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Exploratory toxicology as an integrated part of drug discovery. Part II: Screening strategies

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In an effort to reduce toxicity-related attrition, different strategies have been implemented throughout the pharmaceutical industry. Previously (in Part I), we have outlined our 'integrated toxicology' strategy, which aims to provide timely go/no-go decisions (fail early) but also to show a direction to the drug discovery teams (showing what will not fail). In this review (Part II of the series) we describe our compound testing strategies with respect to cardiovascular safety, hepatotoxicity, genotoxicity, immunotoxicity and exploratory *in vivo* toxicity. We discuss the *in vitro*, *ex vivo* and *in vivo* assays and models we employ to assess safety risks and optimize compound series during the drug discovery process, including their predictivity and the decisions they generate.

Introduction

To reduce attrition of drug candidates during clinical development as a result of safety issues, toxicology and risk assessment should be an integrated part of the drug discovery process. We have argued that a successful 'integrated toxicology' strategy should include safety assessment of novel drug targets, selection of chemical series without inherent safety issues, designing out risk factors and a broad toxicological profiling of potential drug candidates with the aim not only to provide timely go/no-go decisions (fail early) but also a direction to the drug discovery teams (what will not fail) [1]. Here, we discuss Lundbeck's discovery toxicology screening strategy which focuses on: (i) serious adverse drug reactions that most frequently impact drug development or lead to drug withdrawal (cardiovascular safety, hepatotoxicity) [1,2]; (ii) toxicities that usually have an immediate impact on further development (genotoxicity, immunotoxicity); and (iii) overt toxicities that present acutely in animals. We discuss the predictive assays and models that are employed, including conclusions and decisions in project teams that can be based on the data, and potential ways to follow up with further in-depth studies.

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Cardiovascular safety

Because cardiovascular (CV) safety issues are a major cause for attrition of drug development projects and for market withdrawals, we aim to catch and mitigate the most overt CV issues early in drug discovery projects, and provide an integrated risk assessment toward candidate selection. Initially, we evaluate the target in relation to the CV system, including possible activity on closely related off-targets. The assays and models employed aim to evaluate the cardiac conduction system [including the QT interval of the electrocardiogram (ECG)] and other well-known CV risks such as hypotension leading to syncope [3] and increases in blood pressure and/or cardiac contractility, which are linked to increased patient mortality [4,5] (Fig. 1). The relation between inhibition of the hERG channel (the rapid delayed rectifier potassium channel, Kv11.1), preclinical QT models, human QT effects and cardiac arrhythmia is well known [6] and is covered in two ICH guidelines specifically addressing the risk assessment of QT prolongation [7,8]. Therefore, we screen for hERG inhibition during the hit-to-lead process (to prioritize series) and lead optimization (to optimize if needed), using an automated population patch clamp platform and we flag all compounds that inhibit hERG (tested up to 30 μM) as potential risk compounds. It has been proposed that a safety margin of 30–45 between the hERG IC_{50} and clinical $C_{max,free}$ provides an optimal balance between correctly identifying QT risk drugs and avoiding too many false

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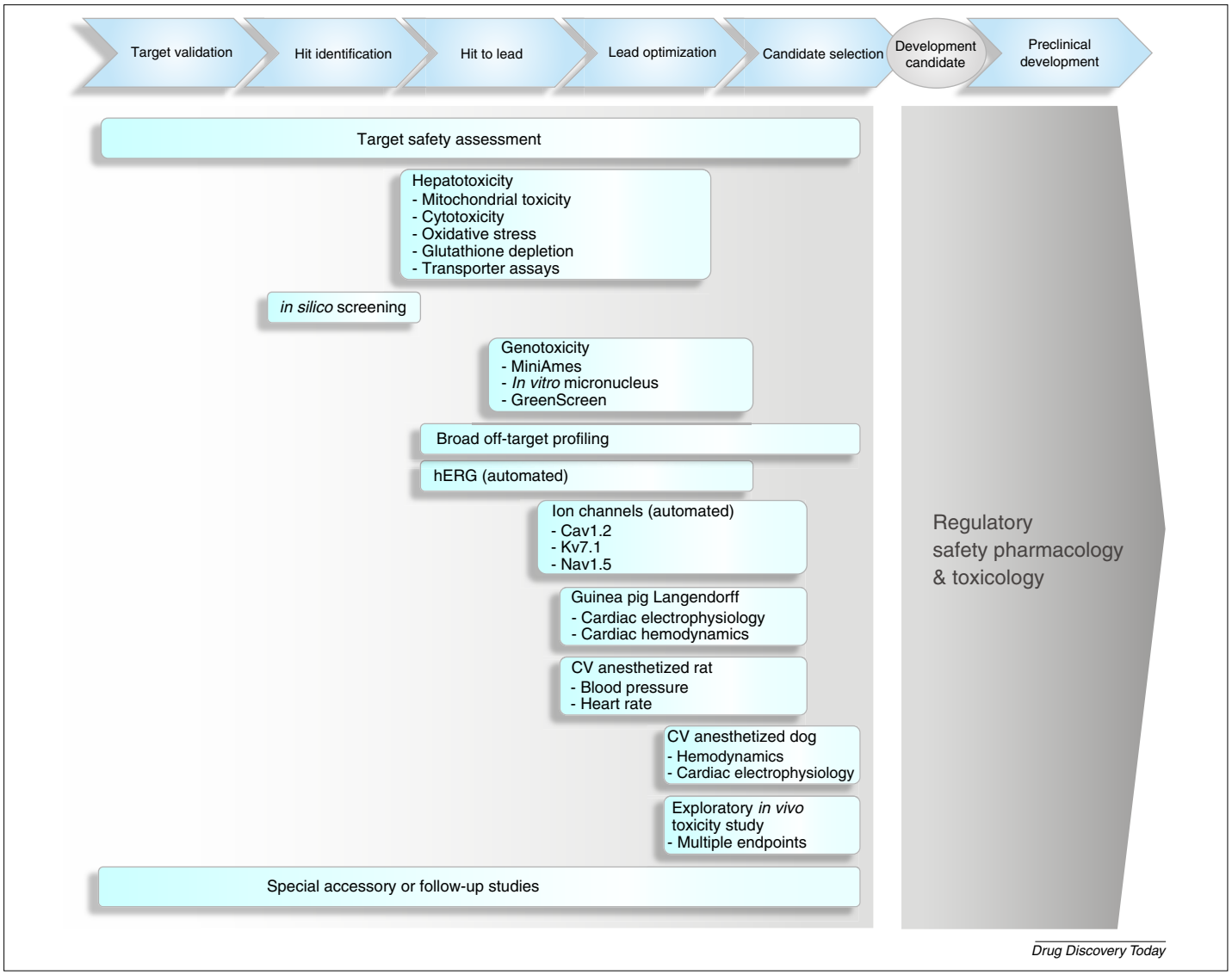

FIGURE 1

Timing of exploratory toxicology assays during the drug discovery project phases. Depicted is an overview of the most commonly used assays and models, along with their timing in terms of at which stage of a project they are employed. The timing is not static and can be adjusted according to the needs for an individual project and issues encountered. The predictivity of the separate assays and models, conclusions and decisions that are based on the data are discussed in the text. Abbreviations: CV, cardiovascular; hERG, rapid delayed rectifier potassium channel (Kv11.1); Cav1.2, the cardiac L-type calcium channel; Kv7.1, slow delayed rectifier potassium channel; Nav1.5, the cardiac sodium channel.

positives [9,10]. Based on these data, we decided to classify compounds into three categories, according to their safety margins (i.e. ratio between hERG IC_{50} and clinical $C_{max,free}$): a margin of >300 is low risk, a margin of 30–300 needs follow-up assessment and a margin of <30 is considered as high risk. To direct synthesis, *in silico* modeling is used in collaboration with computational chemistry in cases where hERG inhibition persists in a particular series.

At the start of each lead optimization program, compounds are screened for effects on the most relevant ion channels: the cardiac L-type calcium channel (Cav1.2), the cardiac sodium channel (Nav1.5) and the slow delayed rectifier potassium channel (Kv7.1) [11–13]. Other, more classical, targets for cardiovascular effects (e.g. alpha and beta receptors) are also evaluated at this stage, as part of routine off-target profiling.

When series mature during lead optimization, compounds are evaluated in guinea pig isolated perfused Langendorff hearts, which provides information on cardiac electrophysiology, contractile capacity and coronary blood flow [14]. The current guideline states that a 5 ms increase in the standard human QT interval is a critical safety threshold [8], which corresponds to a 1.4% increase in a human QT interval of 360 ms. We have shown that even a small increase in the QT interval can be detected in the Langendorff model and this is predictive of QT prolongation in humans (Fig. 2). These data confirm that minor inhibition of hERG can lead to clinically relevant prolongation in the QT interval. Therefore, the Langendorff model is our preferred screening model to detect long QT compared with other models such as dog telemetry, which does not detect a QT increase of less than 10% [15].

Toward candidate selection, compounds are tested at a range of intravenous (i.v.) dosages in anesthetized rats for effects on heart rate and blood pressure. If dose-dependent changes are identified,

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