Once promising compounds have emerged from the early optimisation cycles, animal PK studies will be performed to verify the improvements made in vitro in the whole animal in vivo. Establishing and verifying the link between in vitro and in vivo (IVIVC) is important to ensure that the screening assays have the desired impact and that the screening tree is still relevant. Otherwise, the screening tree needs readjustment to respond to the chemical space and issues of the current compounds and to return on the critical path. While there are simple and convenient tools to examine IVIVCs such as correlation analysis between two or a few more properties, more sophisticated tools such physiologically based PK (PBPK) models offer the advantage to propagate in vitro ADME data through an in silico representation of the whole organism which allows simulation of the impact a given property change may have on the concentration–time profile (Parrott et al. 2005; Lüpfert and Reichel 2005; Peters et al. 2009; Chen et al. 2012; Rostami-Hodjegan 2012; Jones et al. 2015). The purpose of the application of PBPK modelling in this phase is not to be predictive of human but to diagnose ADME liabilities that are key to be improved and subsequently to aid the identification of compounds with an optimal balance of properties (and an acceptable compromise of their insufficiencies) to enable the desired concentration–time and PK profile.

Compounds which are expected to provide sufficient target exposure in animal PD models qualify for efficacy studies to explore their pharmacological profile in vivo. Prior information from the in vivo PK and/or pilot exposure studies supports the design of these studies in terms of suggesting doses and schedules which are most likely to be efficacious and for suggesting suitable time points for sampling of plasma and/or tissue. Exposure measurements which allow to capture a time course throughout the dosing interval are important and allow to dynamically link the concentration–time profile with the effects seen (or not seen). Dedicated dose–response studies with different dosing schedules are particularly powerful for PK/PD modelling and simulation (Gabrielsson et al. 2009, 2010, 2011; Bueters et al. 2013; Tuntland et al. 2014). If the design of these studies has been carefully thought through, important pieces of information can be extracted giving answers to questions such as the following: Can efficacy be linked to the unbound plasma concentrations? What element(s) in the plasma concentration–time profile drives efficacy (i.e. the PK/PD driver)? For how long should the target be exposed/engaged to elicit a certain level of efficacy? What would be the unbound concentration–time profile we like to see in humans to be efficacious in the clinic?

Single-dose studies with MoA readouts in target tissue can be very informative of target exposure, target engagement and subsequent expression of pharmacology when pharmacological readouts are taken dynamically, i.e. followed along a time course after dosing together with plasma and tissue samples to be analysed by means of LCMS/MS for the corresponding compound concentrations. The relation between the compound concentrations in plasma and target tissue and the extent and duration of target inhibition needed for efficacy forms the basis for quantitative PK/PD models which ultimately will be used to estimate the human efficacious dose.