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Symposium 2: Increasing predictive power: exploiting current knowledge to advance preclinical testing

Chairs: Dr Carolin Schramm and Dr Nicholas Coltman

Introduction

Pre-clinical testing is essential in the development of any drug. Proper identification of toxicity and toxic risk at the pre-clinical testing stage is key to reducing in patient toxicity and decreases the risk of late-stage drug withdrawal, which is costly in terms of time and resources. This importance, as well as the need to better stratify use of animals pre-clinically under the 3Rs framework, means there is ongoing research and debate on how to optimize pre-clinical models. A better understanding of pre-clinical models, in terms of differences to humans and relationships between in vitro and in vivo models, can help increase predictive power of pre-clinical testing and in turn enhance the drug development pipeline and reduce clinical toxicity.

Talk 1: Learnings from when preclinical drug development failed to predict adverse events in clinical trials. **Dr Lap Hing Chi**

The first talk of the session was delivered by Dr Lap Hing Chi, who presented a detailed review of previous instances in clinical drug development where pre-clinical toxicity studies did not identify toxicity that later presented in humans. For many studies the first step in preclinical testing is use of an in vitro cell model. Panel screenings of cell models such as proteomics and transcriptomics can prove very useful in toxicity testing but it is often difficult selecting an appropriate cell type. Screening techniques can help understand drug mechanisms and possible points of toxicity, for instance activity-based protein profiling can use reporters to see where probes specifically target and can be incorporated with mass spectrometry techniques.

Differences between preclinical species and humans can represent a major limiting factor in predictive power of preclinical studies. This is especially a concern with gene and immune therapies, which can lead to seemingly random off target effects that are not translated across models due to different species response systems. One of the most infamous examples of where the (then) standard pre-clinical evaluations did not predict potential human toxicity was for the humanised monoclonal antibody, TGN1412, which resulted in patients developing organ failure and life-changing injuries during clinical trials. After TGN1412 was tested in rodents and primates cross-species differences in levels of target receptor binding with humans were not accounted for. This led to development of a new approach incorporating the minimum anticipated biological effect level (MABEL) which included evaluation of binding kinetics. This now allows the option to select a more conservative starting dose in humans, lowering risk of severe toxicity upon first administration.

One potential solution to the difficulties in cross-species differences may be the use of humanized models. Metabolism plays a major role in many toxic events, but differences in metabolism between models can mean toxicity is missed. One notable case for this is a human specific enzyme that causes toxic metabolites from thalidomide to form, where teratogenicity was not seen in pre-clinical models that occurred when given to patients. Humanized rodent models may have more representative metabolic capability and therefore offer a more predictive model. The talk was concluded by highlighting the need to tackle these issues prospectively, rather than retrospectively after a toxic event in humans, which may be more feasible with increasing preclinical predictivity with more advanced models.

Talk 2: Large-scale human genetics in drug target discovery and safety assessment. **Dr Eleanor Wheeler**

The next symposium talk presented by Dr Eleanor Wheeler focused on the importance of genetics in drug development pipelines and the incorporation of genetics into development work at AstraZeneca. Notably drugs with data involving genetic support for selection of potential therapeutic targets are two times more likely to be approved by regulatory bodies. Approaches for testing gene-drug relationships can include GWAS, which arrays genotype, and EXWAS which uses exome sequencing to get a broader overall picture. Implementation of these powerful tools can help find rare variations in patient cohorts. The development of the UK biobank has revolutionized genetic studies and represents a rich pool of phenotypic data. Using these resources researchers developed the AstraZeneca PheWas Portal, a publicly accessible online repository (https://azphewas.com/) for interrogating gene databases.

The portal implements scoring systems which can help spot genes which should not be targeted and can see which genome regions are tolerant to variation. Furthermore, gene targets can be uncovered, for instance loss of a mitogen-activated protein kinase subtype in men causes no detrimental impact on health, and can also reduce diabetes risk by decreasing blood glucose, therefore representing a potential pharmacological target. Implementation of rich genetic datasets can undoubtedly have a huge benefit in predicting drug outcomes in preclinical and clinical testing, however there also needs to be a conscious effort to diversify the source of the genetic information, rather than currently relying on a mostly European cohort which may skew outcomes.

Talk 3: Benefits of humanised mouse models in drug discovery. **Prof Roland Wolf**

Prof Roland Wolf then discussed long-term efforts to develop a suitable humanised mouse model for preclinical testing. One of the major pitfalls in rodent drug testing is the differences in metabolism with humans. Due to this it has long been sought to develop a humanized model with all the advantages of a rodent pre-clinical species whilst also having a more translatable metabolism. With this in mind, researchers developed a humanized mouse model by substituting murine CYP450 enzymes and the upstream transcriptional regulators CAR and PXR with human counterparts. The introduction of human genes related to drug distribution in mouse liver led to an expression level almost identical to that of human liver.

Drug pharmacokinetics in humanized mouse models are significantly closer and can accurately predict drug-drug interactions seen in humans linked to induction and inhibition of the particular CYP enzymes. Through several iterations and genetic implementations/crossovers the group have developed various humanized models with high relevance to in-patient studies. These models can be especially beneficial when looking at polypharmacy, and is relevant across all stages of drug development. Furthermore, data generated from the models is being implemented within in silico programmes, meaning this highly relevant predictive model can be applied to a wide range of studies.

Talk 4: Unravelling species differences in hepatic stress response capacity to inform the selection of animals for use in preclinical drug safety assessment. **Ms. Hannah Coghlan**

Finally, Hannah Coghlan presented an oral communication based on her PhD project research looking into differences in liver stress response between species and the impact of this for clinical testing. Pre-clinical animal models are often selected for drug safety studies due to ease of access or whether a group has used a certain strain or species before, rather than selecting the most appropriate species for the specific pre-clinical test. Furthermore, rodents have typically been used

as a main model of choice, but there are suggestions that models such as the mini pig may be more translatable to humans. Indeed, data from pre-clinical studies often extrapolates across species such as mouse and rat and into humans without considering the species differences that may affect response to drugs. Here it was shown that there was a remarkable difference in hepatic stress response linking to the Nrf2 pathway when comparing mouse and rat. The seemingly higher oxidative stress capacity of rats means that their response to toxins, especially those that cause oxidative stress, could be very different to mice and humans. This has an implication when choosing pre-clinical species for drug testing.

A wider genetic difference was then shown across species using public basal gene expression data. Expression data from human, mouse and rat liver revealed differences in basal gene expression between the three species. These species-specific differences were then investigated using cultured primary hepatocytes. Liver cell mRNA response to model toxins was different between rat, mouse and human for genes including those involved in the Nrf2 pathway and for unfolded protein response, which both have toxicological relevance. This data reinforces the need to be aware of species differences in pre-clinical models and how they translate into human response to a drug, and that it is vital to consider these differences before carrying out pre-clinical tests in order to select the most relevant model that will limit toxicity and reduce study disruption at later endpoints.

Conclusion

Overall this symposium was a fascinating insight into the challenges presented by species differences in response to potential toxins in drug development and the importance of understanding the impact these differences can have on pre-clinical outcomes. Increasing research into novel models, such as humanized mice or mini pigs, which may be more relevant to humans could bridge the gap between pre-clinical and clinical testing, increasing predictive power of potential toxicity before reaching humans and therefore hopefully leading to better outcomes and reduced drug attrition.