

molecules to target particular disease pathways, immune therapies and cellular therapies may act by educating a patient's immune system to fight disease.

[0005] When in a disease state, or during treatment of such disease state, a patient's immune system mobilizes to fight the disease. As a result, the turnover rate of the immune repertoire may change. A measurement of such change may give an indication of the effectiveness of the treatment. Currently, however, no tools or methods are available which would allow scientists or physicians to measure the immune repertoire turnover rate; current methods, such as white blood cell counts and flow cytometry, are not ideal. Measuring a patient's white blood cell count provides only the total number of lymphocytes or the number of cells that belong to lymphocyte category, and flow cytometry allows classification of T and B lymphocytes based on surface markers. Neither of these methods, however, provides an accurate measurement of the immune repertoire turnover rate. As such, current methods are not ideal for the evaluation of therapeutic results of therapies, such as immune therapies, the generation of markers for pre-screening patients, and the identification of those patients best fit for a particular therapy. What is needed, therefore, is a tool to classify T and B lymphocytes at the single cell level based on the differentially-expressed T or B lymphocyte receptors and a method of utilizing this information to determine a change in an individual's immune repertoire.

Summary

[0006] In one aspect, the present invention refers to a method of calculating the change in the immune repertoire of a subject, comprising the steps of: quantifying clonotypes of immune cells in a population or subpopulation of immune cells in a first sample and a second sample collected from the same patient; calculating frequency data for the first sample and the second sample by identifying the frequency of each clonotype; normalizing the frequency data for the first sample and the second sample; determining the absolute difference in the frequency of each clonotype present in the first sample and the second sample; determining a cohort of the clonotypes common to the first sample and the second sample with the largest frequency change; and calculating the average percentage change in expression of clonotypes within the cohort. In another aspect, the

present invention refers to a method of calculating the change in the immune repertoire of a subject, comprising the steps of: separately amplifying polynucleotides from each population of at least two sample populations of white blood cells collected from the subject, wherein the amplifying is in a reaction mix comprising target-specific nested primers to produce a set of first amplicons, at least a portion of the target-specific nested primers comprising additional nucleotides which, during amplification, serve as a template for incorporating into the first amplicons a binding site for at least one common primer; separately transferring a portion of each of the first reaction mixes containing the first amplicons to second reaction mixes comprising at least one common primer; for each population, amplifying, using the at least one common primer, the first amplicons to produce a set of second amplicons; sequencing each of the second amplicons to identify the frequency of specific CDR3 regions present in the sample; normalizing the CDR3 frequency data; comparing the normalized CDR3 frequency data of the first sample and the second sample to determine the absolute difference between the frequency of each CDR3 sequence; determining a cohort of the CDR3 sequences, common to the first sample and the second sample, with the largest frequency changes; and calculating the average percentage change in expression of clonotypes within the cohort. Also described herein is a method of calculating the change in the immune repertoire of an individual, wherein such change is a measurement of the turnover rate of the most dynamic immune cells. The method may comprise quantifying clonotypes (i.e., clonal types) in populations of immune cells from two or more samples collected from the same patient. In one example, frequency data is calculated for two or more samples by identifying the frequency of each clonotype, wherein the frequency data are normalized to correct for sample differences. The absolute qualitative and quantitative differences in the frequency of each clonotype in the patient samples may then be calculated to determine the clonotypes, common to each sample, with the largest frequency change. The method may then comprise determining

the percentage of the clonotypes with the highest degree of change between samples. The samples may be collected at the same time point to determine an individual's baseline immune repertoire, or collected at different time points to calculate the immune repertoire change rate. In certain examples, the immune repertoire change rate is determined at time point before, during or after a health event. The presently disclosed methods allow for the analysis of a vast amount of sequencing data while eliminating sampling and sequencing inconsistencies that have previously made the calculation of immune repertoire changes impossible.

Brief Description of the Figures

- [0007] The disclosure can be better understood with reference to the following figures.
- [0008] FIG. 1 is an illustration of raw CDR3 sequencing data obtained from patients at different time points.
- [0009] FIG. 2 illustrates the calculations utilized in determining normalized sequence data: Column A represents raw sequence data and columns B and C represent normalized data.
- [0010] FIG. 3 represents the calculation utilized in determining the Delta Index of two samples collected from the same patient: Column D represents the absolute difference between the frequencies of each CDR3 read count in each sample; Column E represents the calculation of the impact range of the two samples; and Column F represents the calculation of the Delta Index for the two samples.
- [0011] FIG. 4 represents signal optimization for the samples: Column H represents calculation of the signal to noise ratio for an impact range of 100; Column I represents calculation of the signal to noise ratio for an impact range of 1,000; and Column J represents the calculation of the signal to noise ratio for an impact range of 10,000.
- [0012] FIG. 5 illustrates the calculated Delta Index for several sample groups: Group A represents a single sample taken from one patient, where the sample is split into two subsamples; Group B represents multiple samples taken from the same individual at the same time point; Group C represents samples taken from the same individual at two different time points with an interval of one or two days; Group D represents samples taken from the same individual at two different time points with an interval of three

CLAIMS

Now, therefore, the following is claimed:

1. A method of calculating the change in the immune repertoire of a subject, comprising the steps of:
 - quantifying clonotypes of immune cells in a population or subpopulation of immune cells in a first sample and a second sample collected from the same patient;
 - calculating frequency data for the first sample and the second sample by identifying the frequency of each clonotype;
 - normalizing the frequency data for the first sample and the second sample;
 - determining the absolute difference in the frequency of each clonotype present in the first sample and the second sample;
 - determining a cohort of the clonotypes common to the first sample and the second sample with the largest frequency change; and
 - calculating the average percentage change in expression of clonotypes within the cohort.
2. The method of claim 1, wherein clonotypes of immune cells are quantified using arm-PCR and next generation sequencing.
3. The method of claim 1, wherein the clonotypes are CDR3 clonotypes.
4. The method of claim 1, wherein the first sample and the second sample are collected at the same time point.
5. The method of claim 1, wherein the first sample and the second sample are collected at different time points.
6. The method of claim 1, wherein the cohort comprises at least 100 clonotypes with the greatest frequency change.
7. The method of claim 1, wherein each sample comprises white blood cells.

8. The method of claim 1, wherein the subject is healthy.
9. The method of claim 1, wherein the subject has been diagnosed with, or is a suspected of having, a disease.
10. The method of claim 1, wherein the subject is treated with an immune therapy.
11. The method of claim 10, wherein the subject receives immune therapy after the first sample is collected.
12. A method of calculating the change in the immune repertoire of a subject, comprising the steps of:
 - separately amplifying polynucleotides from each population of at least two sample populations of white blood cells collected from the subject, wherein the amplifying is in a reaction mix comprising target-specific nested primers to produce a set of first amplicons, at least a portion of the target-specific nested primers comprising additional nucleotides which, during amplification, serve as a template for incorporating into the first amplicons a binding site for at least one common primer;
 - separately transferring a portion of each of the first reaction mixes containing the first amplicons to second reaction mixes comprising at least one common primer;
 - for each population, amplifying, using the at least one common primer, the first amplicons to produce a set of second amplicons;
 - sequencing each of the second amplicons to identify the frequency of specific CDR3 regions present in the sample;
 - normalizing the CDR3 frequency data;
 - comparing the normalized CDR3 frequency data of the first sample and the second sample to determine the absolute difference between the frequency of each CDR3 sequence;
 - determining a cohort of the CDR3 sequences, common to the first sample and the second sample, with the largest frequency changes; and

calculating the average percentage change in expression of clonotypes within the cohort.

13. The method of claim 12, wherein the first sample and the second sample are to be collected at the same time point.

14. The method of claim 12, wherein the first sample and the second sample are to be collected at different time points.

15. The method of claim 12, wherein the subject is healthy.

16. The method of claim 12, wherein the subject has been diagnosed with, or is suspected of having, a disease.

17. The method of claim 12, wherein the cohort comprises at least 100 clonotypes with the greatest frequency change.