

WHAT IS CLAIMED IS:

1. A method of determining the efficacy of a glucosyl ceramide synthase (GCS) inhibitor in a polycystic kidney disease (PKD) patient, the method comprising:

(a) providing a first sample comprising urine obtained from the PKD patient at a first time point;

(b) concentrating the first sample for exosomes;

(c) determining the level(s) of at least one marker selected from the group consisting of Proliferating Cell Nuclear Antigen (PCNA), cyclin D1, cyclin D3, MAPK-ERK kinase 1 (MEK), ribosomal protein S6 (S6), phosphorylated ribosomal protein S6 (pS6), extracellular signal-regulated kinase (ERK), phosphorylated extracellular signal-regulated kinase (pERK), protein kinase B (Akt), phosphorylated protein kinase B (pAkt), caspase-2, total ribosomal protein S6, and retinoblastoma binding protein (RBBP) in the concentrated first sample of step (b);

(d) providing a second sample comprising urine obtained from the PKD patient at a second time point and performing steps (b) and (c) on the second sample, wherein the PKD patient received a GCS inhibitor between the first and second time points; and

(e) identifying the GCS inhibitor as being effective if the level of at least one marker is decreased at the second time point as compared to the first time point.

2. The method of claim 1, wherein the GCS inhibitor is selected from the group consisting of:

(S)-quinuclidin-3-yl (2-(4'-(2-methoxyethoxy)-[1,1'-biphenyl]-4-yl)propan-2-yl)carbamate;

4-fluoro-1-(5-fluoro-4-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-(4-(methoxymethyl)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-(4-(methoxymethyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-(4-(2-methoxyethoxy)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-(4-(2-methoxyethoxy)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-(4-(2-fluoroethoxy)phenyl)pyrimidin-2-yl)-N-(quinuclidin-3-yl)piperidine-4-carboxamide; and

4-fluoro-1-(4-(4-(2-fluoroethoxy)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide.

3. The method of claim 1, wherein after step (e), additional doses of the GCS inhibitor identified as being effective is selected for the PKD patient.

4. The method of claim 3, wherein the GCS inhibitor identified as being effective is selected from the group consisting of:

(S)-quinuclidin-3-yl (2-(4'-(2-methoxyethoxy)-[1,1'-biphenyl]-4-yl)propan-2-yl)carbamate;

4-fluoro-1-(5-fluoro-4-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-(4-(methoxymethyl)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-(4-(methoxymethyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-(4-(2-methoxyethoxy)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-(4-(2-methoxyethoxy)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-(4-(2-fluoroethoxy)phenyl)pyrimidin-2-yl)-N-(quinuclidin-3-yl)piperidine-4-carboxamide; and

4-fluoro-1-(4-(4-(2-fluoroethoxy)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide.

5. The method of claim 1, wherein steps (c) and (d) comprise determining the levels of at least two markers selected from the group consisting of PCNA, cyclin D1, cyclin D3, MEK, S6, and pS6, and the GCS inhibitor is identified as being effective if at least one of the two levels is decreased at the second time point as compared to the first time point.

6. The method of claim 5, wherein the GCS inhibitor is identified as being effective if both levels are decreased at the second time point as compared to the first time point.

7. The method of claim 1, wherein steps (c) and (d) comprise determining the levels of at least three markers selected from the group consisting of PCNA, cyclin D1, cyclin D3, MEK, S6, and pS6, and the GCS inhibitor is identified as being effective if at least one of the three levels is decreased at the second time point as compared to the first time point.

8. The method of claim 7, wherein the GCS inhibitor is identified as being effective if all three levels are decreased at the second time point as compared to the first time point.

9. The method of claim 1, wherein steps (c) and (d) comprise determining the levels of at least four markers selected from the group consisting of PCNA, cyclin D1, cyclin D3, MEK, S6, and pS6, and the GCS inhibitor is identified as being effective if at least one of the four levels is decreased at the second time point as compared to the first time point.

10. The method of claim 9, wherein the GCS inhibitor is identified as being effective if all four levels are decreased at the second time point as compared to the first time point.

11. The method of claim 1, wherein the concentrating in (b) and (d) includes ultracentrifuging the first and second samples, respectively.

12. The method of claim 1, wherein determining the level(s) of at least one marker in (c) and (d) comprise determining the level of protein of the at least one marker.

13. The method of claim 12, wherein (c) and (d) comprise contacting the sample with antibodies that bind specifically to the protein of the at least one marker.

14. The method of claim 1, wherein (c) and (d) comprises determining the level of one or both of cyclin D1 and MEK.

15. The method of claim 1, wherein after (e), the PKD patient received additional doses of the treatment identified as being effective.

16. A method of diagnosing polycystic kidney disease (PKD) in a patient comprising:
(a) providing a sample comprising urine from a patient suspected of having PKD;
(b) concentrating the sample for exosomes;
(c) determining the level(s) of at least one marker selected from the group consisting of Proliferating Cell Nuclear Antigen (PCNA), cyclin D1, cyclin D3, MAPK-ERK kinase 1 (MEK), and ribosomal protein S6 (S6) in the concentrated sample; and
(d) identifying the patient as having PKD if the level is elevated as compared to a control level.

17. The method of claim 16, wherein step (c) comprises determining the levels of at least two markers selected from the group consisting of PCNA, cyclin D1, cyclin D3, MEK, and S6 and the patient is identified as having PKD if at least one of the two levels are elevated as compared to control level(s).

18. The method of claim 17, wherein the patient is identified as having PKD if both levels are elevated as compared to control levels.

19. The method of claim 16, wherein step (c) comprises determining the levels of at least three markers selected from the group consisting of PCNA, cyclin D1, cyclin D3, MEK, and S6, and the patient is identified as having PKD if at least one of the three levels are elevated as compared to control levels.

20. The method of claim 19, wherein the patient is identified as having PKD if all three levels are elevated as compared to control levels.

21. The method of claim 16, wherein step (c) comprises determining the levels of at least four markers selected from the group consisting of PCNA, cyclin D1, cyclin D3, MEK, and S6, and the patient is identified as having PKD if at least one of the four levels are elevated as compared to control levels.

22. The method of claim 21, wherein the patient is identified as having PKD if all four levels are elevated as compared to control levels.

23. The method of claim 16, wherein concentrating in (b) includes ultracentrifuging the sample.

24. The method of claim 16, wherein determining the level(s) of at least one marker in (c) comprises determining the level of protein of the at least one marker.

25. The method of claim 24, wherein (c) comprises contacting the concentrated sample with antibodies that bind specifically to the protein of the at least one marker.

26. The method of claim 16, wherein (c) comprises determining the level of at least two of PCNA, cyclin D1, cyclin D3, MEK, and S6.

27. The method of claim 16, wherein after step (d), the identified patient receives a glucosyl ceramide synthase (GCS) inhibitor-treatment for PKD.

28. The method of claim 27, wherein the GCS inhibitor is selected from the group consisting of:

(S)-quinuclidin-3-yl (2-(4'-(2-methoxyethoxy)-[1,1'-biphenyl]-4-yl)propan-2-yl)carbamate;

4-fluoro-1-(5-fluoro-4-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-(4-(methoxymethyl)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-(4-(methoxymethyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-(4-(2-methoxyethoxy)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-(4-(2-methoxyethoxy)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-(4-(2-fluoroethoxy)phenyl)pyrimidin-2-yl)-N-(quinuclidin-3-yl)piperidine-4-carboxamide; and

4-fluoro-1-(4-(4-(2-fluoroethoxy)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide.

29. The method of claim 16, further comprising after (d): (e) imaging one or both kidney(s) in a patient identified as having PKD.

30. The method of claim 16, wherein the control level is a threshold level or a level in a healthy subject or a population of healthy subjects.

31. A method of determining the stage of polycystic kidney disease (PKD) in a patient comprising:

- (a) providing a sample comprising urine from a patient suspected of having PKD or identified as having PKD;
- (b) concentrating the sample for exosomes;
- (c) determining the level(s) of at least one marker selected from the group consisting of Proliferating Cell Nuclear Antigen (PCNA), cyclin D1, cyclin D3, MAPK-ERK kinase 1 (MEK), and ribosomal protein S6 (S6) in the concentrated sample; and
- (d) determining the stage of PKD in the patient from the level.

32. The method of claim 31, wherein step (c) comprises determining the levels of at least two markers selected from the group consisting of PCNA, cyclin D1, cyclin D3, MEK, and S6, and the stage of PKD in the patient is determined from at least one of the two levels.

33. The method of claim 32, wherein the stage of PKD in the patient is determined from both levels.

34. The method of claim 31, wherein step (c) comprises determining the levels of at least three markers selected from the group consisting of PCNA, cyclin D1, cyclin D3, MEK, and S6, and the stage of PKD in the patient is determined from at least one of the three levels.

35. The method of claim 34, wherein the stage of PKD in the patient is determined from all three levels.

36. The method of claim 31, wherein step (c) comprises determining the levels of at least four markers selected from the group consisting of PCNA, cyclin D1, cyclin D3, MEK, and S6, and the stage of PKD in the patient is determined from at least one of the four levels.

37. The method of claim 36, wherein the stage of PKD in the patient is determined from all four levels.

38. The method of claim 31, wherein concentrating in (b) includes ultracentrifuging the sample.

39. The method of claim 31, wherein determining the level(s) of at least one marker in (c) comprises determining the level of protein of the at least one marker.

40. The method of claim 39, wherein (c) comprises contacting the concentrated sample with antibodies that bind specifically to the protein of the at least one marker.

41. The method of claim 31, wherein (c) comprises determining the level of at least two of PCNA, cyclin D3, MEK, and S6.

42. The method of claim 31, further comprising after (d), a patient identified to have stage I, stage II, stage III, stage IV, or stage V PKD receives a treatment for stage I, stage II, stage III, stage IV, or stage V PKD, respectively.

43. The method of claim 31, further comprising after (d): (e) imaging one or both kidney(s) in a patient after (d) to confirm the stage of PKD in the patient.

44. A method of monitoring polycystic kidney disease (PKD) patient, the method comprising:

(a) providing a first sample comprising urine obtained from the PKD patient at a first time point;

(b) concentrating the first sample for exosomes;

(c) determining the level(s) of at least one marker selected from the group consisting of Proliferating Cell Nuclear Antigen (PCNA), cyclin D1, cyclin D3, MAPK-ERK kinase 1 (MEK), and ribosomal protein S6 (S6) in the concentrated first sample;

(d) providing a second sample comprising urine obtained from the PKD patient at a second time point after the first time point, and performing steps (b) and (c) on the second sample; and

(e) identifying the patient as having improving or static PKD if the level is not elevated at the second time point as compared to the level at the first time point.

45. The method of claim 44, wherein steps (c) and (d) comprise determining the levels of at least two markers selected from the group consisting of PCNA, cyclin D1, cyclin D3, MEK, and S6, and the patient is identified as having improving or static PKD if at least one of the two levels is not elevated at the second time point as compared to the first time point.

46. The method of claim 45, wherein the patient is identified as having improving or static PKD if both levels are not elevated at the second time point as compared to the first time point.

47. The method of claim 44, wherein steps (c) and (d) comprise determining the levels of at least three markers selected from the group consisting of PCNA, cyclin D1, cyclin D3, MEK, and S6, and the patient is identified as having improving or static PKD if at least one of the three levels is not elevated at the second time point as compared to the first time point.

48. The method of claim 47, wherein the patient is identified as having improving or static PKD if all three levels are not elevated at the second time point as compared to the first time point.

49. The method of claim 44, wherein steps (c) and (d) comprise determining the levels of at least four markers selected from the group consisting of PCNA, cyclin D1, cyclin D3, MEK, and S6, and the patient is identified as having improving or static PKD if at least one of the four levels are not elevated at the second time point as compared to the first time point.

50. The method of claim 49, wherein the patient is identified as having improving or static PKD if all four levels are not elevated at the second time point as compared to the first time point.

51. The method of claim 44, wherein concentrating in (b) and (d) includes ultracentrifuging the sample.

52. The method of claim 44, wherein determining the level(s) of at least one marker in (c) and (d) comprise determining the level of protein of the at least one marker.

53. The method of claim 52, wherein (c) and (d) comprise contacting the concentrated sample with antibodies that bind specifically to the protein of the at least one marker.

54. The method of claim 44, wherein (c) and (d) comprise determining the level of at least two of PCNA, cyclin D3, MEK, and S6.

55. The method of claim 44, wherein the PKD patient received a treatment prior to the second time point.