areas for nonclinical safety *screening* of small-molecule drug candidates during discovery phase before start of nonclinical regulatory GLP studies. For large molecules ("biologics"), other screening strategies may be applied (e.g., immunogenicity screening, human tissue cross-reactivity studies). Toxicity of biologics is mostly related to the mode of action with classical off-target effects not frequently observed. The basic requirements for nonclinical safety assessment are described in ICH S6 (R1) (2011). However, these compounds are beyond the scope of this overview.

2 Molecular Off-Target Interaction

Screening for off-target interaction at a relatively early stage of the drug discovery process is important. The binding affinity and interaction of the candidate with structures closely related to the intended target (e.g., other receptors or enzymes of the same family) are determined as part of the primary pharmacodynamics characterization in order to define the specificity of a targeted pharmacological approach. An important aspect of secondary pharmacodynamics is the understanding of the expression profile of the pharmacological target in humans and nonclinical models used in safety evaluation. This can be done by literature mining; biochemical, cellular, genomic, and proteomic profiling; and systems biology approaches (MacDonald et al. 2006; Prokop and Michelson 2012). These areas of drug evaluation are evolving, but there are still significant gaps in knowledge and technologies.

In addition, evaluation of the interaction with other important protein targets is needed as part of pharmacological characterization. Off-target effects may result from interaction of the drug candidate with receptors, ion channels, transporters, enzymes, or other protein targets, which may cause significant adverse effects. Although the interaction of a drug candidate with the target protein at the intended binding site can be modeled and the 3D structure of the drug candidate is mostly available, the prediction of possible off-target binding based on a proteome-wide drug-target network using in silico tools is not yet routinely done because of the complexity of the analysis (Xie et al. 2011). Today, in vitro screening is still the method of choice by testing binding to and interaction with a broad panel of important human receptors, transporters, ion channels, and enzymes (Denton et al. 2012). Standard panels for this type of test are offered by various vendors. As a first step, a simple binding assay is generally sufficient. When binding is observed at relevant concentrations (IC_{50}-value), the potential functional impact on the target structure can be assessed – inhibitory or antagonistic activity vs. activating or agonistic activity. Such data may support compound selection in various phases of drug discovery; can also guide decisions on the need for additional nonclinical safety testing, for example, by expanding the safety pharmacology testing panel to check the in vivo relevance of such findings (see ICH S7A 2000); and can also be helpful in mechanistic explanation of possible effects found in subsequent in vivo studies.