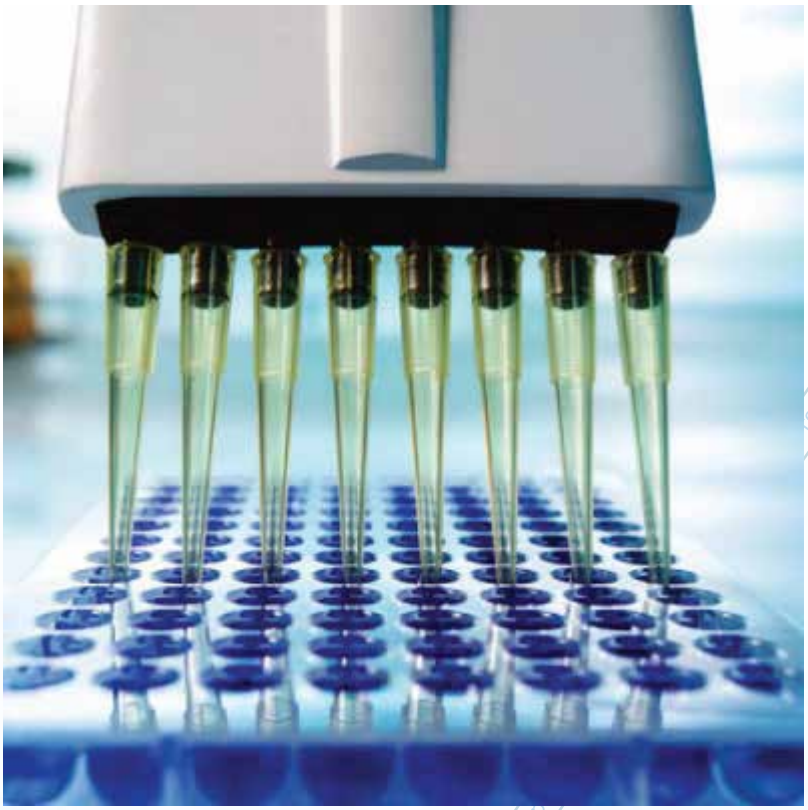


Understanding Drug-Induced Phospholipidosis: Mechanistic and Screening Insights

Key Highlights

Drug-induced phospholipidosis (PLD) can affect multiple organs and considered an important safety concern in the drug development process. Screening for PLD in the early stage of discovery may lower the risk of late-stage attrition and enhance compound safety. TCG Lifesciences offers *in vitro* assays specialised in assessing drug-induced PLD using mechanistically validated methodologies. Our assay platforms provide robust, reproducible, and sensitive detection of PLD, enabling pharmaceutical partners to make informed decisions with confidence

Overview

Phospholipidosis (PLD) is a lipid storage disorder characterised by the excessive accumulation of phospholipids within cellular lysosomes. The condition is most commonly associated with exposure to cationic amphiphilic drugs (CADs), a structural class that includes several widely used antibiotics, antidepressants, antihistamines and many others.

PLD may affect various organs, including the lungs, heart, liver, kidneys, and it is considered an important health concern. Mechanistic studies suggest that CADs either directly inhibit

lysosomal phospholipases and other catabolic enzymes, or they form indigestible drug–lipid complexes that resist degradation, leading to intracellular phospholipid accumulation.

Mechanistic Understanding

The pathogenesis of drug-induced PLD involves the interaction between CADs and negatively charged phospholipids in the lysosomal membrane. This interaction can disrupt phospholipid catabolism, resulting in the formation of lamellar bodies observable under electron microscopy.

Traditional detection relies on transmission electron microscopy (TEM), which remains the gold standard for confirming PLD through visualisation of these lamellar inclusions. However, TEM is time-consuming, labour-intensive, and unsuitable for high-throughput compound screening.
To address these limitations, image-based screening methods using fluorescent dyes have been introduced, offering faster and more quantitative assessment of PLD induction during early-stage drug discovery.

Experimental Approach

Image-Based High-Content Screening (HCS) Assay

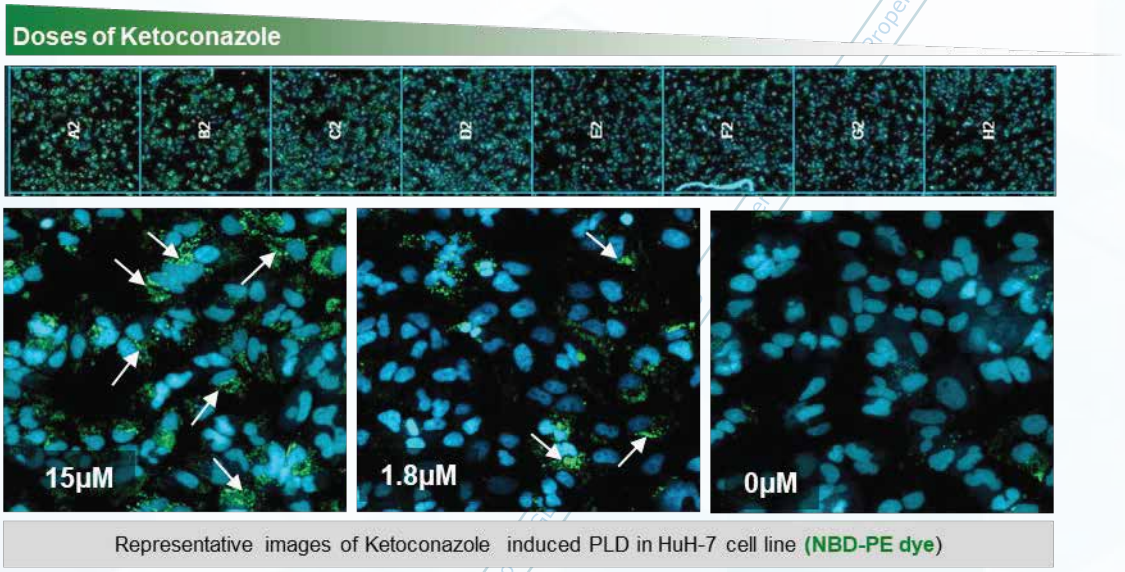
An image-based HCS assay was established to characterise compound-induced alterations in lipid metabolism in mammalian cell lines using two fluorescent dyes. The assay enables visual detection and quantification of intracellular phospholipid accumulation.

Assay Model and Conditions

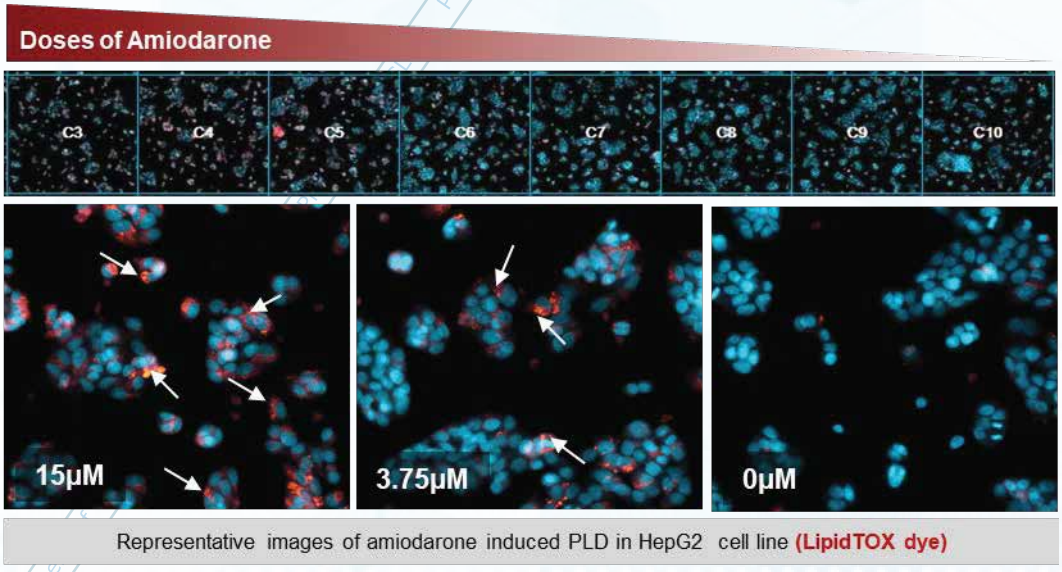
- **Cell lines:** HepG2 and HuH-7 (human hepatocyte-derived)
- **Plate format:** 96 well
- **Vehicle control:** 0.5% DMSO
- **Inducers tested:** Amiodarone and Ketoconazole
- **Dose response & replicate:** 8-point dose, 2-fold serial dilution in duplicate
- **Compound incubation:** 24 hours
- **Z:** > 0.5
- **Assay method:** high content imaging (HCI)

- **Instrument:** ImageXpress Cell Imaging System
- **Dyes used:** NBD-PE & Hoechst 33342 (Nuclear stain); LipidTOX Red & Hoechst 33342
- **Endpoints:** Puncta formation and intensity in the cytosol as indicators of phospholipid accumulation, and nuclear count (using Hoechst 33342) considered as viable cells for viability calculation

Data Representation

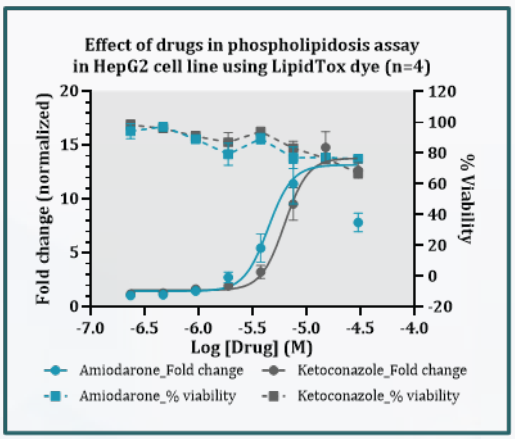


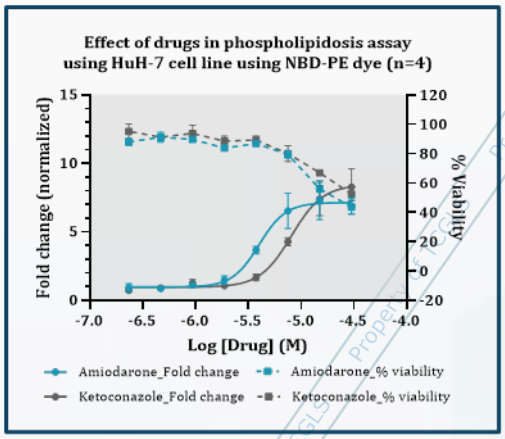
Representative images of Ketoconazole induced PLD in HuH-7 cell line (NBD-PE dye)



Representative images of amiodarone induced PLD in HepG2 cell line (LipidTOX dye)

Representative images demonstrated that both Amiodarone and Ketoconazole induced cytosolic puncta formation, indicative of phospholipid accumulation. Puncta intensity increased in a dose-dependent manner and was proportional to the drug concentrations.





Fold change was expressed as the ratio of the percentage of phospholipidosis normalised with percentage viability, allowing correlation between the phospholipid accumulation and cytotoxicity.

Test compounds	EC_{50} (µM)	
	HuH-7 cell line	HepG2 cell line
Amiodarone	4.0	4.4
Ketoconazole	8.1	6.2

The EC_{50} values for Amiodarone and Ketoconazole in HepG2 cells were consistent with literature data (Shahane et al., *J. Biomol. Screen.*, 2014, Vol. 19(1): 66–76*).

Relevance and Application

Early detection of phospholipidosis is a critical parameter in preclinical drug safety evaluation. The combination of fluorescent lipophilic dyes with high-content imaging provides a scalable, efficient approach to assess the potential of compounds to induce PLD.

By integrating such image-based screening into discovery workflows, researchers can reduce late-stage failures and enhance compound selection for safety and efficacy.

This assay framework aligns with contemporary toxicological evaluation practices and supports predictive safety pharmacology through mechanistic and quantitative analysis of lipid dysregulation.