Claims

1. A method of monitoring a lymphoid neoplasm in a patient with defined cancer clonotypes comprising the steps of:

(a) determining a profile of recombined DNA sequences in T-cells and/or B-cells comprising the steps of:

(i) obtaining a sample from the patient comprising at least 10,000 T-cells and/or B-cells;

(ii) amplifying molecules of recombined DNA sequences from the T-cells and/or B-cells of the sample;

(iii) spatially isolating individual molecules of the amplified recombined DNA sequences; and

(iv) sequencing the amplified recombined DNA sequences to provide at least 10,000 sequence reads from which to form a clonotype profile; and

(b) evaluating the clonotype profile to determine levels of the defined cancer clonotypes and clonotypes evolved therefrom.

2. The method of claim 1 further including the step of repeating steps (a) and (b) to monitor said lymphoid neoplasm in the patient.

3. The method of claim 1 or claim 2 wherein the clones are evolved from the defined cancer clonotypes by VH replacement.

4. The method of claim 3 wherein the VH replacement is identified by detecting the D-J junction.

5. The method of claim 1 or claim 2 wherein said clones are evolved from the defined cancer clonotypes by somatic hypermutation.

6. The method of claim 1 or claim 2 wherein said clones are evolved from the defined cancer clonotypes by one or more insertions and/or deletions of from 1-10 bases.

7. The method of claim 1 or claim 2 wherein the clones evolved from the defined cancer clonotypes are computed based on having identically mutated V region and J region and a different NDN region.

8. The method of claim 1 or claim 2 wherein the clones evolved from the defined cancer clonotypes are computed based on:

a) the number of differences in the NDN region,

b) the length of the NDN region, and

c) the position of the NDN region between the V-NDN boundary and J-NDN boundary.

9. The method of any one of claims 1 to 8 wherein said lymphoid neoplasm is a malignant lymphoid neoplasm.

10. The method of any one of claims 1 to 9 wherein the nucleic acid is genomic DNA.

11. The method of any one of claims 1 to 10 wherein said recombined sequences comprise a genomic rearrangement selected from the group consisting of a VDJ rearrangement of IgH, a DJ rearrangement of IgH, a VJ rearrangement of IgK, a VJ rearrangement of IgL, a VDJ rearrangement of TCR β, a DJ rearrangement of TCR β, a VJ rearrangement of TCR α, a VJ rearrangement of TCR λ, a VDJ rearrangement of TCR δ, and a VD rearrangement of TCR δ.

12. The method of any one of claims 1 to 11 wherein said step of sequencing includes generating at least 10^4 clonotypes in a range of from 20 to 400 nucleotides.

What is claimed is:

1. A method of monitoring a disease in a patient, the method comprising the steps of:

(a) identifying one or more patient-specific clonotypes correlated with a disease by determining a clonotype profile from a sample of lymphocytes in a disease-related tissue, the sample comprising a repertoire of clonotypes from the disease-related tissue; and

(b) determining a clonotype profile from a sample of peripheral blood cells to identify a presence, absence and/or level of the one or more patient-specific clonotypes correlated with the disease, such peripheral blood sample comprising a repertoire of clonotypes.

2. The method of claim 1 further including the step of repeating said step (b) to monitor the disease or condition in the patient.

3. The method of claim 1 wherein said step of identifying further includes determining a clonotype profile from a sample of lymphocytes in a non-disease-related tissue in the same patient and comparing such clonotype profile with that from said disease-related tissue to identify said one or more patient-specific clonotypes.

4. The method of claim 3 wherein said disease-related tissue is a tissue from a peak state of said disease and wherein said non-disease-related tissue is a tissue from a non-peak state of said disease.

5. The method of claim 4 wherein said disease is an autoimmune disease.

6. The method claim 5 wherein said autoimmune disease is systemic lupus erythematosus and said disease-related tissue is kidney, skin, peripheral blood or bone marrow; multiple sclerosis and said disease-related tissue is cerebrospinal fluid; or rheumatoid arthritis and said disease-related tissue is synovial fluid.

7. The method of claim 5 further including the step of repeating said step (b) to monitor the disease or condition in the patient.

8. The method of claim 1 wherein said disease is a lymphoma and said disease-related tissue is bone marrow or a lymphoid tissue; or a leukemia and said disease-related tissue is bone marrow or peripheral blood.

9. The method of claim 8 further including the step of repeating said step (b) to monitor the disease or condition in the patient.

10. The method of claim 8 wherein said step of identifying further includes determining a clonotype profile from a sample of lymphocytes in a non-disease-related tissue in the same patient and comparing such clonotype profile with that from said disease-related tissue to identify said one or more patient-specific clonotypes.

11. The method of claim 1 wherein said step of identifying further includes determining a clonotype profile from a sample of lymphocytes in a non-disease-related tissue in the same patient and comparing such clonotype profile with that from said disease-related tissue to identify said one or more patient-specific clonotypes.

12. The method of claim 11 further including the step of repeating said step (b) to monitor the disease or condition in the patient.

13. The method of claim 11 wherein said one or more patient-specific clonotypes are identified by having a different frequency in said disease-related tissue than in said non-disease-related tissue; or by having a different degree of somatic hypermutation in IgH-based clonotypes in said disease-related tissue than in said non-disease-related tissue; or by having different frequencies of V region and J region usage in said disease-related tissue than in said non-disease-related tissue; or by having NDN regions of different average lengths in said disease-related tissue than in said non-disease-related tissue; or by having different levels of BCR and/or TCR expression in said disease-related tissue than in said non-disease-related tissue; or by having different numbers of clonotypes encoding identical or similar proteins in said disease-related tissue than in said non-disease-related tissue.

14. The method of claim 13 further including the step of repeating said step (b) to monitor the disease or condition in the patient.

15. The method of claim 13 wherein said one or more patient-specific clonotypes are identified by having a greater frequency in said disease-related tissue than in said non-disease-related tissue.

16. The method of claim 13 wherein said one or more patient-specific clonotypes are identified by having different frequencies of V region and J region usage in said disease-related tissue than in said non-disease-related tissue.

17. The method of claim 13 wherein said one or more patient-specific clonotypes are identified by having NDN regions of different average lengths in said disease-related tissue than in said non-disease-related tissue.

18. The method of claim 13 wherein said one or more patient-specific clonotypes are identified by having different levels of BCR and/or TCR expression in said disease-related tissue than in said non-disease-related tissue.

19. The method of claim 1 wherein said one or more patient-specific clonotypes are identified by matching determined clonotypes with clonotypes known to be correlated with said disease or by matching frequencies of V region and J region usage with V region and J region usage known to be correlated with said disease.

20. The method of claim 19 wherein said matching comprises finding indentity between an amino acid sequence encoded by said determined clonotype and that of an amino acid sequence encoded by a clonotype known to be correlated to said disease, or a substantially identical variant thereof.

21. The method of claim 19 wherein said matching comprises finding identity between said determined clonotype and a nucleic acid sequence of a clonotype known to be correlated to said disease, or a substantially identical variant thereof.

22. The method of claim 19 wherein said matching comprises finding identity between said determined clonotype and a nucleic acid sequence of a clonotype known to be correlated to said disease, or a substantially identical variant thereof.

23. The method of claim 1 wherein each of said repertoires includes every clonotype present at a frequency of .01 percent or greater with a probability of ninety-nine percent.

24. The method of claim 1 wherein each of said repertoires includes a sample of at least 10^4 clonotypes.

25. The method of claim 1 wherein each of said repertoires includes a sample of at least 10^5 clonotypes.

26. The method of claim 1 wherein said disease is selected from the group consisting of lymphoid proliferative disorders, solid tumors, infectious diseases, and autoimmune diseases.

27. The method of claim 26 wherein said step of determining said repertoire from said sample of blood cells further comprises including as said one or more patient-specific clonotypes any previously unrecorded phylogenic clonotypes of said one or more patient-specific clonotypes.

28. The method of claim 26 wherein said phylogenic clonotypes include previously unrecorded clonotypes that are ninety percent identical to any of said one or more patient-specific clonotypes.

29. The method of claim 26 wherein said phylogenic clonotypes include previously unrecorded clonotypes that are related to said one or more patient-specific clonotypes by somatic hypermutation.

30. The method of claim 26 wherein said disease is a lymphoproliferative disorder and wherein said phylogenic clonotypes include previously unrecorded clonotypes that are related to said one or more patient-specific clonotypes by somatic rearrangement.

31. The method of claim 30 wherein said somatic rearrangement comprises a VH replacement.

32. The method of claim 30 wherein said somatic rearrangement comprises a clonotype having identically mutated V region and J region as any of said one or more patient-specific clonotypes but having a different NDN region as such patient-specific clonotype.

33. The method of claim 27 wherein said step of identifying further includes determining a clonotype profile from a sample of lymphocytes in a non-disease-related tissue in the same patient and comparing such clonotype profile with that from said disease-related tissue to identify said one or more patient-specific clonotypes.

34. The method of claim 27 wherein said disease is a lymphoma or a leukemia and wherein said step (b) of determining further includes computing a measure of clonality from said clonotype profile of said sample from said peripheral blood.

35. A method of monitoring a disease in a patient, the method comprising the steps of:

(a) comparing a clonotype profile from a sample of lymphocytes of an individual afflicted with a disease and a clonotype profile from the same individual from a sample of lymphocytes enriched on the basis of cell surface markers associated with the disease to identify one or more patient-specific clonotypes correlated with the disease; and

(b) determining a level of each of the one or more patient-specific clonotypes in a clonotype profile from a sample of peripheral blood cells, such sample comprising a repertoire of clonotypes thereof

.

36. The method of claim 35 wherein said step of determining said repertoire from said sample of blood cells further comprises including as said one or more patient-specific clonotypes any previously unrecorded phylogenic clonotypes of said one or more patient-specific clonotypes.

37. The method of claim 35 wherein said disease is a lymphoid proliferative disorder.

38. The method of claim 35 wherein said method further includes the step of repeating said step (b) to monitor said disease or condition and wherein each of said repertoires includes every clonotype present at a frequency of .01 percent or greater with a probability of ninety-nine percent.

39. The method of claim 35 wherein said method further includes the step of repeating said step (b) to monitor said disease or condition and wherein each of said repertoires includes a sample of at least 10^4 clonotypes.

40. The method of claim 35 wherein said disease is an autoimmune disease.

41. The method of claim 40 wherein said step of determining said repertoire from said sample of blood cells further comprises including as said one or more patient-specific clonotypes any previously unrecorded phylogenic clonotypes of said one or more patient-specific clonotypes.

42. The method of claim 40 wherein said method further includes the step of repeating said step (b) to monitor said disease or condition and wherein each of said repertoires includes every clonotype present at a frequency of .01 percent or greater with a probability of ninety-nine percent.

43. The method of claim 40 wherein said method further includes the step of repeating said step (b) to monitor said disease or condition and wherein each of said repertoires includes a sample of at least 10^4 clonotypes.

44. The method of claim 40 wherein said autoimmune disease is systemic lupus erythematosus.

45. A method of simultaneously measuring lymphocyte numbers and clonotype expression levels in a sample, the method comprising the steps of:

obtaining from an individual a sample comprising T cells and/or B cells;

sequencing spatially isolated individual molecules derived from genomic DNA of said cells, such spatially isolated individual molecules comprising a number of clonotypes corresponding to a number of lymphocytes in the sample;

sequencing spatially isolated individual molecules derived from RNA of said cells, such spatially isolated individual molecules comprising numbers of clonotypes corresponding to expression levels thereof in the lymphocytes of the sample; and

determining clonotype expression levels in lymphocytes of the sample by comparing for each clonotype the number determined from isolated individual molecules derived from genomic DNA of said cells and the number determined from isolated individual molecules derived from RNA of said cells.

46. The method of claim 45 wherein said step of determining further includes determining said number of lymphocytes in said sample by adding a known quantity of an internal standard to said genomic DNA.

47. The method of claim 45 wherein said sample is a peripheral blood sample having a defined volume and wherein said step of determining includes determining a concentration of said lymphocytes in said sample.

48. The method of claim 45 wherein said step of determining said expression levels includes for each of said clonotypes dividing said number of clonotypes determined from said isolated individual molecules derived from said RNA by said number of clonotypes determined from said isolated individual molecules derived from said genomic DNA.

49. The method of claim 45 wherein said step of sequencing said spatially isolation individual molecules derived from said RNA includes labeling each of said spatially isolated individual molecules with a first label indicating its RNA origin and wherein said step of sequencing said spatially isolation individual molecules derived from said genomic DNA includes labeling each of said spatially isolated individual molecules with a second label indicating its genomic DNA origin such that the first label is distinguishable from the second label.

50. The method of claim 45 wherein said sample comprises a repertoire of said number of said clonotypes corresponding to said number of said lymphocytes comprises a repertoire of clonotypes.

51. The method of claim 50 wherein said sample comprises B cells.

52. The method of claim 50 wherein said sample comprises T cells.

53. A method of simultaneously measuring lymphocyte numbers and lymphocyte clonality in a sample, the method comprising the steps of:

obtaining from an individual a sample comprising T cells and/or B cells;

sequencing spatially isolated individual molecules derived from nucleic acid of said cells, such spatially isolated individual molecules comprising a number of clonotypes corresponding to a number of lymphocytes in the sample;

determining the number of lymphocytes from the number of spatially isolated individual molecules;

determining abundances of different sequences of the spatially isolated individual molecules to generate a clonotype profile and a measure of clonality based thereon.

54. The method of claim 53 wherein said step of determining said number further includes determining said number of lymphocytes in said sample by adding a known quantity of an internal standard to said genomic DNA.

55. The method of claim 54 wherein said sample is a peripheral blood sample having a defined volume and wherein said step of determining said number includes determining a concentration of said lymphocytes in said sample.

56. The method of claim 53 wherein said sample comprises a repertoire of said number of said clonotypes corresponding to said number of said lymphocytes comprises a repertoire of clonotypes.

57. The method of claim 56 wherein said sample comprises B cells.

58. The method of claim 56 wherein said sample comprises T cells.

59. The method of claim 53 wherein said disease is a lymphoma and said disease-related tissue is bone marrow or a lymphoid tissue or wherein said disease is a leukemia and said disease-related tissue is bone marrow or peripheral blood.

60. A method of monitoring responsiveness to a vaccination of an individual, the method comprising the steps of:

enriching after a vaccination a sample of lymphocytes from peripheral blood of an individual to obtain a sample of vaccine-responsive lymphocytes;

determining a clonotype profile from the sample of vaccine-responsive lymphocytes to identify one or more patient-specific clonotypes correlated with vaccine response;

determining a level of each of the one or more patient-specific clonotypes in a clonotype profile from a sample of peripheral blood cells obtained at one or more subsequent times to monitor the responsiveness of the individual to the vaccination.

61. A method of monitoring a lymphoproliferative disorder in a patient, the method comprising the steps of:

(a) identifying one or more patient-specific clonotypes correlated with a lymphoproliferative disorder by determining a clonotype profile from lymphocytes from a sample of bone marrow, the sample comprising a repertoire of clonotypes; and

(b) determining at a later time a clonotype profile from a sample of bone marrow or peripheral blood cells to identify a presence, absence and/or level of the one or more patient-specific clonotypes correlated with the disease, such latter bone marrow sample or peripheral blood sample comprising a repertoire of clonotypes.