

### CLAIMS

We claim:

1. An *in vitro* blood-brain barrier (BBB) system comprising E4ORF1+ endothelial cells.
2. The system of claim 1, wherein the endothelial cells are mouse endothelial cells.
3. The system of claim 1, wherein the endothelial cells are human endothelial cells.
4. The system of claim 1, wherein the endothelial cells are brain endothelial cells.
5. The system of claim 4, wherein the brain endothelial cells are brain microvascular endothelial cells.
6. The system of claim 4, wherein the brain endothelial cells are derived from a brain tumor.
7. The system of claim 4, wherein the brain endothelial cells are derived from a glioma.
8. The system of claim 4, wherein the brain endothelial cells are derived from a glioblastoma (GBM).
9. The system of claim 1, wherein the E4ORF1+ endothelial cells are disposed upon a permeable solid support.
10. The system of claim 9, wherein the permeable solid support is a membrane or filter.
11. The system of claim 9, wherein the permeable solid support comprises polyester, polycarbonate, nitrocellulose, cellulose, or fiberglass.
12. The system of claim 9, wherein the permeable solid support is coated with one or more extracellular matrix components.
13. The system of claim 12, wherein the extracellular matrix components are selected from the group consisting of astrocyte extracellular matrix, laminin, vitronectin, fibronectin, collagen, and Matrigel.

14. The system of claim 1, further comprising an additional cell type selected from the group consisting of astrocytes, pericytes, and neural stem/progenitor cells (NSPCs).
15. The system of claim 14, wherein the astrocytes are mouse astrocytes.
16. The system of claim 14, wherein the astrocytes are human astrocytes.
17. The system of claim 14, wherein the astrocytes are in direct contact with the endothelial cells.
18. The system of claim 1, wherein the endothelial cells are present in an essentially confluent endothelial monolayer.
19. The system of claim 1, comprising an essentially confluent monolayer of astrocytes.
20. The system according to claim 18, wherein the endothelial cell monolayer has a trans endothelial electrical resistance (TEER) of greater than about  $25 \Omega \cdot \text{cm}^2$ , or greater than about  $50 \Omega \cdot \text{cm}^2$ , or greater than about  $75 \Omega \cdot \text{cm}^2$ , or greater than about  $100 \Omega \cdot \text{cm}^2$ , or greater than about  $125 \Omega \cdot \text{cm}^2$ , or greater than about  $150 \Omega \cdot \text{cm}^2$ , or greater than about  $175 \Omega \cdot \text{cm}^2$ , or greater than about  $200 \Omega \cdot \text{cm}^2$ .
21. The system according to claim 18 wherein the endothelial cell monolayer has a Lucifer Yellow permeability coefficient (Pc) of less than about  $2.5 \times 10^{-6} \text{ cm/s}$ .
22. Use of a system according to claim 1 in a method for assessing the blood-brain barrier permeability of a candidate agent.
23. A method for assessing the blood-brain barrier permeability of a candidate agent, the method comprising contacting the endothelial cells in the system of claim 1 with a candidate agent.
24. A method for assessing the blood-brain barrier permeability of a candidate agent, the method comprising: (a) contacting one side of an endothelial cell monolayer in a BBB system according to claim 1 with a candidate agent, and (b) determining whether the candidate agent is present on the other side of the endothelial cell monolayer, wherein if

the candidate agent is present on the other side of the endothelial cell monolayer the agent is blood-brain-barrier permeable, and wherein if the candidate agent is not present on the other side of the endothelial cell monolayer the agent is not blood-brain-barrier permeable.

25. Use of a system according to any one of claims 1 in a method for assessing the effect of an agent on the permeability of the blood-brain barrier.
26. A method for assessing the effect of an agent on the permeability of the blood-brain barrier, the method comprising: contacting the endothelial cells in a system according to claim 1 with a candidate agent.
27. A method for assessing the effect of an agent on the permeability of the blood-brain barrier, the method comprising: (a) contacting the endothelial cells in a BBB system according to claim 1 with a candidate agent, and (b) determining the trans endothelial electrical resistance (TEER) of the BBB system, wherein if the candidate agent decreases the TEER value of the BBB system as compared to the TEER value in the absence of the candidate agent, then the candidate agent increases the permeability of the BBB, and if the candidate agent increases the TEER value of the BBB system as compared to the TEER value in the absence of the candidate agent, then the candidate agent reduces the permeability of the BBB.
28. A method for assessing the effect of an agent on the permeability of the blood-brain barrier, the method comprising: contacting the endothelial cells in a BBB system according to claim 1 with a candidate agent, and determining the permeability coefficient (Pc) of the BBB system, wherein if the candidate agent decreases the Pc value of the BBB system as compared to the Pc value in the absence of the candidate agent, then the candidate agent decreases the permeability of the BBB, and if the candidate agent increases the Pc value of the BBB system as compared to the Pc value in the absence of the candidate agent, then the candidate agent increases the permeability of the BBB.
29. A composition comprising E4ORF1+ endothelial cells and astrocytes, and optionally also comprising (a) pericytes and/or (b) neural stem/progenitor cells (NSPCs).

30. The composition of claim 29, wherein the endothelial cells are mouse E4ORF1+ endothelial cells.
31. The composition of claim 29, wherein the E4ORF1+ endothelial cells are human E4ORF1+ endothelial cells.
32. The composition of claim 29, wherein the E4ORF1+ endothelial cells are brain endothelial cells.
33. The composition of claim 32, wherein the brain endothelial cells are brain microvascular endothelial cells.
34. The composition of claim 32, wherein the brain endothelial cells are derived from a brain tumor.
35. The composition of claim 32, wherein the brain endothelial cells are derived from a glioma.
36. The composition of claim 32, wherein the brain endothelial cells are derived from a glioblastoma.
37. The composition of claim 29, wherein the astrocytes are mouse astrocytes.
38. The composition of claim 29, wherein the astrocytes are human astrocytes.
39. The composition of claim 29, wherein the astrocytes are in direct contact with the endothelial cells.
40. The composition according to any of the preceding claims wherein the endothelial cells are present in an essentially confluent endothelial monolayer.
41. The composition according to any of the preceding claims wherein the astrocytes are present in an essentially confluent monolayer.
42. A composition according to any of claims 29-41 wherein the composition is a therapeutic composition.

43. A composition according to claim 42, wherein the composition further comprises one or more pharmaceutically acceptable diluents.
44. A method of treating a defect, disease, or disorder of the blood-brain barrier, the method comprising administering to a subject in need thereof a composition comprising E4ORF1+ endothelial cells.
45. The method of claim 44, wherein the subject is a mouse and the endothelial cells are mouse endothelial cells.
46. The method of claim 44, wherein the subject is human and the endothelial cells are human endothelial cells.
47. The method of claim 44, wherein the endothelial cells are brain endothelial cells.
48. The method of claim 47, wherein the brain endothelial cells are brain microvascular endothelial cells.
49. The method of claim 47, wherein the brain endothelial cells are derived from a brain tumor.
50. The method of claim 47, wherein the brain endothelial cells are derived from a glioma.
51. The method of claim 47, wherein the brain endothelial cells are derived from a glioblastoma.
52. The method of claim 44, wherein the endothelial cells are autologous endothelial cells.
53. The method of claim 44, wherein the endothelial cells are allogeneic endothelial cells.
54. The method of claim 44, further comprising administering to the subject a composition comprising astrocytes.
55. The method of claim 54 wherein the astrocytes and the endothelial cells are present in the same composition.

- 56. The method of claim 54 wherein the astrocytes and the endothelial cells are present in separate compositions.
- 57. The method of claim 54, wherein the subject is a mouse and the astrocytes are mouse astrocytes.
- 58. The method of claim 54, wherein the subject is human and the astrocytes are human astrocytes.
- 59. The method of claim 54, wherein the astrocytes are autologous astrocytes.
- 60. The method of claim 54, wherein the astrocytes are allogeneic astrocytes.