DeeTox: Single-Cell Mechanistic AI Predicting Toxicity Mechanisms for Immune Cells

Jeany Delafiori[1], Mohammed Shahraz[2], Katja Ovchinnikova[1], Shawn Owens[1], Theodore Alexandrov[12]

[1]DeepCyte, Inc. [2]Department of Pharmacology, University of California San Diego

Immunotoxicology requires assessment of both immune-mediated and cell-intrinsic toxicity, where cells sustain direct molecular damage. Conventional assays report cytotoxicity without resolving mechanisms and are optimized for adherent cells, leaving suspension-cultured immune cells poorly served. Regulatory demand is increasing for New Approach Methodologies (NAMs) providing mechanistic, human-relevant data without animal testing. A further challenge is that most compounds engage multiple mechanisms: a primary insult triggers cascading downstream effects, as formalized in the Adverse Outcome Pathway (AOP) framework, thus making mechanistic assignment at a single timepoint inherently difficult.

Here we present DeeTox, an AI-driven, NAM-aligned solution for cytotoxicity prediction and mechanism inference in immune cells using single-cell metabolomics (SCM). SCM captures the metabolic state of individual cells, providing an unbiased, high-dimensional readout of cell-intrinsic damage. SCM is compatible with suspension cells and low cell numbers, and supports the throughput required for predictive toxicology.

Jurkat T cells were exposed to compounds spanning seven toxicity classes including proteostasis stress, DNA damage, DNA synthesis inhibition, mitochondrial dysfunction, oxidative stress, lipid metabolism stress, and central carbon deprivation. SCM data were acquired using MetaCore, our high-throughput platform for single-cell metabolomics. For toxicity and mechanism prediction, we used a deep learning framework with a neural network for data representation and classification and a custom loss function. The evaluation was done using repeated 5-fold CV leaving test compounds out of training.

DeeTox achieved 52% top-1 accuracy (four times better than random), 0.41 F1 score, and 61% top- 2 accuracy. Moreover, single-cell prediction outperformed pseudobulk data-based prediction by 10 percentage points — confirming that single-cell resolution preserves toxicological information lost through averaging.

Single-cell resolution revealed within-sample mechanistic heterogeneity invisible to bulk assays. For example, for plumbagin, an investigational anti-cancer compound, the prediction identified several mechanisms including ROS & electrophilic stress (reported primary mediator of plumbagin toxicity), nucleotide synthesis, and ETC/mitochondrial dysfunction. These mechanisms represent a complex yet biologically plausible AOP cascade. Such predictions illustrate DeeTox's potential to resolve primary mechanisms and downstream cascade effects within a single experiment. Dose-response analyses further showed that predictions can detect toxicity signatures below viability-affecting concentrations.

These results establish DeeTox as a NAM-aligned AI solution for scalable in vitro predictive toxicology, resolving mechanistic heterogeneity in single cells, supporting preclinical safety assessment and AOP-informed regulatory toxicology.