

WHAT IS CLAIMED IS:

1. An *in vitro* method for predicting renal proximal tubular cell-specific toxicity of a compound in humans *in vivo*, the method comprising:

contacting a test compound for which renal proximal tubular cell-specific toxicity in humans *in vivo* is to be predicted, with an *in vitro* test population of renal proximal tubular cells grown attached to a support; and

measuring the following features of the renal proximal tubular cells in the test population: cell area and cell roundness, wherein the cell area and cell roundness are measured based on the horizontal dimensions of individual cells in the test population;

comparing the measured features of the test population with the corresponding features of a control population that has not been contacted with the test compound; and

predicting the test compound is specifically toxic for renal proximal tubular cells in humans *in vivo*, based on a decrease in cell area and increase in cell roundness of the test population relative to the control population.

2. The method of claim 1, wherein the measuring comprises use of automated image analysis techniques.

3. The method of claim 1 or 2, further comprising:

examining arrangement of tubulin; and

comparing the arrangement of tubulin of the test population with arrangement of tubulin of a control population that has not been contacted with the test compound;

wherein the predicting the test compound as specifically toxic for renal proximal tubular cells in humans *in vivo* is further based on a change in the arrangement of tubulin of the test population relative to the control population.

4. The method of any one of claims 1 to 3, further comprising:

determining cell numbers of the renal proximal tubular cells in the test population; and

comparing cell count of the test population with cell count of a control population that has not been contacted with the test compound;

wherein the predicting the test compound as specifically toxic for renal proximal tubular cells in humans *in vivo* is further based on a decrease in cell numbers of the test population relative to the control population.

5. The method of any one of claims 1 to 4, further comprising:

examining arrangement of F-actin; and

comparing the arrangement of F-actin of the test population with arrangement of F-actin of a control population that has not been contacted with the test compound;

wherein the predicting the test compound as specifically toxic for renal proximal tubular cells in humans *in vivo* is further based on a change in the arrangement of F-actin of the test population relative to the control population.

6. The method of any one of claims 1 to 5, further comprising:

measuring area of nucleus: area of whole cell ratio; and

comparing the area of nucleus: area of whole cell ratio of the test population with area of nucleus: area of whole cell ratio of a control population that has not been contacted with the test compound;

wherein the predicting test compound as specifically toxic for renal proximal tubular cells in humans *in vivo* is further based on an increase in area of nucleus: area of whole cell ratio of the proximal tubular cells of the test population relative to the control population.

7. The method of any one of claims 1 to 6, further comprising prior to said measuring, examining and/or said determining, labeling the test population with a detectable label.

8. The method of claim 7, further comprising labeling the control population with the detectable label.

9. The method of claim 7 or claim 8, wherein the detectable label is a fluorescent label, or a coloured label.

10. The method of claim 7 or claim 8, wherein the detectable label is a nuclear label, a whole cell label, a cytoplasmic label, a label for cytoskeletal proteins or a cell surface label.

11. The method of any one of claims 1 to 10, wherein the renal proximal tubular cells are derived from somatic cells or from stem cells.

12. The method of claim 11, wherein the renal proximal tubular cells are derived from somatic cells and are primary cells or are cells from a stable cell line.
13. The method of claim 12, wherein the renal proximal tubular cells are human primary renal proximal tubular cells, HK-2 cells, or LLC-PK1 cells.
14. The method of claim 11, wherein the renal proximal tubular cells are derived from stem cells and are differentiated from embryonic stem cells, mesenchymal stem cells, or induced pluripotent stem cells.
15. The method of any one of claims 1 to 14, wherein the renal proximal tubular cells are human renal proximal tubular cells.
16. The method of any one of claims 1 to 14, wherein the renal proximal tubular cells are non-human renal proximal tubular cells.
17. The method of any one of claims 1 to 16, wherein said contacting is performed over a period of time of about 8 hours or longer.
18. The method of any one of claims 1 to 17, wherein said contacting is repeated one or more times in a period of from about 3 to about 14 days.
19. The method of any one of claims 1 to 18, wherein said contacting comprises adding the test compound to the test population of renal proximal tubular cells at a concentration of about 0.001 to about 1000 $\mu\text{g/ml}$.

WHAT IS CLAIMED IS:

1. An *in vitro* method of ~~screening for~~ predicting renal proximal tubular cell-specific toxicity of a compound in humans *in vivo*, the method comprising:

~~contacting a test compound that is to be screened for~~ which renal proximal tubular cell-specific toxicity in humans *in vivo* is to be predicted, with an *in vitro* test population of renal proximal tubular cells grown attached to a support; and

~~measuring one or both of the following features of the renal proximal tubular cells in the test population: cell area; and cell roundness, area of nucleus; area of whole cell ratio~~ wherein the cell area and cell roundness are measured based on the horizontal dimensions of individual cells in the test population;

~~comparing the one or more measured~~ features of the test population with the corresponding ~~one or more~~ features of a control population that has not been contacted with the test compound; and

~~identifying-predicting the test compound as is~~ specifically toxic for renal proximal tubular cells in humans *in vivo*, based on ~~one or both of a decrease in cell area and increase in cell roundness, and an increase in area of nucleus; area of whole cell ratio~~ of the test population relative to the control population.

2. The method of claim 1, wherein the measuring comprises use of automated image analysis techniques.

3. The method of claim 1 or 2, further comprising:

examining arrangement of tubulin; and

comparing the arrangement of tubulin of the test population with arrangement of tubulin of a control population that has not been contacted with the test compound;

wherein the ~~identifying-of-predicting~~ the test compound as specifically toxic for renal proximal tubular cells in humans *in vivo* is further based on a change in the arrangement of tubulin of the test population relative to the control population.

4. The method of any one of claims 1 to 3, further comprising:

determining cell numbers of the renal proximal tubular cells in the test population; and

comparing cell count of the test population with cell count of a control population that has not been contacted with the test compound;

wherein the ~~identifying of~~predicting the test compound as specifically toxic for renal proximal tubular cells in humans in vivo is further based on a decrease in cell numbers of the test population relative to the control population.

5. The method of any one of claims 1 to 4, further comprising:

examining arrangement of F-actin; and

comparing the arrangement of F-actin of the test population with arrangement of F-actin of a control population that has not been contacted with the test compound;

wherein the ~~identifying of~~predicting the test compound as specifically toxic for renal proximal tubular cells in humans in vivo is further based on a change in the arrangement of F-actin of the test population relative to the control population.

6. The method of any one of claims 1 to 5, further comprising:

~~examining cell roundness~~measuring area of nucleus: area of whole cell ratio; and

comparing the ~~cell roundness~~ area of nucleus: area of whole cell ratio of the test population with ~~cell roundness~~ area of nucleus: area of whole cell ratio of a control population that has not been contacted with the test compound;

wherein the ~~identifying of the~~predicting test compound as specifically toxic for renal proximal tubular cells in humans in vivo is further based on an increase in area of nucleus: area of whole cell ratio ~~rounding~~ of the proximal tubular cells of the test population relative to the control population.

7. The method of any one of claims 1 to 6, further comprising prior to said measuring, examining and/or said determining, labeling the test population with a detectable label.

8. The method of claim 7, further comprising labeling the control population with the detectable label.

9. The method of claim 7 or claim 8, wherein the detectable label is a fluorescent label, or a coloured label.

10. The method of claim 7 or claim 8, wherein the detectable label is a nuclear label, a whole cell label, a cytoplasmic label, a label for cytoskeletal proteins or a cell surface label.

11. The method of any one of claims 1 to 10, wherein the renal proximal tubular cells are derived from somatic cells or from stem cells.

12. The method of claim 11, wherein the renal proximal tubular cells are derived from somatic cells and are primary cells or are cells from a stable cell line.
13. The method of claim 12, wherein the renal proximal tubular cells are human primary renal proximal tubular cells, HK-2 cells, or LLC-PK1 cells.
14. The method of claim 11, wherein the renal proximal tubular cells are derived from stem cells and are differentiated from embryonic stem cells, mesenchymal stem cells, or induced pluripotent stem cells.
15. The method of any one of claims 1 to 14, wherein the renal proximal tubular cells are human renal proximal tubular cells.
16. The method of any one of claims 1 to 14, wherein the renal proximal tubular cells are non-human renal proximal tubular cells.
17. The method of any one of claims 1 to 16, wherein said contacting is performed over a period of time of about 8 hours or longer.
18. The method of any one of claims 1 to 17, wherein said contacting is repeated one or more times in a period of from about 3 to about 14 days.
19. The method of any one of claims 1 to 18, wherein said contacting comprises adding the test compound to the test population of renal proximal tubular cells at a concentration of about 0.001 to about 1000 $\mu\text{g/ml}$.