Challenge 18: Targeting off-targets Surgery Q and As

Q. Could uncharacterised/unstructured receptors be considered?

A. Yes if they are relevant from a toxicity perspective, but Sponsors would need to know the level of confidence in the prediction if receptors are not fully characterised

Q. Are there a particular set of adverse outcomes that are of particular concern to your industry? (e.g. cardiovascular risks are of more concern in pharmaceuticals)

A. Yes, for example local skin effects, or other relevant routes of exposure (e.g., oral, inhalation). For risk assessment Sponsors consider the exposure scenario and how the product is used. If the exposure assessment suggests that it is systemically available, there will be further implications and therefore broadly the same organ target toxicity as for pharmaceuticals is investigated. For agrochemicals, common targets include liver, kidney, thyroid, and testes.

Q. In terms of using a QSAR approach to link chemicals to a biological outcome, there is adverse event reporting for pharmaceuticals, but is there similar data documentation for chemicals that could be used to develop a QSAR?

A. Yes, cosmetovigilance is equivalent to pharmacovigilance but data is very limited. For agro-chemicals there is limited human data available and therefore it is difficult to link exposure to an adverse outcome in humans as the data is predominantly epidemiological. In this project we want to link any predicted adverse outcome to human relevance.

Sponsors could provide some data, but applicants would also need to check what data is publicly available. Publicly available human and animal data is limited but there is a raft of *in vitro* data available. What Sponsors want to achieve is an understanding of an interaction at a particular receptor at a quantitative level to ensure application in a real life risk assessment context.

Q. *In silico* modelling. This is mentioned in Phase 1 but is there also emphasis on *in silico* in Phase 2?

A. We envisage a comprehensive model backed up with data (this can be *in silico* or *in vitro*). In Phase 1 we would want a reasonable amount of data to support the model and indicate that the approach is likely to be successful. More data will be required in Phase 2 to provide the model with greater breadth and validation.

Q. What comprehensive models are currently used to characterise receptor binding to substances?

A. For *in vitro* assays there is a reasonable amount of data publicly available (e.g. ToxCast). For *in silico* approaches, there are docking studies, but there is currently not much confidence in this because of the training sets used in the algorithm are not relevant to our chemical space or they are not designed to be used in the single ligand-multiple receptor mode.

Q. What level of readiness for an *in silico* approach would be needed to launch this project?

A. Sponsors would need some kind of confidence in meeting the Phase 1 deliverables provided by

some worked examples of chemicals/receptors.

Q. Are you primarily looking for a computational model?

A. Not necessarily. *In vitro* models may be acceptable.

Q. What kind of *in vitro* model are you looking for? E.g. Organotypic?

A. Sponsors don't know yet – whatever kind is necessary to link the Molecular Initiating Event (MIE) to the adverse outcome. We ultimately would like to consider biological adaption vs. adversity but identifying the most appropriate pathways is a first step in achieving the aim of being able to conduct risk assessments based on mechanistic information.

Q. What level of data can you share for your animal models?

A. Currently, all agrochemicals have an extensive data packages. e.g. four species with full toxicity packages including acute, chronic and carcinogenicity data, but there is limited gene expression data from these same studies. Sponsors would be happy to share data to help with validation. However, publicly available receptor binding data e.g. ToxCast, ChEMBL may be more useful. Sponsor data could be used for validation purposes to prove the assumptions made from publicly available data. It may be appropriate to partner with data providing companies to strengthen the team. It will be important for applicants to focus on linking the MIE to the adverse outcome.

Q. How involved are Sponsors in Phase 1?

A. Sponsors want to be as involved as possible and are fully committed to a collaborative project

Q. What about the emphasis on discovery of new targets?

A. Ideally focus on known receptors initially and then potentially add new targets to characterise later otherwise the project becomes too expansive. It would be appropriate to begin by looking at public data sources (e.g. ToxCast) and the targets that they focus on.

Q. In terms of developing a representative panel of receptors, how do we determine what is a necessary size?

A. Sponsors need a breadth of receptors that represent the diversity of biological interactions to identify the adverse outcome of concern. Sponsors need higher confidence (lower risks) in the targets being hit as well as the unknown targets. However, it will be impossible to test for everything; models need to be pragmatic. A combination of *in silico* and *in vitro* approaches is most likely to be successful. A smaller range of receptors with high confidence would be preferred to a large number of receptors and low confidence in the results.

Q. Skin exposure - would it be useful to explore dermatology- kinetic models to consider exposure?

A. Exposure is not a key aim or a focus of the Challenge, but would be nice as a bonus.

Q. From a gene pathway analysis perspective, what tools are you currently using?

A. This area is new to Sponsors, so we are not familiar with the tools available.

Q. If we are considering exposure in humans, presumably heterogeneity in human genotypes and phenotype should be considered. What about sensitive populations?

A. Sensitive populations and consideration of intra-species variation are very important for the Sponsors. The beauty of working at the MIE level is that the knowledge can apply to multiple species/populations where the biology is conserved. This brings in the question of how much confidence we have in the prediction.

Q. What level of throughput is needed?

A. A very low level of throughput screening is needed. Unilever are looking to put <100 chemicals per year but this has a potential to increase if we have the right screen. However, Unilever is just one company and there is wider application for screening tools of this kind outside of Unilever and in other sectors. Lower throughput allows you to take more time to characterise the interactions more thoroughly and to potentially build in higher throughput in the future.

Q. What metric are you trying to predict?

A. An *in vitro* receptor screen which provides an IC_{50} can currently be used in decision making so something along these lines is sought. The predictive tool needs to be quantitative and deliver value(s) that can be used to make a decision.

Q. Will relative information work (rather than being fully quantitative)?

A. Relative information on a panel of receptors would be useful as long as the comparisons are transparent.

Q. Is there a scale of what types of adverse outcomes are of more concern? e.g. a scale of severity?

A. This is something that could come out of the project. We may find that we get scales of severity within different receptor types. We need a quantitative aspect within this challenge and comparative benchmarking may work to achieve this.

Q. Is the MIE or adverse outcome more important?

A. A balance is needed for use in decision making in a risk assessment context. A potency scale is important and will be different for different receptors. We are seeking to understand adverse outcomes on a mechanistic level to be able to predict toxic pathways and the MIE may not always be identifiable, but in most cases it is a good anchor for prediction hence the focus of this challenge

Q. Presumably we would want to test the receptor in a range of cell types (as this may affect the adverse outcome). What types of cells should be tested?

A. This would need to be considered during the project, but cells of human relevance will be important.

Q. Also, which organ types should be considered, as different receptors are expressed to differing extents in different organs? What data do the Sponsors have on this? Are there relevant cell lines to test?

A. This will require the use of publicly available data alongside your own experience and expertise. We would recommend using whichever cell lines are needed to give a high level of confidence for prediction in humans. This could be dependent on what is in the publicly available data or is widely accepted.

Q. Have you got high quality structural data on the receptors? We would need this to build *in silico* models.

A. No, we don't know all of the receptors we are interested in yet.

Q. What is the time-frame for the *in silico* approach?

A. Phase 1 is six months (starting late December 2014). Sponsors would need proof of concept within this time.

Q. How many receptors would be required?

A. Using an *in silico* approach to replicate the recent Bowes paper (Bowes et al, 2012) would include 45+ receptors. This would be a good place to start as there are currently no reliable predictive tools to cover these. However this paper was published from the pharmaceutical sector rather than a chemicals sector perspective. The number of receptors for the final tool is undefined - it will depend on the approach as to how many are feasible and can be predicted confidently.

Q. What is the diversity of your small molecules?

A. Sponsors have a diverse list of compounds which we can share with applicants during Phase 1. Sponsors would want to span a wide range of receptors.

Q. Do you have interactive data set you can share?

A. Yes and ToxCast has ~2,500 overlap with chemicals that Unilever has investigated. Even if we only end up with a predictive panel of receptors for liver toxicity (or other focused panels), it would be a good outcome from the Challenge.

Q. Are the Sponsors wanting an entire receptor model (crystal characterised structure etc.) without setting parameters around binding?

A. No, Sponsors would ideally want that as well, but see how far you can get. We know this is a big challenge.

Q. What are your interests around chronic vs. acute side effects? For example, carcinogenicity predictions are difficult mechanistically.

A. This will be one tool amongst many. To take into account bystander and operating exposures we would want a tool that will help risk assess both chronic and acute effects. The focus should not be on exposure but building a quantitative element into the receptor binding prediction so that exposure can be built in retrospectively.

Q. Have you developed your own scoring system for your chemical panels?

A. No. Currently Sponsors are reliant on commercial *in vitro* screening tools which have their own quantitative metrics based on vendors' experience.

Q. Can you determine why existing tools aren't working?

A. They have either been developed in the wrong mode (i.e. single receptor and multiple ligands for efficacy screening) or they have been trained on limited/irrelevant data sets. However, the algorithms behind the tools haven't been looked at thoroughly. Sponsors haven't had enough experimental data and chemical overlap to examine/validate this properly.

Q. Do you not want docking tools?

A. Not necessarily. Please use whatever tools you need to show your system works.

Q. For real-life adverse effects, metabolism and exposure can have an effect. Should these be taken into account?

A. No, this is not within the scope of this Challenge.

Q. Will you be sharing data/information on proprietary compounds? And would this have an impact on what an academic could publish coming out of this project?

A. Some data is proprietary but most is not. Unilever encourages publication and will actively find ways to ensure that we can publish any results.

Q. Could we use Sponsor data as well as literature data?

A. Yes to both. Unilever data is limited, but we have collaboration subscriptions to databases which we may be able to share and there is publicly available data which could be used

Q. Are you confident that enough data will be available?

A. Yes, there are large amounts of *in vitro* data available

Q. Can we use pharmaceutical data to benchmark chemicals and look at overlap to train the model?

A. Yes. That would be okay and the Sponsors can help characterise the overlap.

Q. We would have to do this without the use of animals?

A. Yes. *In vivo* studies are out of scope for this Challenge.

Q. Who should we email with questions?

A. General questions can be sent to the NC3Rs. Questions regarding a specific challenge can be sent to the Sponsors, but enquiries should be sent to ALL Sponsor parties for a particular Challenge. Please email the NC3Rs to facilitate interaction with the Sponsors at CRACKITenquiries@nc3rs.org.uk.

References:

J. Bowes *et al,* (2012). Reducing safety-related drug attrition: the use of *in vitro* pharmacological profiling. *Nature reviews: Drug Discovery*, 11: 909-922.