

CLAIMS

WHAT IS CLAIMED IS:

1. A kit for the diagnosis and/or treatment of a health condition in an individual, the kit comprising (i) reagents for assessing an expression level of CD58 or the presence and/or absence of one or more molecular alterations of a CD58-encoding gene or a product thereof in a biological sample from the individual, and (ii) instructions for use thereof.
2. The kit of claim 1, wherein the kit is further configured for determining the responsiveness of the individual to a CAR-T cell therapy, wherein the determining comprises:
 - a) detecting whether the expression level of CD58 is decreased or lost or one or more molecular alterations in the CD58-encoding gene or a product thereof is present in a biological sample obtained from the individual, wherein said detecting comprises contacting the biological sample with a detection reagent and detecting an interaction between the detection reagent with a CD58 encoding gene or a product thereof in the sample; and
 - b) identifying the individual as having decreased responsiveness to treatment with the CAR-T cell therapy if the expression level of CD58 is decreased compared to a reference expression level of CD58 or lost or at least one of the one or more molecular alterations in CD58 activity is detected in the sample.
3. The kit of claim 1, wherein the kit is further configured for identifying an individual who has an increased unresponsiveness to a CAR-T cell therapy, wherein the identifying comprises:
 - a) detecting whether the expression level of CD58 is decreased or lost or one or more molecular alterations in the CD58-encoding gene or a product thereof is present in a biological sample obtained from the individual, wherein said detecting comprises contacting the biological sample with a detection reagent and detecting an interaction between the detection reagent with the CD58 encoding gene or a product thereof in the sample; and
 - b) selecting the individual as having increased unresponsiveness to treatment with the CAR-T cell therapy if the expression level of CD58 is decreased compared to a reference expression level of CD58 or lost or at least one of the one or more molecular

alterations in CD58 activity is detected in the sample; or

selecting the individual as not having increased unresponsiveness to treatment with the CAR-T cell therapy if the expression level of CD58 is not decreased compared to a reference expression level of CD58 nor lost or none of the one or more molecular alterations in CD58 activity is detected in the sample.

4. The kit of claim 1, wherein the kit is further configured for optimizing the therapeutic efficacy of a CAR-T cell therapy in an individual, wherein the optimizing comprises:

- a) detecting whether the expression level of CD58 is decreased compared to a reference expression level of CD58 or lost or one or more molecular alterations in the CD58-encoding gene or a product thereof is present in a biological sample obtained from the individual, wherein said detecting comprises contacting the biological sample with a detection reagent and detecting an interaction between the detection reagent with a CD58 encoding gene or a product thereof in the sample; and
- b) identifying a therapeutically effective amount of the CAR-T cell therapy based on the detected interaction between the detection reagent with the CD58 encoding gene or a product thereof.

5. The kit of any one of claims 1 to 4, wherein the individual has or is suspected of having a health condition associated with a decreased level or loss of CD58 expression compared to the reference expression level of CD58 or with one or more molecular alterations in the CD58-encoding gene or a product thereof.

6. The kit of any one of claims 1 to 5, wherein the health condition is a proliferative disorder selected from the group consisting of a solid tumor cancer, a non-solid tumor cancer, and a hematological malignancy.

7. The kit of any one of claims 1 to 5, wherein the health condition is a cancer, optionally non-Hodgkin's lymphoma, Burkitt's lymphoma, small lymphocytic lymphoma, large B-cell lymphoma (LBCL), primary effusion lymphoma, diffuse large B-cell lymphoma, splenic marginal zone lymphoma, MALT (mucosa-associated lymphoid tissue) lymphoma, hairy cell leukemia, chronic lymphocytic leukemia, B-cell prolymphocytic leukemia, B cell lymphoma, Hodgkin's disease, B cell non-Hodgkin's lymphoma (NHL), leukemias, acutelymphoblastic

leukemia (ALL), chronic lymphocytic leukemia (CLL), B-cell chronic lymphocytic leukemia (B-CLL), hairy cell leukemia, chronic myoblastic leukemia, or myeloma.

8. The kit of any one of claims 1 to 7, wherein the one or more molecular alterations in the CD58-encoding gene or a product thereof is selected from the group consisting of an increased RNA/protein expression, a reduced RNA/protein expression, a loss of expression, an aberrant RNA/protein expression, a single nucleotide point mutation (SNP), a single-nucleotide variation (SNV), a gene amplification, a gene rearrangement, a gene fusion, a deletion, a frameshift deletion, an insertion, an InDel mutation, an epigenetic alteration, an amino acid substitution, and combinations of any thereof.

9. The kit of any one of claims 1 to 8, wherein the one or more molecular alterations comprises a loss of CD58 expression, a reduced expression of CD58 compared to the reference expression level of CD58, or expression of a mutated form of CD58.

10. The kit of any one of claims 1 to 9, wherein the one or more molecular alterations comprises an amino acid substitution at a position corresponding to K60 of SEQ ID NO: 1.

11. The kit of claim 10, wherein the amino acid substitution is a Lys-to-Glu substitution (K60E).

12. The kit of any one of claims 1 to 11, wherein the one or more molecular alterations comprises an amino acid substitution at a position corresponding to C187 of SEQ ID NO: 1.

13. The kit of claim 12, wherein the amino acid substitution is a Cys-to-Arg substitution (C187R).

14. The kit of any one of claims 1 to 13, wherein the one or more molecular alterations in the CD58-encoding gene or a product thereof comprises a reduced binding affinity of a CD58 protein product to its ligand CD2.

15. The kit of any one of claims 1 to 14, wherein said assessing the presence and/or absence of one or more molecular alterations of a CD58-encoding gene or a product thereof comprises using a nucleic-acid-based analytical assay selected from the group consisting of cancer personalized profiling by deep sequencing (CAPP-seq), nucleic acid sequencing, circulating tumor nucleic acid assessment, next generation sequencing (NGS), nucleic acid amplification-based assays, loop-mediated isothermal amplification (LAMP), rolling circle

amplification (RCA), polymerase chain reaction (PCR), real-time PCR, quantitative reverse transcription PCR (qRT-PCR), PCR-RFLP assay, HPLC, mass-spectrometric genotyping, nucleic acid hybridization assay, comparative genomic hybridization, fluorescent in-situ hybridization (FISH), restriction digestion, capillary electrophoresis, and combinations of any thereof.

16. The kit of any one of claims 1 to 15, wherein said assessing the presence and/or absence of one or more molecular alterations of a CD58-encoding gene or a product thereof comprises using a protein-based analytical assay selected from the group consisting of immunohistochemistry (IHC), protein-microarray, western blotting, mass spectrometry, flow cytometry, enzyme-linked immunosorbent assay (ELISA), immunofluorescence staining, multiplex detection assay, and combinations of any thereof.

17. The kit of any one of claims 1 to 16, further configured for treatment of the health condition.

18. The kit of any one of claims 2 to 17, wherein the CAR-T cell therapy is to be administered to individual as single therapy or in combination with one or more additional therapies.

19. The kit of claim 18, wherein the CAR-T cell therapy and/or at least one additional therapy comprises a CAR construct comprising a CD2 signaling domain.

20. The kit of any one of claims 2 to 19, wherein the CAR-T cell therapy targets an antigen that is expressed at low density compared to a density in a wild-type cell.

21. A genetic-based system for diagnosis and/or treatment of health conditions comprising:

a) a logic processor;

b) a stored program code that is executable by the logic processor, which when executed by the processor provides operations for performing one or more of the following:

i) determining the responsiveness of an individual to a CAR-T cell therapy;

ii) identifying an individual as having increased unresponsiveness to treatment with a CAR-T cell therapy;

iii) optimizing the therapeutic efficacy of a CAR-T cell therapy in an individual; and

iv) calculating ~~or administering~~ a therapeutically effective amount of a CAR-T cell therapy to be administered to an individual.

22. The system of claim 21, further comprising a report engine communicatively coupled to the logic processor, wherein reports produced by the report engine depend upon results from execution of the program code, wherein the program code configures the logic processor to receive a preselected set of data input pertaining to an expression level of CD58 or the presence and/or absence of one or more molecular alterations of a CD58-encoding gene or a product thereof in a biological sample obtained from an individual in order to assign a relative performance score to the individual's responsiveness to the CAR-T cell therapy based at least in part on the preselected set of data input, and optionally:

- a) determining the responsiveness of the individual to the CAR-T cell therapy;
- b) identifying the individual as having increased unresponsiveness to treatment with the CAR-T cell therapy;
- c) optimizing the therapeutic efficacy of the CAR-T cell therapy in the individual; and/or
- d) ~~calculating or administering a therapeutically effective amount of the CAR-T cell therapy~~ to be administered the individual.

23. The system of any one of claims 21 to 22, further comprising generating a report that contains information relevant to the individuals identified as having increased unresponsiveness to the CAR-T cell therapy and/or relevant to the CAR-T cell therapy identified as being effective for treatment of health conditions.

24. The system of claim 23, wherein the profile report is characterized as having an encoding selected from the group consisting of “.doc”; “.pdf”; “.xml”; “.html”; “.jpg”; “.aspx”; “.php”, and a combination of any thereof.

25. A non-transitory computer readable medium containing machine executable instructions that when executed cause a processor to perform operations comprising:

- receiving a profile comprising a preselected set of data input;
- assigning, based at least in part on the profile, a relative performance score to the identified CAR-T cell therapy; and
- outputting a report for the CAR-T cell therapy based upon the assigned performance score.

26. A non-transitory computer readable medium containing machine executable instructions that when executed cause a processor to perform operations comprising:
- receiving a profile comprising a preselected set of data input;
 - assigning, based at least in part on the profile, a relative unresponsiveness score to the identified individual; and
 - outputting a report for the individual based upon the assigned unresponsiveness score.
27. A report generated by the system or medium of any one of claims 23 to 26.
28. A method for determining the responsiveness of an individual to a CAR-T cell therapy, the method comprising:
- a) detecting whether an expression level of CD58 is decreased compared to a reference expression level of CD58 or lost or one or more molecular alterations in CD58 activity is present in a biological sample obtained from the individual, wherein said detecting comprises contacting the biological sample with a detection reagent and detecting an interaction between the detection reagent with a CD58 encoding gene or a product thereof in the sample; and
 - b) identifying the individual as having decreased responsiveness to treatment with the CAR-T cell therapy if the expression level of CD58 is decreased compared to a reference expression level of CD58 or lost or at least one of the one or more molecular alterations in CD58 activity is detected in the sample.
29. A method for identifying an individual who has an increased unresponsiveness to a CAR-T cell therapy, the method comprising:
- a) detecting whether an expression level of CD58 is decreased compared to a reference expression level of CD58 or lost or one or more molecular alterations in CD58 activity is present in a biological sample obtained from the individual, wherein said detecting comprises contacting the biological sample with a detection reagent and detecting an interaction between the detection reagent with the CD58 encoding gene or a product thereof in the sample; and
 - b) selecting the individual as having increased unresponsiveness to treatment with a CAR-T cell therapy if the expression level of CD58 is decreased compared to a reference expression level of CD58 or lost or at least one of the one or more molecular alterations in CD58 activity is detected in the sample; or
- selecting the individual as not having increased unresponsiveness to treatment with the

CAR-T cell therapy if the expression level of CD58 is not decreased compared to a reference expression level of CD58 nor lost or none of the one or more molecular alterations in CD58 activity is detected in the sample.

30. A method for optimizing the therapeutic efficacy of a CAR-T cell therapy in an individual, the method comprising:

a) detecting whether an expression level of CD58 is decreased compared to a reference expression level of CD58 or lost or one or more molecular alterations in CD58 activity is present in a biological sample obtained from the individual, wherein said detecting comprises contacting the biological sample with a detection reagent and detecting an interaction between the detection reagent with a CD58 encoding gene or a product thereof in the sample; and

b) identifying a therapeutically effective amount of the CAR-T cell therapy to be administered to the individual based on the detected interaction between the detection reagent with the CD58 encoding gene or a product thereof.

~~31. A method for administering a CAR-T cell therapy to an individual, the method comprising:~~

~~a) detecting whether an expression level of CD58 is decreased compared to a reference expression level of CD58 or lost or one or more molecular alterations in CD58 activity is present in a biological sample obtained from the individual, wherein said detecting comprises contacting the biological sample with a detection reagent and detecting an interaction between the detection reagent with a CD58 encoding gene or a product thereof in the sample;~~

~~b) administering a therapeutically effective amount of the CAR-T cell therapy based on the detected interaction between the detection reagent with the CD58 encoding gene or a product thereof.~~

~~32.~~31. The method of any one of claims 28 to- ~~30~~31, the individual has or is suspected of having a health condition associated with a decreased level or loss of CD58 expression compared to a reference expression level of CD58 or with one or more molecular alterations in CD58 activity.

~~33.~~32. The method of claim- ~~31~~32, wherein further comprising treating the health condition is to be treated.

~~34.33.~~ The method of any one of claims 28 to- ~~3233~~, wherein the health condition is a proliferative disorder selected from the group consisting of a solid tumor cancer, a non-solid tumor cancer, and a hematological malignancy.

~~35.34.~~ The method of any one of claims 28 to- ~~3233~~, wherein the health condition is a cancer, optionally non-Hodgkin's lymphoma, Burkitt's lymphoma, small lymphocytic lymphoma, large B-cell lymphoma (LBCL), primary effusion lymphoma, diffuse large B-cell lymphoma, splenic marginal zone lymphoma, MALT (mucosa-associated lymphoid tissue) lymphoma, hairy cell leukemia, chronic lymphocytic leukemia, B-cell prolymphocytic leukemia, B cell lymphoma, Hodgkin's disease, B cell non-Hodgkin's lymphoma (NHL), leukemias, acutelymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), B-cell chronic lymphocytic leukemia (B-CLL), hairy cell leukemia, chronic myoblastic leukemia, or myeloma.

~~36.35.~~ The method of any one of claims 28 to- ~~3435~~, wherein the one or more molecular alterations in CD58 activity is selected from the group consisting of an increased RNA/protein expression, a reduced RNA/protein expression, a loss of expression, an aberrant RNA/protein expression, a single nucleotide point mutation (SNP), a single-nucleotide variation (SNV), a gene amplification, a gene rearrangement, a gene fusion, a deletion, a frameshift deletion, an insertion, an InDel mutation, an epigenetic alteration, an amino acid substitution, and combinations of any thereof.

~~37.36.~~ The method of any one of claims 28 to ~~3536~~ wherein the one or more molecular alterations comprises a loss of CD58 expression, a reduced expression of CD58 compared to a reference expression level of CD58, or expression of a mutated form of CD58.

~~38.37.~~ The method of any one of claims 28 to- ~~3637~~, wherein the one or more molecular alterations comprises an amino acid substitution at a position corresponding to K60 of SEQ ID NO: 1.

~~39.38.~~ The method of claim- ~~3738~~, wherein the amino acid substitution is a Lys-to-Glu substitution (K60E).

~~40.39.~~ The method of any one of claims 28 to- ~~3839~~, wherein the one or more molecular alterations comprises an amino acid substitution at a position corresponding to C187 of SEQ ID NO: 1.

41.40. The method of claim- 3940, wherein the amino acid substitution is a Cys-to-Arg substitution (C187R).

42.41. The method of any one of claims 28 to- 4041, wherein the one or more molecular alterations in the CD58-encoding gene or a product thereof comprises a reduced binding affinity of a CD58 protein product to its ligand CD2.

43.42. The method of any one of claims 28 to- 4142, wherein said detecting interaction between the detection reagent with the CD58 encoding gene or a product thereof comprises a nucleic-acid-based analytical assay selected from the group consisting of nucleic acid sequencing, circulating tumor nucleic acid assessment, next generation sequencing (NGS), nucleic acid amplification-based assays, loop-mediated isothermal amplification (LAMP), rolling circle amplification (RCA), polymerase chain reaction (PCR), real-time PCR, quantitative reverse transcription PCR (qRT-PCR), PCR-RFLP assay, HPLC, mass-spectrometric genotyping, nucleic acid hybridization assay, comparative genomic hybridization, fluorescent in-situ hybridization (FISH), restriction digestion, capillary electrophoresis, and combinations of any thereof.

44.43. The method of any one of claims 28 to- 4243, wherein said detecting interaction between the detection reagent with the CD58 encoding gene or a product thereof comprises a protein-based analytical assay selected from the group consisting of immunohistochemistry (IHC), protein-microarray, western blotting, mass spectrometry, flow cytometry, enzyme-linked immunosorbent assay (ELISA), immunofluorescence staining, multiplex detection assay, and combinations of any thereof.

45.44. The method of any one of claims 28 to- 4344, ~~further comprising administering the CAR-T cell therapy to the individual,~~ wherein the CAR-T cell therapy is to be administered to the individual as single therapy or in combination with one or more additional therapies.

46.45. The method of claim- 4445, wherein the CAR-T cell therapy and/or at least one additional therapy comprises a CAR construct comprising a CD2 signaling domain.

47.46. The method of any one of claims 28 to 4546 wherein the CAR-T cell therapy targets an antigen that is expressed at low density compared to a density in a wild-type cell.

48.47. Use of a CAR construct comprising a CD2 signaling domain in the manufacture of a medicament for treating ~~A method of treating an individual with a health condition in an individual, wherein the health condition is~~ characterized by at least one of: a decreased or lost expression of CD58 or one or more molecular alterations in the CD58-encoding gene, wherein the CAR construct comprising a CD2 signaling domain is to be administered to an individual identified in a method as likely to respond to treatment with a CAR construct comprising a CD2, wherein the method comprisesing:

- a) detecting whether expression level of CD58 is decreased compared to a reference expression level of CD58 or lost or one or more molecular alterations in the CD58-encoding gene or a product thereof is present in a biological sample obtained from the individual, wherein said detecting comprises contacting the biological sample with a detection reagent and detecting an interaction between the detection reagent with a CD58 encoding gene or a product thereof in the sample;
- b) identifying the individual as likely to respond to a treatment with a CAR construct comprising a CD2 signaling domain if the expression level of CD58 is decreased or lost compared to a reference expression level of CD58 or at least one of the one or more molecular alterations in CD58 activity is detected in the sample;~~and~~
- ~~e) administering the treatment with a CAR construct comprising a CD2 signaling domain to the individual identified in step (b) as likely to respond to treatment with a CAR construct comprising a CD2.~~

49.48. Use of a CAR construct comprising a CD2 signaling domain in the manufacture of a medicament for ~~A method of treating a health condition in an individual, wherein the CAR construct comprising a CD2 signaling domain is to be administered to the individual based on the detection of a decreased or lost expression level of CD58 or one or more molecular alterations in the CD58-encoding gene in the method, wherein the method comprisesing:~~

- a) detecting whether expression level of CD58 is decreased or lost compared to a reference expression level of CD58 or one or more molecular alterations in the CD58-encoding gene or a product thereof is present in a biological sample obtained from the individual, wherein said detecting comprises contacting the biological sample with a detection reagent and detecting an interaction between the detection reagent with a CD58 encoding gene or a product thereof in the sample;~~and~~
- ~~b) administering a treatment with a CAR construct comprising a CD2 signaling domain to the individual based on the detection of a decreased or lost expression level of CD58 or one or more molecular alterations in the CD58-encoding gene in step (a).~~

50.49. The use~~method~~ of claim 47~~48~~ or 48~~49~~, wherein the CAR construct comprising a CD2 signaling domain further comprises an anti-CD19 scFv domain.

~~51.50.~~ The ~~method-use~~ of any one of claims ~~48-50~~47-49, wherein the CAR construct comprising a CD2 signaling domain comprises the amino acid sequence of SEQ ID NO: 4.

~~52.51.~~ The ~~method-use~~ of any one of claims ~~48-51~~47-50, wherein the health condition is a proliferative disorder selected from the group consisting of a solid tumor cancer, a non-solid tumor cancer, and a hematological malignancy.

~~53.52.~~ The ~~method-use~~ of any one of claims ~~48-51~~47-50, wherein the health condition is a cancer.

~~54.53.~~ The ~~method-use~~ of claim ~~53~~52, wherein the cancer is selected from the group consisting of: non-Hodgkin's lymphoma, Burkitt's lymphoma, small lymphocytic lymphoma, large B-cell lymphoma (LBCL), primary effusion lymphoma, diffuse large B-cell lymphoma, splenic marginal zone lymphoma, MALT (mucosa-associated lymphoid tissue) lymphoma, hairy cell leukemia, chronic lymphocytic leukemia, B-cell prolymphocytic leukemia, B cell lymphoma, Hodgkin's disease, B cell non-Hodgkin's lymphoma (NHL), leukemias, acutelymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), B-cell chronic lymphocytic leukemia (B-CLL), hairy cell leukemia, chronic myoblastic leukemia, and myeloma

~~55.54.~~ The ~~method-use~~ of any one of claims ~~48-54~~47-53, wherein the one or more molecular alterations in CD58 activity is selected from the group consisting of an increased RNA/protein expression, a reduced RNA/protein expression, a loss of expression, an aberrant RNA/protein expression, a single nucleotide point mutation (SNP), a single-nucleotide variation (SNV), a gene amplification, a gene rearrangement, a gene fusion, a deletion, a frameshift deletion, an insertion, an InDel mutation, an epigenetic alteration, an amino acid substitution, and combinations of any thereof.

~~56.55.~~ The ~~method-use~~ of any one of claims ~~48-55~~47-54, wherein the one or more molecular alterations comprises a loss of CD58 expression, a reduced expression of CD58 compared to a reference expression level of CD58, or expression of a mutated form of CD58.

~~57.56.~~ The ~~method-use~~ of any one of claims ~~48-56~~47-55, wherein the one or more molecular alterations comprises an amino acid substitution at a position corresponding to K60 of SEQ ID NO: 1.

~~58.57.~~ The ~~method-use~~ of claim ~~57~~56, wherein the amino acid substitution is a Lys-to-Glu substitution (K60E).

~~59-58.~~ The ~~method-use~~ of any one of claims ~~48-58~~47-57, wherein the one or more molecular alterations comprises an amino acid substitution at a position corresponding to C187 of SEQ ID NO: 1.

~~60-59.~~ The ~~method-use~~ of claim ~~59~~58, wherein the amino acid substitution is a Cys-to-Arg substitution (C187R).

~~61-60.~~ The ~~method-use~~ of any one of claims- ~~47-59~~48-60, wherein the one or more molecular alterations in the CD58-encoding gene or a product thereof comprises a reduced binding affinity of a CD58 protein product to its ligand CD2.

~~62-61.~~ The ~~method-use~~ of any one of claims ~~48-61~~47-60, wherein said detecting interaction between the detection reagent with the CD58 encoding gene or a product thereof comprises a nucleic-acid-based analytical assay selected from the group consisting of nucleic acid sequencing, circulating tumor nucleic acid assessment, next generation sequencing (NGS), nucleic acid amplification-based assays, loop-mediated isothermal amplification (LAMP), rolling circle amplification (RCA), polymerase chain reaction (PCR), real-time PCR, quantitative reverse transcription PCR (qRT-PCR), PCR-RFLP assay, HPLC, mass-spectrometric genotyping, nucleic acid hybridization assay, comparative genomic hybridization, fluorescent in-situ hybridization (FISH), restriction digestion, capillary electrophoresis, and combinations of any thereof.

~~63-62.~~ The method of any one of claims ~~48-61~~47-60, wherein said detecting interaction between the detection reagent with the CD58 encoding gene or a product thereof comprises a protein-based analytical assay selected from the group consisting of immunohistochemistry (IHC), protein-microarray, western blotting, mass spectrometry, flow cytometry, enzyme-linked immunosorbent assay (ELISA), immunofluorescence staining, multiplex detection assay, and combinations of any thereof.