SCOPE AND DEFINITIONS

According to its aim of being a leading source of concise knowledge of structural alerts for reactive metabolite formation, the expert database SpotRM provides several attractive features:

- A comprehensive set of structural alerts, currently numbering 80, for reactive metabolite (RM) formation that you can match your planned test compounds against.
- Facile and unprecedented access to the most relevant background information on RM mechanisms without you having to sift through piles of scientific articles; currently, there are 140+ short summaries/monographs and seven micro-reviews. This readily available material should vastly strengthen your knowledge base for ranking test substances.
- Continuous updates of alerts, drugs, experimental compounds, and new references keep you at the knowledge front-line.

Designed as a didactic tool, SpotRM with a solid knowledge base will hopefully lead to a deeper understanding of potential toxicity risks with planned test compounds and thus result in a more efficient lead selection and optimization. Background is further discussed in this perspective paper: A Claesson & A Minidis. *Chem. Res. Toxicol*. 2018.

Disclaimer

We caution that users of this tool should appreciate the large limitations of our current understanding of structure-toxicity in the area of RMs. One should be careful not to under-estimate the difficulties involved. This regards several aspects: which reactive species are actually formed, the ways of their formation, and in particular what linkage there is of a certain transformation to the observed adverse effects. To add to this, it is generally agreed that many drugs have side-effects caused by multiple types of metabolic conversions. Most importantly, many of the adverse effects mediated via RMs are idiosyncratic and hence unpredictable. The majority of this type adverse reactions appear to be immune mediated.
A few general considerations regarding drugs with toxicity issues emanating from RMs are found in the end of this document.

Classification of drug examples is based on perceived severity of adverse drug effects

In the SpotRM database all alerts are linked to selected examples of drugs that have the alert in their structures, which far from guarantees its association with major adverse effects. The drug examples have been chosen to reflect in a didactic way on a certain structural alert while avoiding too much speculation on alternative examples. Drug structures with several potential structural alerts are therefore largely avoided although we do not hesitate to propose hypotheses.

For a rapid visual impression of the hazard associated with a given alert, the drugs are classified as Red, Yellow, Green or Neutral. Definitions of these classes are:

Red
The drug has shown clinical adverse effects that have a proven or highly probable association

with activation to RMs or, regarding preclinical compounds, experiments have shown extensive formation of RMs, usually demonstrated by GSH trapping.
A drug used clinically has been withdrawn or bears regulatory warnings.
Examples: *sudoxicam, felbamate, amodiaquine, sitaxentan, lamotrigine* and *halothane.*

Yellow
The drug contains an alert included in SpotRM, and there have been some reports of adverse effects that have been discussed in terms of RM formation.
Preclinical compounds have displayed RM formation.
Examples: *mirtazapine, paroxetine, atorvastatin, tolmetin* and *phenazone*.

Green
Despite of having triggered a RM alert, the drug has been used clinically without reported findings of adverse effects that can be associated with RM formation. The explanations for vindication include low typical dosage or a very low degree of metabolism that involves the relevant substructure. The last-mentioned case often depends on metabolism in other parts of the molecule.
Examples: *rivaroxaban, cefuroxime, aripiprazole* and *tolterodine*.

Neutral
In spite of having a RM alert in the structure the clinical information is insufficient to classify the drug into any of the other categories. This might be because the drug has only recently been introduced or is not widely used, and therefore sufficient safety data have not accumulated. For experimental compounds the relevant in vitro experiments might not have been run.
Examples: *formoterol, fluorofelbamate* and *ranolazine*.

Bioactivation is required, chemical reactivity per se is excluded

SpotRM, with very few exceptions, focuses on drugs that require metabolic activation to form reactive intermediates. It is well-known that there are quite a number of drugs on the market that give rise to toxic effects, including hepatotoxicity and IADR, by being chemically reactive per se. This type of intrinsically reactive compounds are normally filtered away early in the drug design process but might occasionally have beneficial effects. Examples of important drugs of this class range from beta-lactams, like amoxicillin, to cytotoxic anticancer agents, for example busulfan. Intriguingly, such intrinsic reactivity has since several years been a focus of new kinase inhibitors used for cancer treatment; here, a specific amino acid in the active site is targeted with help of unsaturated carbonyl compounds.

For a metabolic intermediate to interfere with certain proteins, it does not have to be a reactive electrophile or radical: for example, we have also included the infamous, simple chemical fluoroacetic acid, which blocks the enzyme aconitase in the Krebs cycle by tight binding after having been converted into its CoA derivative, an example of ‘lethal synthesis’.

Cytotoxic anticancer agents are largely neglected in SpotRM

A large number of anticancer agents require metabolic activation to achieve *cytotoxic therapeutic activity*, a principle that entails many well-known and feared side-effects. For the purpose of SpotRM, which is to help spotting hidden alerts in “normal” drugs, this kind of overt toxicity is

of minor interest and therefore the majority of these drugs, for example dacarbazine and cyclophosphamide, have been excluded. Nevertheless, some anticancer agents may well offer guidance regarding what safety-focused medicinal chemists ought to keep out of their drug design tool-box. We have tried to incorporate some of this type of learning in SpotRM.

A comment on irreversible CYP enzyme inhibition

Many drugs interfere with the metabolism of other drugs, causing drug-drug interactions. This can take place in several ways, but the most common mechanism, which is also relevant in the current context, is irreversible inhibition of a CYP enzyme that is responsible for metabolizing another drug. The most common mechanism of such inhibition is via a reactive species that is formed *in situ* at the enzyme's active site, also called mechanism-based inhibition (MBI). The broader concept of time-dependent inhibition (TDI) can also occur via other mechanisms. Many drugs that have a reported significant evidence of MBI are included in SpotRM, since effects collateral with RMs formed *in situ* most often cause other types of toxicity. However, there is a need to systematically include even more drugs that inhibit CYPs; future updates of SpotRM will gradually fill the gap in order to ensure comprehensiveness.

Selection of alerts - focus on the less well-known

It is a delicate task to select the really useful substructures (RM alerts), which will be represented by a well-written SMARTS string for matching in target structures. The dilemma can be illustrated by including a string that corresponds to a benzene ring with nitrogen attached to it. This would encompass very many drugs. We have instead chosen to split this substructure class into many smaller classes; a few examples are indoles, *o*-methyl-benzeneamines, N-acyl-benzeneamines, benzimidazoles, and more. In this way it is possible to fine-tune the examples that are most appropriate for the input structure.

SpotRM is not complete regarding reported examples of RM formation: the more widespread the knowledge of the hazard of a certain substructure is the less is the marginal gain to the user of having it included. Many alerts in SpotRM are of this type, for example a nitroarene, which is more or less taboo in drug candidates of today though we choose to include several examples of this for the sake of reasonable comprehensiveness. Regarding the number of examples of drugs (in the red/yellow/green/neutral scale) we find less value in listing in SpotRM all the drugs that are anilines (including "hidden" anilines), which have displayed hepatotoxicity. Besides, many of these are hugely obsolete. On the other hand, safe anilines and an explanation as to why they may be considered safe are certainly within the scope of SpotRM.

In line with the notion of SpotRM not being comprehensive: the exploding drug class of kinase inhibitors, which from a RM perspective contains many dubious members, has not been given full coverage even though new ones can exhibit clear structural alerts. Kinase inhibitors are also fraught with complicated pharmacology.

To increase the usefulness of SpotRM we have made considerable efforts to include as alerts substructures that have not been mentioned explicitly as such in the literature but probably have contributed to adverse effects. In particular, our selection of alerts focuses on the less well-studied hazards of certain combinations of ring systems and substituents. Here you may find, for example a range of substructures that ultimately result in various types of *activated benzylic alcohols* such as derivatives of 2- or 4-methyl-benzeneamine, or five-membered heterocycles having alkyl groups that can be oxidised to (benzylic type) alcohols.

Since there is a huge lack of exact knowledge of how adverse effects might be associated with RM formation, we have been generous with hints of potential compound liabilities and offer many hypotheses regarding less obvious reaction pathways based on sound chemical reasoning and guessing of routes of metabolism. Our sincere hope is that our users will be involved in the continuous improvement of SpotRM by communicating their points of view to us.

Delimiting an alert/toxicophore is a delicate task as well. We hope to work together with the users of SpotRM in a process to refine the SMARTS definitions and the linking of alerts to the most illustrative example drugs.

General considerations regarding drugs with toxicity issues

The number of drugs that have been on the market and then withdrawn due to *toxicity associated with their metabolism* is frustratingly large, well over one hundred. The dominant problem here is metabolic activation to form *reactive metabolites* leading to modifications of proteins and DNA, or to inexpedient influence on oxidation processes. Since this kind of toxicity exhibited by candidate drugs usually leads to termination of their development, early identification of compounds that likely exhibit a risky metabolic conversion cannot be stressed enough (*cf.* reviews collated here). It is our hope and belief that SpotRM can contribute in facilitating the process of paying proper attention to these drug structures.

The precise biochemical mechanisms of adverse events of many drugs, in particular no longer-marketed ones, have rarely been investigated. However, in many cases involvement of RMs might be considered when the clinical symptoms involve:

- Hepatotoxicity (drug-induced liver injury, DILI)
- Effects on blood cells (blood dyscrasias), e.g. agranulocytosis
- Anaphylaxis and hypersensitivity reactions, which are immune-mediated; they most often manifest as cutaneous reactions
- Carcinogenicity
- Mitochondrial toxicity
- Drug-drug interaction caused by time-dependent inhibition of cytochrome P450 enzymes (via mechanism-based inhibition, MBI, a.k.a. time-dependent inhibition, TDI)

Furthermore, many unknowns are involved in estimating the clinical consequences of RM formation since the reasons for discontinuation of clinical trials are often unknown to outsiders. Such terminated trials also naturally produce limited amounts of safety data. Additionally, it is important to keep in mind that experts generally consider side-effects of licensed drugs to be underreported.

In SpotRM we try to draw examples of drugs from the first four of the above-mentioned types of clinical adverse effects. Naturally, our conclusions on drugs where the literature is scarce (as hinted, a very common situation) should be regarded as provisional hypotheses.

Another growing set of compounds consists of those described in the medicinal chemistry/ pharmacology literature with *in vitro* data on adduct formation with trapping agents, usually glutathione. Illustrative examples of such compounds are included where pertinent. Updates of SpotRM will include even more examples of this type.

Abbreviations used within SpotRM

ADR, adverse drug reaction; AE, adverse event; CYP, cytochrome P450; DILI, drug induced liver injury; DRESS, Drug reaction with eosinophilia and systemic symptoms; EMA, European Medicines Agency; FDA, the Food and Drug Administration (USA); GSH, glutathione; GST, glutathione transferase; HLM, human liver microsomes; IADR, idiosyncratic adverse drug reaction; MAO, monoamine oxidase; MBI, mechanism-based inhibition; MoA, mode of action; RLM, rat liver microsomes; RM, reactive metabolite; SULT, sulfotransferase; TDI, time-dependent inhibition; ULN, upper level of normal.