CASE STUDY

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Accurate Prediction of Genotoxic Mechanisms by Phenomics

In drug discovery and development, genotoxicity testing is an essential safety evaluation that must be conducted at a specific stage within the design, make, test, and analyze cycle. Traditional "golden-standard" methods, such as the AMES assay for mutagenicity or DNA damage assessment by micronuclei quantification in mammalian cells using high-content imaging or flow cytometry, are time-consuming, low throughput, costly and often require large amounts of valuable compounds. Therefore, there is a clear need for alternative genotoxicity assessment methods. In collaboration with AstraZeneca, pixlbio has developed a novel approach that combines automated Cell Painting with Artificial Intelligence to provide a comprehensive assessment of various genotoxicities. This innovative method offers a new way to mechanistically understand on- and off-target toxicities, which in turn reduces the resources and time required for pharmaceutical development. This approach also minimizes animal use and ultimately supports the creation of safer medicines.

Our Phenomics-based predictive model for Genotoxicity accurately classifies genotoxic mechanisms. This high-throughput approach significantly reduces the cost and time associated with genotoxicity analysis. As a result, it enables faster safety assessments and more efficient compound triaging at any stage of drug development.

Document No 123456a

Study design and methods

Study design

- Compound library: 153 genotoxic compounds were jointly selected and labeled either as Aneugens, Clastogens, Mutagens and Not-Genotoxic. Doses: 1, 3 and 10µM
- Replicates: 3 technical replicates, 2 biological replicates
- Cell line: A549 lung adenocarcinoma
- Plate format: 384 wells

Methods

Cell culture: A549 lung adenocarcinoma cells were cultured in 384 well plates for high throughput automated processing. pixlbio's automated Cell Painting platform and proprietary optimized protocols and pipelines were used to process a total of **20x384 multiwell plates**, generating a total of circa **280.000 images** (3.3Tb), segmenting **5.6M cells**, and extracting **8.4B cellular features** at single cell resolution. Image analysis: Cell Profiler was used for Quality Control, as well as image analysis, cell segmentation and feature extraction. Post-processing: Single cell features were aggregated per field of view within a well using the median. Feature selection involved removing highly correlated, low variance and constant features. Compounds were annotated at the concentration level using endpoints from the image-based *in vitro* Micronucleus (IVM) assay. A genotoxic label was assigned only to compound doses that exceeded the lowest in vitro micronucleus (mn)-inducing concentration observed in the IVM assay.

Predicting genotoxic mechanisms:

Predicting genotoxic mechanisms with conformal prediction: A mutli-class cross-conformal classifier was trained using random forest with genotoxic mechanisms as labels. Model calibration was performed using a class-conditioned prediction using margin as non-conformity function. For evaluation, the p-values from the test set were inferred and aggregated across all folds.

Predicting lowest micronucleus-inducing concentration: A random forest classifier was trained using binary labels for genotoxicity. Predicted probabilities were obtained from the test set and aggregated at the compound concentration level. For each compound, the predicted lowest concentration was defined as the first dose at which the mean predicted probability exceeds a threshold of 0.5.

Results

Phenotypic reference compounds induce distinct phenotypes

As expected, and as a proof of excellent experimental conditions, the phenotypic reference compounds Etoposide and Paclitaxel, Clastogen and Aneugen respectively, induced morphological changes specific to their mechanism of action.

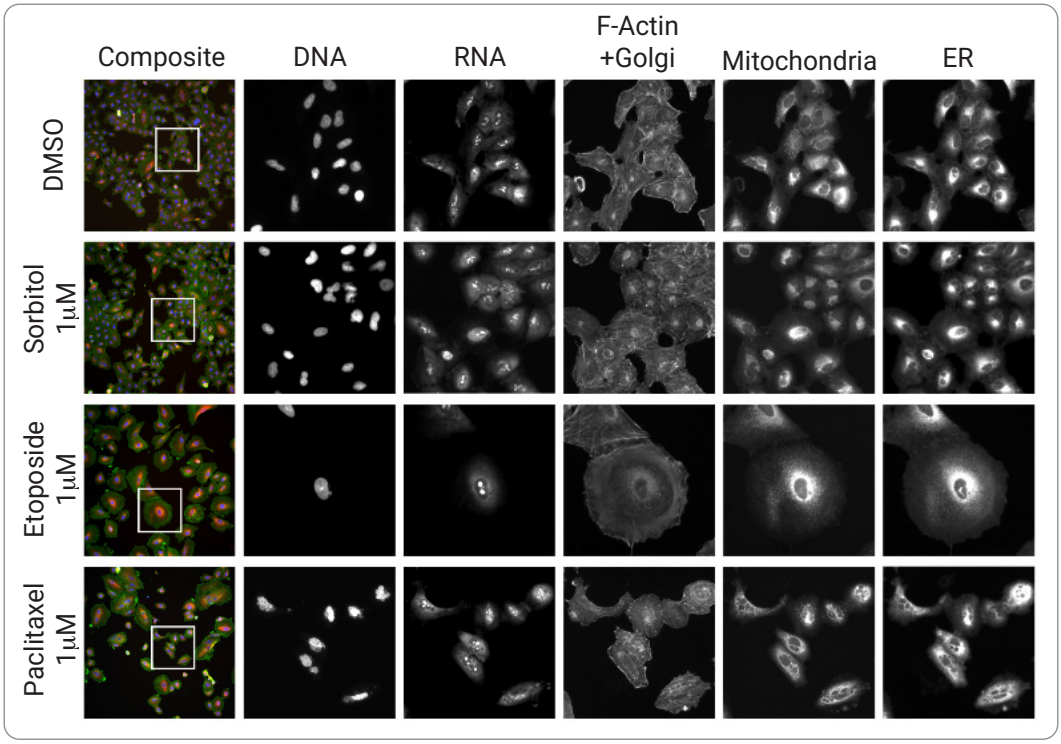

Figure 1: Representative images of the phenotypic reference controls. Clastogenic (Etoposide) and Aneugenic (Paclitaxel) controls exhibit distinct cellular phenotypes. Images obtained with CEPHLA SQUID microscope. Magn.: 20x.

The two major classes of genotoxic compounds, namely Clastogens and Aneugens, induce distinct and unique Cell Painting signatures

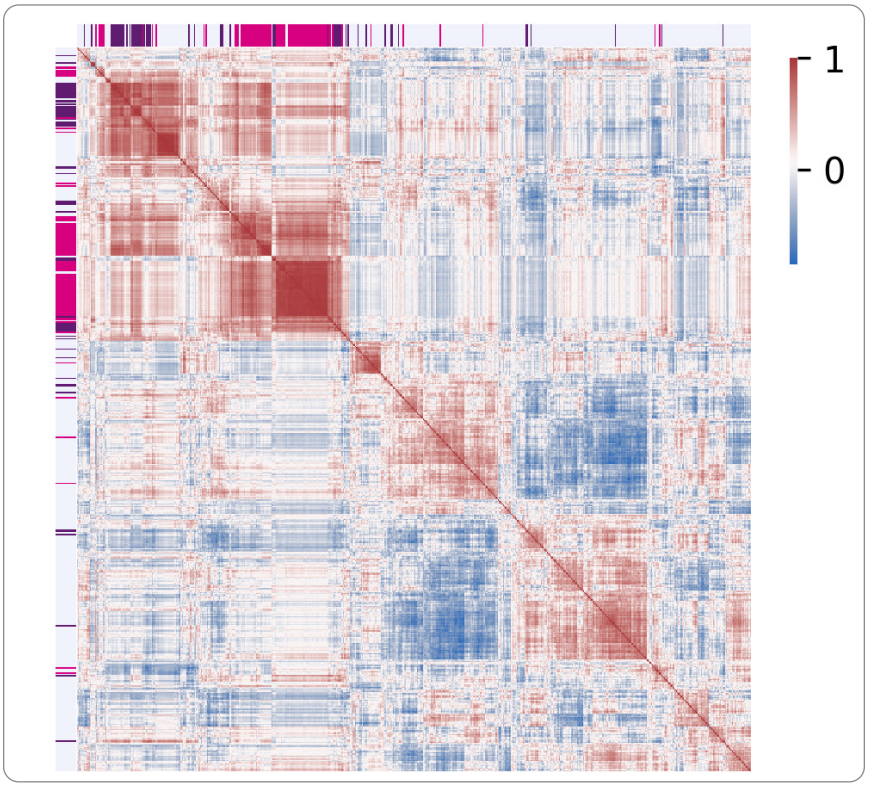

Figure 2: Cosine similarity of compound profiles. Clusters of Clastogenic and Aneugenic compounds colored in magenta and purple, respectively.

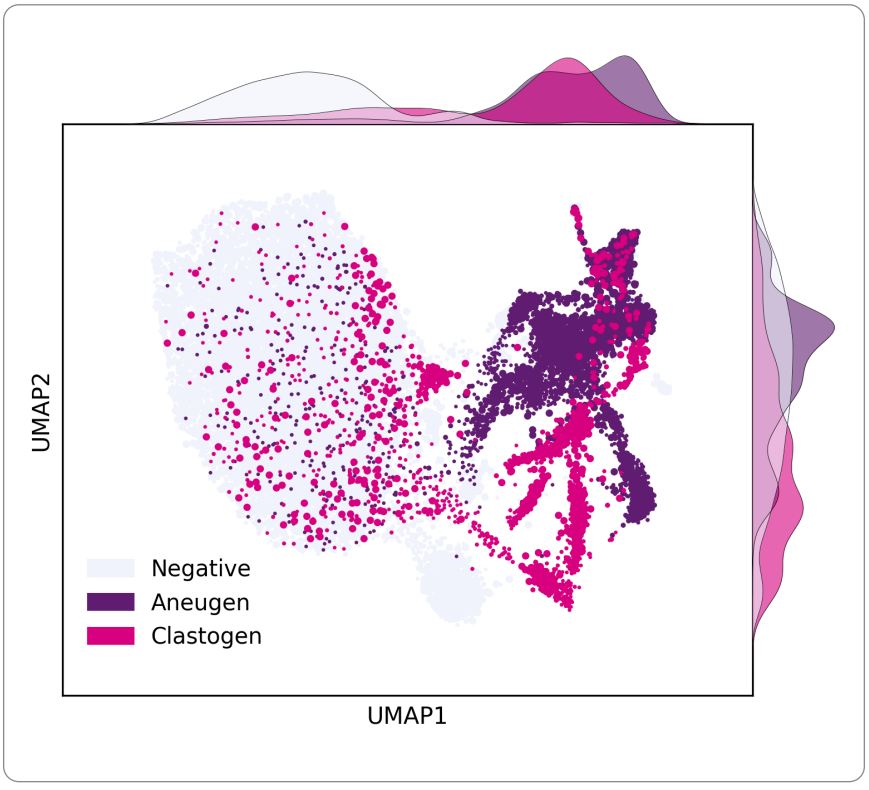

Figure 3: UMAP feature representation. Feature analysis shows separation of Clastogenic and Aneugenic, as well as negative compounds, in the morphological space. Low concentrations of the compounds cluster together with negative compounds and DMSO controls. Increasing concentration is indicated by increasing size of the markers.

Predicting genotoxicity from Phenomics data.

The combination of automated morphological profiling and AI, facilitate the prediction of *in vitro* genotoxicity from Cell Painting data. In many cases, this approach allows for the prediction of genotoxicity at one lower concentration than the reported by the micronuclei assay as ground truth.

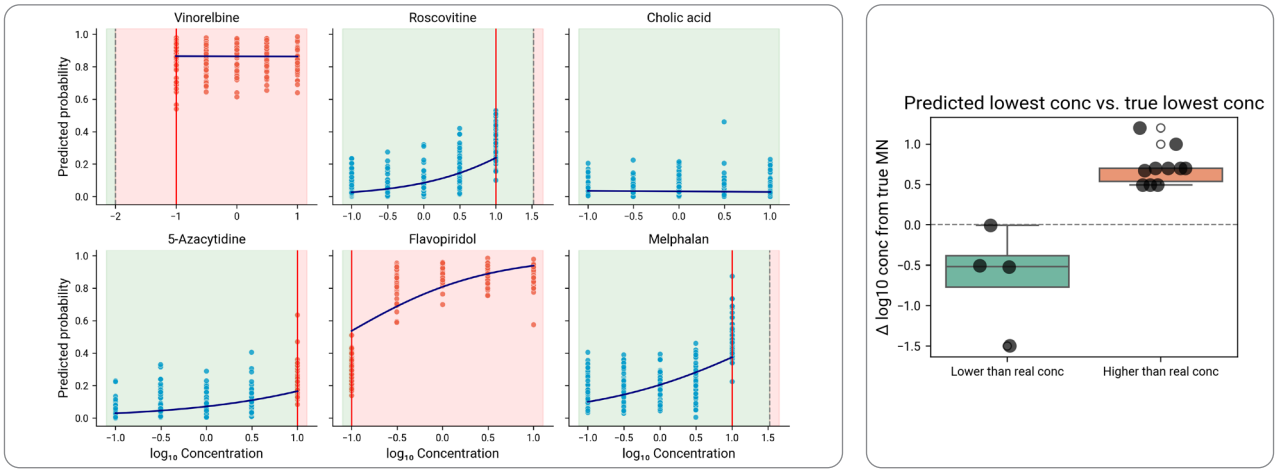

Figure 4: Prediction of lowest mn-inducing concentration. Predictive modeling of the micronucleus (mn) assay demonstrates that Cell Painting can be used to predict lowest genotoxic concentration. Predictivity of dose response with a fitted curve, red line indicates predicted lowest mn-inducing concentration and grey dotted line indicates ground truth mn-positive. Green and red dots represent predicted probabilities (left). Number and distance of predicted lowest concentration compared to true lowest concentration (right).

Conformal prediction allows for accurate classification of genotoxic mechanisms

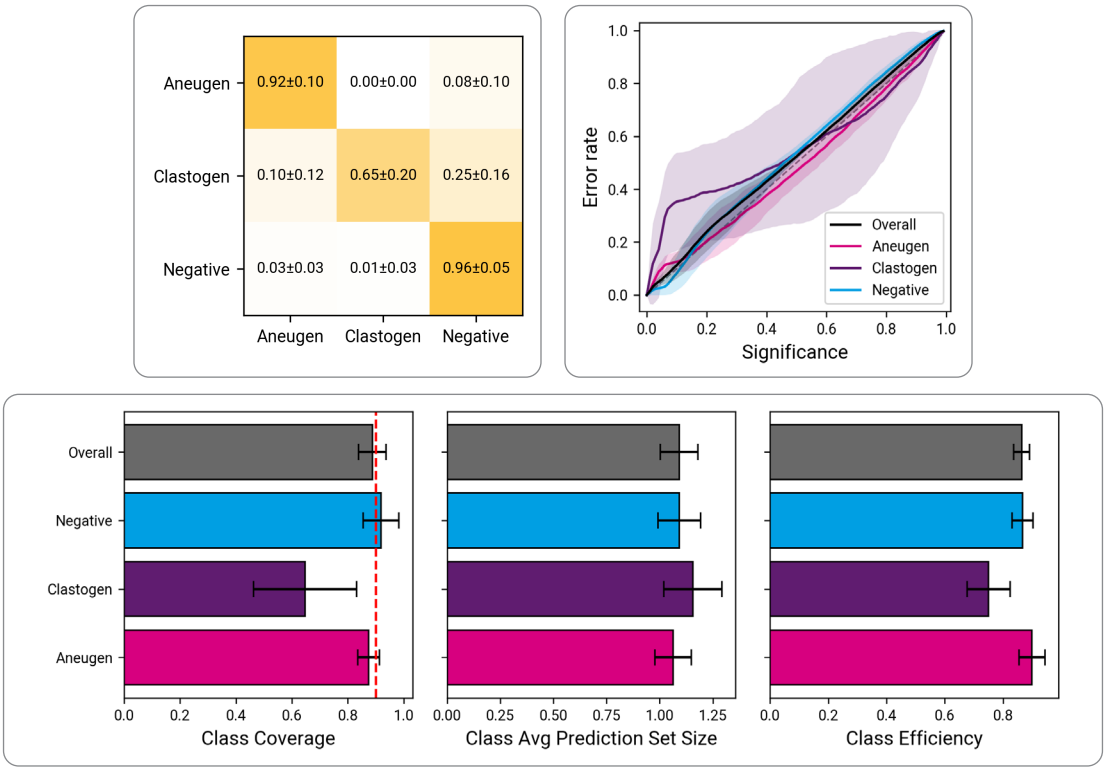

Figure 5: Classification of genotoxic mechanisms. Classification using well calibrated conformal prediction accurately predicts genotoxic mechanisms with high efficiency. Confusion matrix to evaluate the predictive model (top left). Calibration plots showing the observed error rate compared to the significance level of the conformal predictor (top right). Histograms of mean coverage, prediction set size and efficiency calculated per class and overall evaluated at a significance level of 0.1 over all 5 splits, error bars indicate the standard deviation across splits. The red line represents the significance level (bottom).