

WHAT IS CLAIMED IS:

1. A method of determining the efficacy of treatment for polycystic kidney disease (PKD) in a PKD patient, the method comprising:

(a) providing a first sample comprising a biological fluid 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 first sample;

(d) administering a treatment for PKD to the PKD patient;

(e) providing a second sample comprising a biological fluid obtained from the PKD patient at a second time point after step (d) and performing steps (b) and (c) on the second sample; and

(f) identifying the administered treatment as being effective if the level is decreased at the second time point as compared to the first time point.

2. The method of claim 1, wherein the administered treatment is administration of a glucosyl ceramide synthase (GCS) inhibitor.

3. The method of claim 2, 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.

4. The method of claim 2, further comprising after (f): (g) administering additional doses of the administered GCS inhibitor identified as being effective to the PKD patient.

5. The method of claim 4, wherein in step (g) the PKD patient is administered additional doses 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; or

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.

6. The method of claim 1, wherein steps (c) and (e) 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 administered treatment 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.

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

8. The method of claim 1, wherein steps (c) and (e) 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 administered treatment 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.

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

10. The method of claim 1, wherein steps (c) and (e) 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 administered treatment 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.

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

12. The method of claim 1, wherein the administered treatment is administration of a CDK inhibitor to the PKD patient.

13. The method of claim 12, wherein the CDK inhibitor is R-roscovitine.

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

15. The method of claim 1, wherein the first and second samples comprise urine.

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

17. The method of claim 16, wherein (c) and (e) comprise contacting the sample with antibodies that bind specifically to the protein of the at least one marker.

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

19. The method of claim 1, further comprising after (f): (g) administering additional doses of the administered treatment identified as being effective to the PKD patient.

20. The method of claim 19, wherein the administered treatment identified as being effective is a CDK inhibitor, and in step (g) the PKD patient is administered additional doses of the CDK inhibitor.

21. The method of claim 20, wherein in step (g) the PKD patient is administered additional doses of S-CR8.

22. A method of diagnosing polycystic kidney disease (PKD) in a patient comprising:

- (a) providing a sample comprising a biological fluid 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), 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 S6, and retinoblastoma binding protein (RBBP) in the sample; and
- (d) identifying the patient as having PKD if the level is elevated as compared to a control level.

23. The method of claim 22, wherein step (c) comprises 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 patient is identified as having PKD if at least one of the two levels are elevated as compared to control level(s).

24. The method of claim 23, wherein the patient is identified as having PKD if both levels are elevated as compared to control levels.

25. The method of claim 22, wherein step (c) comprises 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 patient is identified as having PKD if at least one of the three levels are elevated as compared to control levels.

26. The method of claim 25, wherein the patient is identified as having PKD if all three levels are elevated as compared to control levels.

27. The method of claim 22, wherein step (c) comprises 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 patient is identified as having PKD if at least one of the four levels are elevated as compared to control levels.

28. The method of claim 27, wherein the patient is identified as having PKD if all four levels are elevated as compared to control levels.

29. The method of claim 22, wherein concentrating in (b) includes ultracentrifuging the sample.

30. The method of claim 22, wherein the sample comprises urine.

31. The method of claim 22, wherein determining the level(s) of at least one marker in (c) comprises determining the level of protein of the at least one marker.

32. The method of claim 31, wherein (c) comprises contacting the sample with antibodies that bind specifically to the protein of the at least one marker.

33. The method of claim 22, wherein (c) comprises determining the level of at least two of PCNA, cyclin D1, cyclin D3, MEK, S6, and phosphorylated S6.

34. The method of claim 22, further comprising after step (d): (e) administering a treatment for PKD to a patient identified as having PKD.

35. The method of claim 34, wherein the treatment is administering a glucosyl ceramide synthase (GCS) inhibitor to the patient.

36. The method of claim 35, 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.

37. The method of claim 34, wherein the treatment is administering a CDK inhibitor to the patient.

38. The method of claim 37, wherein the CDK inhibitor is S-CR8.

39. The method of claim 22, further comprising after (d): (e) imaging one or both kidney(s) in a patient identified as having PKD.

40. The method of claim 22, wherein the control level is a threshold level or a level in a healthy subject or a population of healthy subjects.

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

(a) providing a sample comprising a biological fluid 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), 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 S6, and retinoblastoma binding protein (RBBP) in the sample; and

(d) determining the stage of PKD in the patient from the level.

42. The method of claim 41, wherein step (c) comprises 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 stage of PKD in the patient is determined from at least one of the two levels.

43. The method of claim 42, wherein the stage of PKD in the patient is determined from both levels.

44. The method of claim 41, wherein step (c) comprises 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 stage of PKD in the patient is determined from at least one of the three levels.

45. The method of claim 44, wherein the stage of PKD in the patient is determined from all three levels.

46. The method of claim 41, wherein step (c) comprises 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 stage of PKD in the patient is determined from at least one of the four levels.

47. The method of claim 46, wherein the stage of PKD in the patient is determined from all four levels.

48. The method of claim 41, wherein concentrating in (b) includes ultracentrifuging the sample.

49. The method of claim 41, wherein the sample comprises urine.

50. The method of claim 41, wherein determining the level(s) of at least one marker in (c) comprises determining the level of protein of the at least one marker.

51. The method of claim 50, wherein (c) comprises contacting the sample with antibodies that bind specifically to the protein of the at least one marker.

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

53. The method of claim 41, further comprising after (d): (e) administering a treatment for stage I, stage II, stage III, stage IV, or stage V PKD to a patient identified to have stage I, stage II, stage III, stage IV, or stage V PKD, respectively.

54. The method of claim 41, 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.

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

(a) providing a first sample comprising a biological fluid 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 S6, and retinoblastoma binding protein (RBBP) in the first sample;

(d) providing a second sample comprising a biological fluid 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.

56. The method of claim 55, 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 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.

57. The method of claim 56, 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.

58. The method of claim 55, 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 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.

59. The method of claim 58, 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.

60. The method of claim 55, 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 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.

61. The method of claim 60, 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.

62. The method of claim 55, wherein concentrating in (b) and (d) includes ultracentrifuging the sample.

63. The method of claim 55, wherein the first and second samples comprise urine.

64. The method of claim 55, 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.

65. The method of claim 64, wherein (c) and (d) comprise contacting the sample with antibodies that bind specifically to the protein of the at least one marker.

66. The method of claim 55, wherein (c) and (d) comprise determining the level of at least two of PCNA, cyclin D3, MEK, and phosphorylated S6.

67. The method of claim 55, further comprising after (e): (f) administering the same treatment to a patient identified as having improving or static PKD.