the probability of a real safety problem. The key to either of these scenarios is first, a clear strategy behind the decision-making tools such that results from a simple test (SAR, QSAR, in vitro) can be followed up and confirmed in a relevant in vivo test and second, adequate throughput to facilitate iterative design. Without this confirmation mechanism very little about the *real* risk of a compound or series can be concluded and without adequate throughput the medicinal chemist will be mired in indecision.

What kinds of safety-related decisions can be made by the medicinal chemist? There are actually many. First and foremost one should always be cognisant of the area of chemistry in which one is working, the therapeutic class of compounds, and any similarities they may

have, both in their basic structure and in their pharmacophoric makeup, to compounds with known pharmacologies. Polypharmacology, whilst desirable in some special cases (1) carries the potential to add an unnecessary burden to the drug development project and in many cases, can be identified and avoided by early searching of pharmacology datasets like PubChem (2), GVKBio (3) or BioPrint (4) or by performing broad 'secondary pharmacology' screening at highly skilled and efficient contract organisations like Cerp (5) or MDS Pharma (6). This sort of characterisation hardly ever leads to the decision to stop a chemical series, but rather aids in the identification of potential problems early enough to guide confirmatory experimentation (monitoring of blood pressure in early preclinical species for compounds active at the alpha1 adrenergic receptor, for example) and allows chemistry to be changed such that liabilities can be designed out if they are judged to be serious enough.

A specific case of unwanted pharmacology, of course, is activity at the cardiac hERG K⁺ channel. Potent blockers of the hERG channel can be found in most therapeutic classes and thus few industrial medicinal chemists have escaped this problem entirely. Fortunately, there have been many advances in our understanding of hERG specificity and the physicochemical properties that drive hERG blockade. Homology models of the channel are now available and scores of papers have been published on hERG structure-activity-relationships (7). Whilst building away from hERG blockade while retaining all other properties remains a huge challenge, the combination of better foundation tools such as SAR and protein models, combined with enhanced screening technologies (8) and a constantly improving picture of the preclinical and clinical testing systems required to assess the ultimate risk of fatal arrhythmias (9) has dramatically improved our ability to successfully manage this once-difficult problem. As mentioned above, high resolution preclinical and clinical studies on causal mechanisms of safety problems allow for identification of the chemical events underlying these mechanisms.

Blockade of hERG is one of these cases and now medicinal chemists, armed with the right tools, can in most cases successfully build out hERG liabilities. A list of the full compliment of hERG-related tests is given in (10).

A few simple rules for avoiding hERG activity

Avoid extended cylinder-shaped molecules with hydrophobic cores at the ends

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Avoid amines with a pKa above 7

*

Avoid compounds with logP or logD values over 2

In most cases, potent activity at the hERG channel is a 'show stopper' for the project and it must be addressed. Another such endpoint is genetic toxicity. As with hERG (11), genetic toxicity (12) testing is part of a mandated set of tests that must be performed before humans are exposed to a new drug candidate. As such it must be effectively dealt with in the early phase of a project to avoid a significant issue later in the more mature project. Several very effective tools are available for the medicinal chemist that range from structural warnings (13,14,15) to SAR and QSAR models with reasonable levels of predictivity (16,17,18,19). Screens for genetic alterations also come in a number of types with the most common for drug development being the Salmonella reverse mutation, or 'Ames' test and the Mouse Lymphoma assay. Several other genetic toxicity test exist and they are listed in (20) along with their intended use and, most importantly, a current view on their ability to actually predict carcinogens.

A few simple rules for avoiding Ames activity

Avoid reactive species

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Avoid aromatic amines or compounds that give aromatic amine metabolites

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Avoid compounds that can produce other forms of reactive metabolites

As the last point above illustrates, reactive metabolites are a problem for drug development. Reactive metabolites (21) can either be relatively 'silent', e.g., though present in significant amounts and not cause overt problems in preclinical and early clinical studies, or they can be more direct and thereby be clearly associated with organ-based toxicities. Silent reactive intermediates like those associated with halothane have the potential to bloom into quite serious problems in the clinic as more and more patients are exposed and may become apparent only after years of use in patients. These reactions are sometimes labelled 'idiosyncratic' although in reality the seeds of a serious safety problem were already sown by the medicinal chemist. Direct, organ-based toxicity by compounds producing reactive intermediates, such as paracetamol, is also possible and is usually easier to detect early because they cause a more dose-related lesion that is seen across species and in nearly all individuals exposed. What about those compounds that produce reactive intermediates but have not been shown to produce either direct or idiosyncratic toxicities? Do these represent a set of compounds or lesions that are benign or are they examples of the safety issues of the future when enough patient experience have accumulated and/or enough overdoses have occurred? It is important for medicinal chemists to appreciate that we do not know! Thus prudence, both to avoid unnecessarily burdening the project and to act responsibly towards our future patients would dictate that we identify those functional groups that can give rise to reactive metabolites and at the very least be aware of them and preferably avoid them. Awareness can also aid the interpretation of safety studies when unexpected pathologies are observed. A catalogue of *in vivo* pathologies that have been associated with reactive intermediates is a very useful tool to be used in combination with a thorough library of the functional groups and substructures that can give rise to reactive intermediates.

A few simple rules for avoiding reactive intermediates

- Actively use a 'warning' substructure list *
- Characterise biotransformation as early as possible *
- Look for warning signs: toxicity in genetic tox assays using metabolic activation, time-dependent inhibition of P450 activity, cytotoxicity in P450-expressing cell lines (22) *
- Assess degree of binding in combination with the intended dose. The higher the dose, the greater the reactive metabolite burden

What advice can be given to the medicinal chemist wishing to assess structures for liabilities outside these few areas? Online structure toxicity resources such as ChemIDPlus (23), IUCLID (24), TOXNET (25) and DSSTox (26) can complement the commercial information/model tools such as MultiCASE (27) and DEREK (28). A recent, potentially useful addition to this group is the Mechanism Based Toxicity database from GVKBio (29), a hand-annotated, structure-searchable database of reported toxicities. The current version of this database contains over 13000 entries.

In vitro tests are too numerous to list here, but several new and useful test have recently been reviewed (30). The general trend is to move away from gross cytotoxicity assays in the target cell/tissue type and to instead focus on either non-lethal measures in target cell types or measurements of general interference in basic cellular processes via the monitoring of several endpoints simultaneously (31). Additionally, recent examples of promising applications of toxicogenomics give hope that this technique will finally begin to live up to the initial promise (32,33). There is an almost universal desire to enhance the predictivity of early toxicology screens and this has led to a number of products and initiatives to improve

the general area of 'Predictive Toxicology'. But do we have the patience to validate these methods?

PREDICTIVE TOXICOLOGY: THE TWILIGHT ZONE

There are several promising technologies that are commonly lumped under the heading of 'predictive toxicology' and are, naturally, under varying stages of development and validation. Often one is exposed to these approaches and technologies too early in their development and a generally negative and sceptical attitude develops toward all predictive toxicology approaches. This is unfortunate as many of these are indeed sound basic ideas that suffer from inadequate validation and testing and/or are used for applications outside their originally intended purpose. Thus one can lament the failure rate of drugs and drug candidates, but the failure rate of predictive technologies is, in practice, probably many times higher. That said, why engage these at all? Because some of them actually appear to yield useful information, particularly if enough data are accumulated within a single chemical series to make series-specific structure-toxicity-relationships. The same risk-benefit argument that is used to justify embarking on a drug treatment can be used when committing to the exploitation of a 'predictive toxicology' technology. It may not work for everyone in all cases, but the risk of failure is worth the benefit of enlightened chemical design and the potential of project progression and/or competitive advantage. Also, the effectiveness of a new method is only truly tested with an adequate (large) number of examples; examples that may take years to accumulate. Few organisations possess this level of patience and thus the demise of potentially useful predictive toxicology approaches is usually swift and unfortunately premature.

WILL IT HELP?

Those who choose only the tried and proven methods of discovery safety assessment are in danger of missing true opportunities in the technologies of the predictive toxicology twilight zone. Conversely, those who do not fully exploit the 'fundamental' methods as a foundation upon which to base a diversity of approaches may not only be doing themselves and their organisations a disservice, but they are also neglecting the fact that toxicology data, whether it be from animals or from humans, has been produced through studies designed to explore the edges of tolerability of a drug and thus a certain degree of adversity accompanied producing the data in the first place. With that consideration, as a species with a capacity for ethical behaviour, we owe it to ourselves and our fellow species to use as much toxicology data as possible to inform and improve as many aspects of our early drug discovery decisions as possible.

With the goal of integrating as much of this rapidly expanding sophistication into chemical design, the potential for *reducing* safety-related failures exists, but is far from certain. One certain effect will be that promising compounds will fail less often for the reasons of yesterday. Rather, the problems of the future will be qualitatively different, but if we have done a conscientious job in addressing the problems of yesterday, we will free up more time and resources within our safety groups for problem-solving around more of tomorrow's problems. That, despite the real potential for continued failure, is progress and thus a success in itself.

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