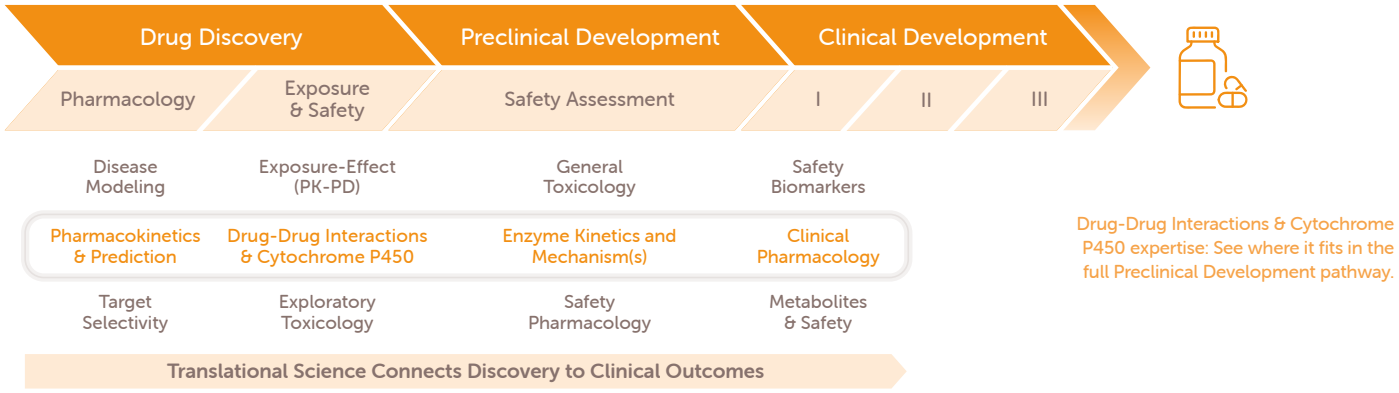
inotiv
analyze. answer. advance.

White Paper

Bridging Bench Science with Clinical Development:

INOTIV'S INTEGRATED SCIENCE APPROACH TO DE-RISKING SMALL MOLECULE DRUG CANDIDATES.



Drug-Drug Interactions & Cytochrome P450 expertise: See where it fits in the full Preclinical Development pathway.

Overview

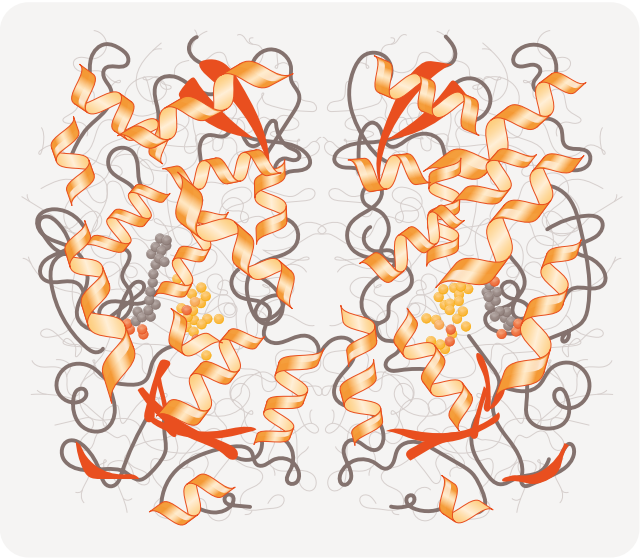
Inotiv's expertise in drug metabolism and drug-drug interactions is critical for partners looking to advance small molecule drug candidates. Our integrated science approach translates routine and advanced assays—including inhibition of CYP enzymes, reaction phenotyping, metabolite identification for both *in vivo* and *in vitro* samples, and targeted proteomics—into actionable insights for our clients.

Using specialized techniques, tucatinib, a highly selective anti-cancer drug, was found to be a mechanism-based inactivator (MBI) of CYP3A4, a key drug-metabolizing enzyme. Our scientists subsequently identified the molecular mechanism behind this inactivation, providing a clear understanding of the drug's safety profile and its potential to interact with other medications.

These results directly support regulatory IND-enabling packages, inform drug-drug interaction risk assessments, and guide clinical development strategies. By bridging bench science with regulatory expectations, Inotiv ensures a smoother and more confident transition from discovery to development and, ultimately, to the clinic.

Cytochrome P450 enzymes are a super-family of drug-metabolizing enzymes, responsible for metabolizing of the majority of all small molecule therapeutic drugs. CYP3A4 is the most significant of these enzymes due to its broad activity and impact on drug clearance. When a new drug inhibits CYP3A4, it can lead to dangerous DDIs, increasing the blood concentration of co-administered drugs and potentially causing adverse effects.

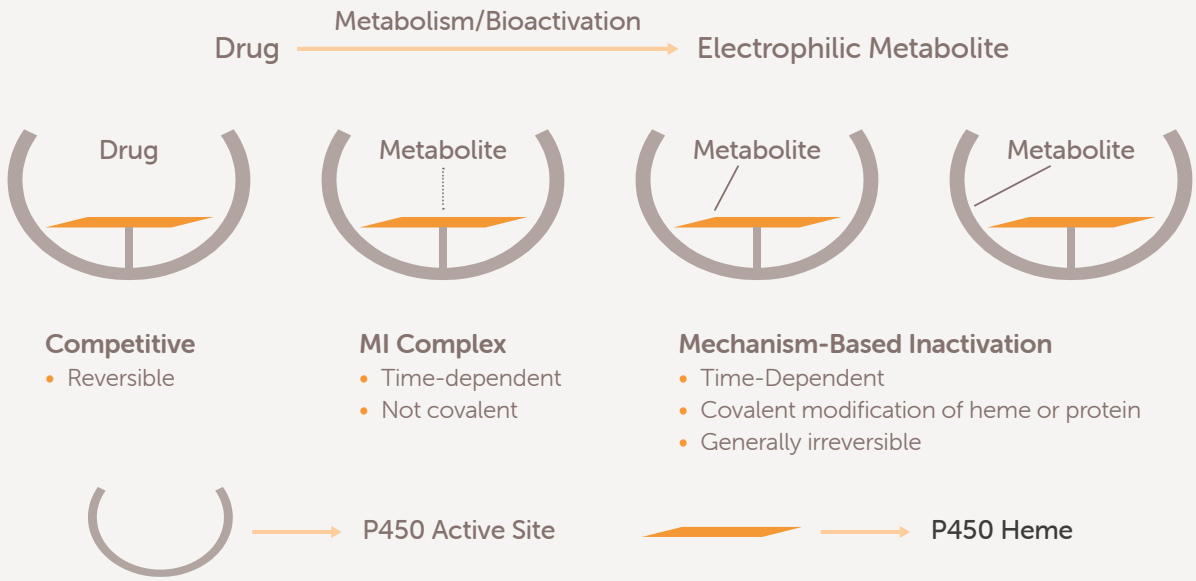
Understanding Drug Metabolism and CYP3A4



Reference: https://www.rcsb.org/structure/6BD7

The Challenge: Differentiating MBI from Reversible Inhibition

Basic P450 Inhibition Mechanisms



While some drug-enzyme interactions are reversible, mechanism-based inactivation (MBI) is a more serious concern in drug development. MBI occurs when an enzyme metabolizes a drug into a highly reactive intermediate that irreversibly or pseudo-irreversibly binds to and inactivates the enzyme. This leads to a permanent loss of enzyme activity, making it crucial to understand and characterize during the overall safety assessment strategy for a drug candidate.

Inotiv's Mechanistic Characterization and Covalent Binding Analysis of Tucatinib

Inotiv's team employed a comprehensive approach to fully understand the mechanism of inactivation by tucatinib on CYP3A4.

The investigations focused on:

- **Mechanistic Characterization:** Understanding the root cause of the inactivation by conducting sophisticated *in vitro* experiments coupled with UV spectral and mass spectrometry analysis to rule out other potential mechanisms of MBI like heme destruction or the formation of a metabolite-intermediate complex.
- **Targeted Proteomics and Covalent Binding Analysis:** Using high resolution mass spectrometry methodology to definitively identify the mechanism of inactivation.

The results of the analysis conclusively demonstrated that tucatinib forms an NADPH-dependent covalent bond with the Cys239 residue in the active site of the CYP3A4 enzyme. This finding provided molecular-level mechanistic understanding of the observed inactivation.