BioCentrum

IN VITRO
PRECLINICAL DEVELOPMENT

Problems with efficacy, absorption, distribution, metabolism and excretion of promising drug candidates may lead to compound failure in clinical trials. To help resolve these issues, BioCentrum offers a comprehensive platform supporting drug discovery projects from hit to preclinical candidate. The data derived from these studies can help our customers to select compounds for further development and validate results from other assays. Our goal is to substantially increase research productivity, while decreasing labor-based expenses. Our cost-conscious approach allows to provide a superior level of quality at prices that are very attractive from a global perspective.

ADME studies

SOLUBILITY TESTING

Solubility of drugs in aqueous media is one of the critical parameters that influences both pharmacokinetic and pharmacodynamic properties of chemicals. It is a key factor highly influencing dissolution rate and bioavailability of substances following oral administration, and therefore can alter the properties responsible for the in vivo performance.

PERMEABILITY ANALYSIS – PAMPA

The Parallel Artificial Membrane Permeability Assay (PAMPA) is a non-cell based assay for predicting passive absorption of drugs in early drug discovery. Most drugs are absorbed through the intestines without using cellular pumps. Therefore, passive permeability assays are useful for screening oral-absorption potential of drug candidates.

PLASMA BINDING

The pharmacokinetic and pharmacodynamic properties of drugs are largely a function of the binding of drugs to plasma proteins. High drug-protein binding can both reduce the amount of free drug available for target sites and prolong the duration of drug activity. For this reason it is crucial to estimate the percentage drug bound to plasma proteins.

METABOLITE PROFILING

Administered drugs are subject to metabolism. Resulting metabolites might have pharmacological effect or even pose a toxicity risk on the organism, it is therefore necessary to identify and evaluate them in terms of safety. Applying biotransformation rules in combination with modern analytical techniques such LC/MSn yields information on metabolic pathways of drugs. This in turn allows for metabolite identification and quantification as well as their synthesis and toxicity assessment.

CYP IHIBITION

The assays for cytochrome P450 inhibition facilitate the identification of drug candidates with lower potential for drug-drug interactions. In vitro experiments conducted to determine whether a drug inhibits a specific CYP enzyme involve incubation of the drug with probe substrates for the CYP enzymes. Recombinant cytochrom P450 isoforms employs the assay: CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4 with various probe substrates enabling fluorescence detection or more specific LC/MS/MS analysis.

IN VITRO CYTOTOXICITY ASSAYS – MTT, MTS, LDH, BRDU

In vitro evaluation of possible cytotoxicity of a drug candidate or its metabolites constitutes the first step in toxicological studies. Both MTT and MTS tetrazolium compounds are bioreduced in living cells only to form a spectrophotometrically determined product. Unlike MTT and MTS, LDH assay is based on quantitatively measurement of lactate dehydrogenase, intracellular cytosolic enzyme that is released upon cell death and lysis. BrdU assay in turn is a colorimetric immunoassay which enables quantitative measurement of DNA synthesis and cell proliferation. This is done through assessment of thymidine analog incorporation into the DNA during the S phase of cell division process.

GENOTOXICITY TESTS

- Ames test (performed according to the OECD TG 471 guideline)
- Micronucleus assay (performed according to the OECD TG 487 guideline)

Molecular cell biology for drug discovery

CYTOMETRIC ANALYSIS OF APOPTOSIS AND NECROSIS (ANXV/PI STAINING)

Apoptosis is a physiological process of controlled cell death that is essential during embryonic development and in maintenance of tissue homeostasis. New drugs inducing apoptosis are expected to be most effective for use in the treatment of cancer. Biocentrum performs cell death identification with the use of fluorescently labeled annexin V (AnxV) that specifically binds with high affinity to phosphatidylserine, the best known apoptotic cells surface marker. Additional use of propidium iodide (PI) and professional flow cytometry analysis enables to characterize type of cell death (apoptotic or necrotic) with high reliability. Analysis of apoptosis/necrosis together with cell cycle arrest identification constitute excellent complement to cytotoxicity research.

CYTOMETRIC ANALYSIS OF CELL CYCLE INHIBITION (PI STAINING)

Studies of the proliferation characteristics of normal and malignant cells provide broad spectrum of information about molecular mechanisms of action of drugs and aid design of effective cancer therapy strategies. Cell cycle arrest analysis similarly with apoptosis identification and cytotoxicity assays becomes basic method used in anti-cancer drug discovery and development pipeline. In this method, fixed and ribonuclease treated cells are stained with propidium iodide and cytometric analysis is performed, in order to characterize number of cells residing in each of three analyzed cell phases (G0/G1, S and G2/M).