Report for BTS

Symposium 3:
The value of non-clinical studies adversity in patients in clinical trials

Abstract

The use of non-clinical studies are seen as first stage of assessing the efficacy and identifying or investigating key events to determine toxicity. They are typically completed using *in vitro* and *in vivo* models. Upon which the results can influence what key events are focused on during clinical trials. This symposium discussed the use, relevance and the developments in non-clinical studies. The presentations covered the positive and negative predictive value between the study types and the development of a database for this. Alongside the development of an *in vitro* model that could reduce the reliance on animal trials and finally a practical approach of how findings during the non-clinical studies will influence clinical studies.

Report

Non-clinical studies are seen as the starting point for researchers and developers to investigate key events (KE) and efficacy to establish if there is an adverse outcome pathway (AOP) of chemicals and drugs alike. These typically include the use of *in vitro* and *in vivo* studies to give an initial indications of KE that need investigating and if the process of development is beneficial and safe to proceed into clinical trials.

In vitro models are used in the first instance with relevant cells types where exposure and target of effects are likely to occur. This can provide initial information regarding cellular response and molecular initiating events (MIE) that could induce negative effects and have larger implications on organisms. This can pinpoint KE and pathways that lead to the development of associated adverse outcomes (AO) that can be specially investigated further. While they are deemed more ethically and socially acceptable to a wide audience. However, these models can only provide localised cellular effects overview and not a systemic view.

The next stage of that is the use of animal testing, of which can bring a boarder view of the potential negative impacts or the efficacy dosage required. This will allow researchers to establish if progression into human trials is worth in regard to safety, clinical impact and cost. However, the use of animal testing has many ethic issues and poor public opinion. Relevance in relation to humans has also been criticised and has been noted that some toxic effects are not applicable to humans. This led to the UK and EU implementing a ban on the use of animal experimentation for cosmetics. The creation of the 3 R's; replacement, reduction and refinement, is an attempt to minimise the reliance of animal testing. Research in alternatives to *in vivo* studies have increased and have led to the creation of more complex *in vitro* models to address this issue.

This symposium discussed the use, relevance and developments of these non-clinical testing approaches to allow drug development and chemical assessment to safely proceed to clinical trials. The first seminar "Assessing the value of non-clinical testing to enable safe entry to first in human clinical trials" by Dr Tom Monticello from Amgen was looking at comparing non-clinical studies results with clinical trials to establish if the conclusions from the non-clinical investigations aligned with findings in the clinical trials. It was highlighted that there is a current lack of research between the non-clinical and clinical concordance databases. The IQ Non-clinical to Clinical Translational

Safety Working Group has looked to address this issue. They complied data covering 182 molecules from animal studies and phase 1 clinical trials, adverse events and animal toxicity studies were then grouped according to organs affected. Statistical analysis was completed to evaluate results from animal toxicity studies and clinical studies to determine positive predictive value (PPV) and negative predictive value (NPV).

PPV relates to proportion of positive non-clinical studies events positively correlates to human adverse events, while NPV shows the proportion of negative non-clinical studies correlates to negative human adverse events. The results showed NPV had a stronger predictive measure between non-clinical and clinical studies, suggesting that negative events documented in animal studies have been documented in human clinical trials. PPV was shown to be varied and was effected by prevalence of disease and the number of different species used. It was shown that high prevalence rates equated to an increase of PPV. This is an important issue for disease states that have a low prevalence among populations and the concordance between animal and clinical studies. The number and type of species used can effect PPV and also NPV, this effects overall specificity and sensitivity. As non-human primate was shown to be the strongest predictor of adverse events, while dogs were the strongest predictor of the lack of adverse events. This highlights the importance of utilising at least 2 different species.

The next stage and future from the group is to investigate sub-chronic and chronic studies, as the database previously discussed was focused on acute events. By providing a similar assessment to obtain PPV and NPV and assess the effective use of the type and number of species. It will be also expanding the database to include adolescents, to understand that animal testing is concordant with clinical trials within this population group.

The next seminar "An overview of the Translational Relevance of Complex *in vitro* models to assess the Safety of New Medicines" by Dr Lorna Ewart from Emulate. This seminar focused on exploring the use of an advanced *in vitro* model, organ-on-a-chip, as a tool used as a predictive tool for toxicity in drug development and improve attrition. But also using these alternatives as a form of modernising the process of development and discovery with incorporating the 3R's. Emulate has developed a Liver-chip using primary hepatocytes including, a microfluid system with flow control, mimic tissue to tissue interface, provide similar mechanical forces and can be enhanced with immune cells and microbiome. The model was then assessed using Innovation and Quality criteria; biological recapitulation, specific tests and endpoints, statistical analysis and experimental hygiene and domains of validity. This was then compared with 3D models and used to see if the Liver-chip was capable of identifying toxicities among 27 molecular drugs.

The treatments that were exposed to the Liver-chip are based on human C_{max} of albumin and covered upto 300x C_{max}. Albumin, urea, and ALT was used to measure the toxicity effects in hepatocytes during the exposures of all models used. The chip showed to have a high predictive value compared to other *in vitro* studies with 87% sensitivity and 100% specificity. This was achieved with the use of 2 cellular donors, as it was found that the use of only one donor had a lower sensitivity and specificity. It is believed this model can be used within the non-clinical stages to prevent hepatotoxic drugs from entering into the clinics but could also lead to a reduction of animal testing or reliance.

The next seminar "A viewpoint of a Phase I/II Clinician" by Professor Duncan Richards from University of Oxford. This seminar focused how clinicians use the information provided from non-

clinical studies to influence how this is integrated into clinical trials. Safety is the main focus during clinical trials and is shown that drug attrition rate is low. But this is also due to efficacy influences drug development, as around 18% of drugs are not able to pass through phase 2. It can also be difficult for safety assessments as there is reduced data on dose response and adverse events in humans. To improve these assessments more data points are required. There needs to be increased specificity on identifying adverse events within nonclinical testing to understand variability of them. While nonclinical testing can be helpful in providing potential data on KEs and adverse events but sometimes these are not noted or different within clinical trials. Also, the efficacy of the drug may be different among species.

An example of a combination drug to treat systemic amyloidosis was discussed to highlight how nonclinical trials are used. Anti SAP and Miridesep was used for the removal of amyloid proteins from blood, via use of macrophages. This showed to work well within the mouse model and provided a mechanistic pathway of adverse events to monitor that were on target and systemic locations. This was then applied to clinical studies and showed to have removal of amyloid proteins in the liver. The next target was assessment in cardiac tissue, as this is the main mortality cause of systemic amyloidosis. However, the drug did not show to have any reduction of amyloid protein, as the anti SAP did not penetrate cardiac tissue. Also, there was an identified case of vasculitis, a known adverse event. The combination of non-desired efficacy and adverse event resulted in the project being terminated. This shows that nonclinical trials are capable of providing mechanistic pathways for safety and efficacy but still shows that animal testing cannot be solely relied on.

The oral communication was about “Statistical analysis of the preclinical inter-species concordance of histopathological findings in the eTOX database” by Peter Wright from University of Cambridge. This short talk was analysing the concordance of inter-species toxicity testing and the prediction to clinical findings. As previously mentioned there is a lack of analysis regarding concordance between nonclinical and clinical findings. This study used eTOX data to complete a comparison with specific focus on histopathological findings using studies that had similar exposure, exposure duration and same sex animal models. It was determined that there was a bias positive concordance over negative but indicated that there were histopathological differences and organ specific toxicities. Overall, this showed that there is association between nonclinical species used but concordance was low, which could have implications on translation to human trials.