CLAIMS

What is claimed is:

1. A method for predicting liver injury induced by a test compound comprising:

acquiring images of fluorescently stained cells obtained from a cell culture in which the cells have been treated with a dose-range of at least the test compound and its vehicle;

segmenting the acquired images;

extracting and analyzing one or more phenotypic features from the segmented images, wherein the one or more phenotypic features are selected from the group of intensity, textural, morphological, or ratiometric features consisting of (a) features of DNA, (b) features of a RELA (NF-κB p65 [*NF-κB p65*]), and (c) features of cellular organelles and their substructures in the segmented images;

normalizing results from the treated samples to vehicle controls; and

predicting a probability of liver injury by the test compound based on test compound-induced normalized changes of the extracted and selected phenotypic features using machine learning methods,

wherein extracting and selecting the one or more phenotypic features from the segmented images comprises extracting and selecting at least (i) the coefficient of variation of the intensity of a DNA marker at chromosomal regions in the segmented images, (ii) an average of the intensity of the RELA (NF-κB p65 [*NF-κB p65*]) marker at chromosomal regions in the segmented images, and (iii) a fraction of the intensity of objects based on the RELA (NF-κB p65 [*NF-κB p65*]) marker at nuclear regions in the segmented images.

2. The method in accordance with Claim 1, wherein extracting and selecting the one or more phenotypic features from the segmented images further comprises extracting and selecting

an average of a sum average of the intensity of a DNA marker determined from a grey-level co-occurrence matrix (GLCM) at nuclear regions in the segmented images.

3. The method in accordance with Claim 1, wherein the cells are selected from the group comprising hepatic cells, induced pluripotent stem cell (iPS)-derived hepatocyte-like cells, primary hepatocytes, an (immortalized) hepatocyte cell line, and hepatoma cells.

4. The method in accordance with Claim 3, wherein the hepatic cells are derived from hepatic progenitor-like cells.

5. The method in accordance with Claims 3 and 4, wherein the cells are human cells.

6. The method in accordance with Claims 3 and 4, wherein the cells are animal cells.

7. The method in accordance with any one of Claims 1 to 6 wherein the cells of the cell culture are cultured in a 2D culture.

8. The method in accordance with any one of Claims 1 to 7 further comprising the step of fluorescently labelling the cells for detection of one or more of DNA, RELA (NF-κB p65 [*NF-κB p65*]), F-actin, gammaH2AX and the whole cells.

9. The method in accordance with any one of Claims 1 to 8 wherein treating the cells comprises treating with the at least one test compound and the vehicle for a predetermined time duration of at least twelve hours or overnight.

10. The method in accordance with Claim 9 wherein treating the cells comprises treating with the at least one test compound and the vehicle for a predetermined time duration of sixteen hours or greater.

11. A method for predicting liver injury induced by a test compound comprising:

acquiring images of fluorescently stained cells obtained from a cell culture in which the cells have been treated with a dose-range of at least the test compound and its vehicle;

segmenting the acquired images;

extracting and analyzing one or more phenotypic features from the segmented images, wherein the one or more phenotypic features are selected from the group of intensity, textural, morphological, or ratiometric features consisting of (a) features of DNA, (b) features of a RELA (NF-κB p65 [*NF-κB p65*]) and (c) features of cellular organelles and their substructures in the segmented images;

normalizing results from the treated samples to vehicle controls; and

predicting a probability of liver injury by the test compound based on test compound-induced normalized changes of the extracted and selected phenotypic features using machine learning methods, wherein predicting the probability of liver injury by the test compound comprises:

treating the cells with a dose-range of the test compound and the vehicle;

determining maximal response values (Δ_{max}) with respect to the phenotypic features; and

using machine learning methods to predict hepatotoxicity based on the maximal response values (Δ_{max}) of the compound-induced feature changes, and

wherein extracting and selecting the one or more phenotypic features from the segmented images comprises extracting and selecting at least (i) the coefficient of variation of the intensity

of a DNA marker at chromosomal regions in the segmented images, (ii) an average of the intensity of the RELA (NF-κB p65 [*NF-κB p65*]) marker at chromosomal regions in the segmented images, and (iii) a fraction of the intensity of objects based on the RELA (NF-κB p65 [*NF-κB p65*]) marker at nuclear regions in the segmented images.

12. The method in accordance with Claim 11 wherein using machine learning methods to predict hepatotoxicity comprises using machine learning methods that use a median of the maximal response values (Δ_{max}) of the selected and normalized phenotypic feature changes of the cells for compound classification.

13. The method in accordance with Claim 11, when the maximal response values comprise response values at a concentration of 1 mM, 2 mM or 5 mM of the test compound.

14. The method in accordance with Claim 1 wherein predicting the probability of liver injury comprises:

normalizing the phenotypic features from different test compounds to a same range when more than one test compound is tested; and

predicting liver toxicity by using a machine learning classification algorithm that has been trained with a training set of compounds that is statistically independent from a test set of compounds.

15. The method in accordance with Claim 14, wherein the machine learning classification algorithm is a random-forest algorithm.

16. The method in accordance with Claim 14, wherein the machine learning classification algorithm is a support vector machine.

17. A system for predicting liver injury induced by at least one test compound comprising:

an apparatus for treating a cell culture with the at least one test compound and its vehicle and staining the cell culture for fluorescent detection;

an imaging device arranged with respect to the apparatus to acquire images of the fluorescently stained cells for detecting the cells and their stained components; and

a computing device coupled to the imaging device for receiving and segmenting the acquired images, the computing device comprising a feature extraction module for extracting and analyzing one or more phenotypic features from the segmented images, wherein the one or more phenotypic features are selected from the group of intensity, textural, morphological, or ratiometric features consisting of (a) features of DNA, (b) features of a RELA (NF-κB p65 [*NF-κB p65*]), and (c) features of cellular organelles and their substructures -in the segmented images, the computing device further comprising a prediction module for normalizing results from the treated samples to vehicle controls and predicting a probability of liver injury by the test compound based on test compound-induced normalized changes of the extracted and selected phenotypic features using machine learning methods,

wherein the feature extraction module extracts and selects a phenotypic feature from the segmented images for analysis by measuring at least (i) a coefficient of variation of the intensity of a DNA marker at chromosomal regions in the segmented images, (ii) an average of the intensity of the RELA (NF-κB p65 [*NF-κB p65*]) at chromosomal regions in the segmented images, and (iii) a fraction of the intensity of objects based on the RELA (NF-κB p65 [*NF-κB p65*]) at nuclear regions in the segmented images.

18. The system in accordance with Claim 17 wherein the feature extraction module extracts and selects a phenotypic feature from the segmented images measuring an average of a sum average of the intensity of a DNA marker determined from a grey-level co-occurrence matrix (GLCM) at nuclear regions in the segmented images.

19. The system in accordance with either Claim 17 or Claim 18 wherein the prediction module of the computing device predicts hepatotoxicity by using machine learning methods based on maximum response values (Δ_{max}) with respect to the phenotypic feature changes of the cells treated with the at least one test compound and the vehicle.

20. The system in accordance with Claim 19 wherein the prediction module of the computing device predicts hepatotoxicity by using machine learning methods that uses a median of the maximum response values (Δ_{max}) of the selected and normalized phenotypic feature changes.

21. The system in accordance with Claim 19, wherein the maximum response values comprise response values at a compound concentration of 1mM, 2mM or 5mM of the test compound.

22. The system in accordance with any one of Claims 17 to 20 wherein the prediction module of the computing device predicts hepatotoxicity based on changes of phenotypic features normalized to a same range when more than one test compound is used and by using a machine learning classification algorithm trained with a statistically independent training set of compounds.

23. The system in accordance with Claim 22 wherein the machine learning classification algorithm is a random forest algorithm.

24. The system in accordance with Claim 22 wherein the machine learning classification algorithm is a support vector machine.