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(54) **Title:** A METHOD FOR ASSESSING PROGNOSIS OF LYMPHOMA

(57) **Abstract:** The invention relates to methods, devices and kits for providing prognosis of subjects having lymphoma. In particular, the methods relate to determining likelihood of survival, recurrence or treatment outcome in B-cell lymphoma patients who have undergone and/or undergoing chemotherapy. In particular, the present invention provides a method for providing a prognosis of a subject having diffuse large B-cell lymphoma responding to a treatment regime including an anthracycline and a B-celldepleting antibody, such as an anti-CD20 antibody, the method comprising: determining an immune score for the subject based upon the ratio of a level of any one or more of CD137, CD4, CD8, CD56, TNF α (alpha) and LMO2 in the subject to a level of any one or more of PD-1, PD-L1, CD163, CD68, PD-L2, LAG3, TIM3 and SCYA3(CCL3) in the subject, comparing the immune score to a reference score; wherein the immune score in comparison with the reference score is indicative of the subject's prognosis of responding to a treatment regime including an anthracycline and a B-cell depleting antibody.



A method for assessing prognosis of lymphoma

Cross reference(s) to related applications

This application claims priority from Australian provisional application no. 2015900625 the entire disclosure of which is incorporated herein by reference.

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Field of the invention

The invention relates to methods, devices and kits for providing prognosis of subjects having lymphoma. In particular, the methods relate to determining likelihood of survival, recurrence or treatment outcome in B-cell lymphoma patients.

Background of the invention

10 Lymphomatous B-cells develop within a complex tumour microenvironment (TME) that influences their growth and spread. Immune-effectors are vital: data strongly implicates T-cell deficiencies in lymphoma pathogenesis and outcome. However Diffuse large B-cell lymphoma (DLBCL) biopsies also display features of an inhibitory host-checkpoint response, which is exploited by malignant B-cells to dampen anti-tumour
15 immune-effectors to foster lymphoma cell survival. In solid-organ cancers, tumour biopsies display 'adaptive resistance' in which checkpoints are triggered in response to immune-effectors. Whether a similar relationship between immune-effectors and checkpoints is present in DLBCL remains to be determined.

Diffuse large B-cell lymphoma (DLBCL) is an aggressive B-cell lymphoma.
20 Approximately ~30% of patients remain refractory or relapse following initial therapy with regimens such as R-CHOP (Coiffier B. *J Clin Oncol* 2005; 23(26): 6387-93). New therapeutic strategies are in development, but their application requires accurate identification of patients likely to fail induction. However, despite the use of established pre-treatment prognosticators such as the cell-of-origin (COO) and international
25 prognostic index (IPI) and/or revised-international prognostic index (R-IPI), (Alizadeh AA, et al. *Nature* 2000; 403(6769): 503-11; Sehn LH et al. *Blood* 2007; 109(5): 1857-61), considerable heterogeneity of outcome persists (Coiffier B. *J Clin Oncol* 2005; 23(26): 6387-93).

To date there has not been a method to predict response to therapy in subjects
30 having DLBCL. To the extent that WO2013037813, WO2014122271 or EP2195347

relate to DLBCL, they include significant deficiencies such as, amongst other things, that none describe a method of prognosis of response to therapy, provide relative low predictive power, and / or involve markers which do not associate with relevant end points.

- 5 There is a need for methods that predict response to therapy in subjects having diffuse large B-cell lymphoma. Preferably, those methods add to the predictive power of conventional prognosticators to more accurately predict response to initial therapy.

Reference to any prior art in the specification is not an acknowledgment or suggestion that this prior art forms part of the common general knowledge in any
10 jurisdiction or that this prior art could reasonably be expected to be understood, regarded as relevant, and/or combined with other pieces of prior art by a skilled person in the art.

Summary of the invention

The present invention provides a method for providing a prognosis of a subject
15 having diffuse large B-cell lymphoma (DLBCL) responding to a treatment regime including an anthracycline and a B-cell depleting antibody, the method comprising:

determining an immune score for the subject based upon the ratio of a level of any one or more of CD137, CD4, CD8, CD56, TNF α (alpha) and LMO2 in the subject to a level of any one or more of PD-1, PD-L1, CD163, CD68, PD-L2, LAG3, TIM3 and
20 SCYA3(CCL3) in the subject,

comparing the immune score to a reference score;

wherein the immune score in comparison with the reference score is indicative of the subject's prognosis of responding to a treatment regime including an anthracycline and a B-cell depleting antibody. Preferably, the B-cell depleting antibody is an anti-
25 CD20 antibody.

The present invention also provides a method for providing a prognosis of a subject having diffuse large B-cell lymphoma responding to a treatment regime including an anthracycline and a B-cell depleting antibody, the method comprising

determining an immune score for the subject based upon the ratio of a level of any one or more of CD137, CD4, CD8, CD56, TNF α (alpha) and LMO2 in the subject to a level of any one or more of PD-1, PD-L1, CD163, CD68, PD-L2, LAG3, TIM3 and SCYA3(CCL3) in the subject,

5 comparing the immune score to a reference score;

wherein the reference score is a score above which correlates with an increased probability of recurrence free survival or overall survival of the subject at a later time, and below which correlates with a decreased probability of recurrence free survival or overall survival of the subject at the later time, thereby providing a prognosis of the
10 subject for responding to a treatment regime including an anthracycline and a B-cell depleting antibody. Preferably, the B-cell depleting antibody is an anti-CD20 antibody.

The present invention also provides a method for predicting the likelihood of overall survival of a subject having diffuse large B-cell lymphoma if treated with a regime including an anthracycline and a B-cell depleting antibody, the method
15 comprising:

determining an immune score for the subject based upon the ratio of a level of any one or more of CD137, CD4, CD8, CD56, TNF α (alpha) and LMO2 in the subject to a level of any one or more of PD-1, PD-L1, CD163, CD68, PD-L2, LAG3, TIM3 and SCYA3(CCL3) in the subject,

20 comparing the immune score to a reference score;

wherein the reference score is a score above which correlates with an increased probability of recurrence free survival or overall survival of the subject at a later time, and below which correlates with a decreased probability of recurrence free survival or overall survival of the subject, thereby providing a prognosis of the subject for
25 responding to a treatment regime including an anthracycline and a B-cell depleting antibody. Preferably, the B-cell depleting antibody is an anti-CD20 antibody.

In any method of the invention described above, determining an immune score for the subject may be based upon the ratio of a level of any one or more of CD137, CD4, CD8 and CD56 in the subject to a level of any one or more of PD-1, PD-L1,
30 CD163 and CD68 in the subject.

The present invention also provides a method for predicting the likelihood of overall survival of a subject having diffuse large B-cell lymphoma if treated with a regime including an anthracycline and a B-cell depleting antibody, the method comprising:

5 analysing levels of at least one of CD137, CD4, CD8, CD56, TNF α (alpha) and LMO2, and at least one of PD-1, PD-L1, CD163, CD68, PD-L2, LAG3, TIM3 and SCYA3(CCL3) in a biological sample obtained from the subject

 determining an immune score for the subject based upon the ratio of a level of any one or more of CD137, CD4, CD8, CD56, TNF α (alpha) and LMO2 in the subject to
10 a level of any one or more of PD-1, PD-L1, CD163, CD68, PD-L2, LAG3, TIM3 and SCYA3(CCL3),

 comparing the immune score to a reference score, wherein the reference score has been derived from a patient cohort of known survival outcome treated with a regime including an anthracycline and a B-cell depleting antibody, and

15 assigning the subject to a risk group based on whether the immune score is higher or lower than the reference score, wherein an immune score higher than the reference score indicates a high likelihood of overall survival and an immune score lower than the reference indicates a low likelihood of overall survival,

 thereby predicting the likelihood of overall survival of a subject. Preferably, the B-
20 cell depleting antibody is an anti-CD20 antibody. Preferably, the method of the invention includes the following steps:

 analysing levels of at least one of CD137, CD4, CD8 and CD56, and at least one of PD-1, PD-L1, CD163 and CD68 in a biological sample obtained from the subject

 determining an immune score for the subject based upon the ratio of a level of
25 any one or more of CD137, CD4, CD8 and CD56 in the subject to a level of any one or more of PD-1, PD-L1, CD163 and CD68.

 In any method of the invention, the immune score for the subject may be based upon the ratio of a level of each of CD137, CD4, CD8, CD56, TNF α (alpha) and LMO2 in the subject to a level of each of PD-1, PD-L1, CD163, CD68, PD-L2, LAG3, TIM3 and
30 SCYA3(CCL3) in the subject.

In any method of the invention, the immune score for the subject may be based upon the ratio of a level of each of CD137, CD4, CD8 and CD56 in the subject to a level of PD-1, PD-L1, CD163 and CD68 in the subject.

The present invention provides a method for providing a prognosis of a subject having diffuse large B-cell lymphoma responding to a treatment regime including an anthracycline and anti-CD20 antibody, the method comprising:

determining an immune score for the subject based upon the ratio of the product of levels of each of CD4, CD8, CD137(TNFR9), TNF α (alpha) and LMO2 in the subject to the product of levels of PD-L1, PD-1, CD163, CD68 and SCYA3(CCL3) in the subject, wherein the ratio is defined by $CD4 \times CD8 \times CD137 \cdot TNFR9 \times TNF \cdot \alpha \cdot LMO2 \cdot \text{div} \cdot M2 \times SCYA3 \cdot CCL3 \times PD \cdot 1 \times PD \cdot L1$, wherein M2 is $(CD163 \cdot \text{div} \cdot CD68)$,

comparing the immune score to a reference score;

wherein the immune score in comparison with the reference score is indicative of the subject's prognosis of responding to a treatment regime including an anthracycline and anti-CD20 antibody.

The present invention provides a method for providing a prognosis of a subject having diffuse large B-cell lymphoma responding to a treatment regime including an anthracycline and anti-CD20 antibody, the method comprising:

determining an immune score for the subject based upon the ratio of the product of levels of each of CD4 and CD8 in the subject to the product of levels of PD-L1, CD163 and CD68 in the subject, wherein the ratio is defined by $CD4 \times CD8 \cdot \text{div} \cdot M2 \times PD \cdot L1$, wherein M2 is $(CD163 \cdot \text{div} \cdot CD68)$,

comparing the immune score to a reference score;

wherein the immune score in comparison with the reference score is indicative of the subject's prognosis of responding to a treatment regime including an anthracycline and anti-CD20 antibody.

In any of the above methods, where the level of more than one of CD137, CD4, CD8, CD56, TNF α (alpha) and LMO2 is used in the ratio, the mathematical product of

their values is used. The same applies for PD-1, PD-L1, CD163, CD68, PD-L2, LAG3, TIM3 and SCYA3(CCL3). However, the level of CD163 and CD68 can be present in the ratio as a ratio of CD163.div.CD68 (i.e. CD163:CD68, or 'M2'). Preferably, the ratio is not the level of CD4 to PD-1, CD56 to PD-1 or CD56 to PDL-1 (shown in Table 3 as the
 5 last 3 rows as CD4.div.PD-1, CD56.div.PD-1 and CD56.div.PD-L1).

In any aspect or embodiment of the invention, a method may be used for providing a prognosis for recurrence free survival, overall survival, four year survival or other clinically or biochemically detectable response to a treatment regime. Preferably, the treatment regime is a chemo-immunotherapy treatment including, or consisting of,
 10 an anthracycline and a B-cell depleting antibody. Preferably, the B-cell depleting antibody is an anti-CD20 antibody.

In any aspect of the invention described herein, the anthracycline may be doxorubicin, daunorubicin, idarubicin or epirubicin, and the B-cell depleting antibody may be inotuzumab or an anti-CD20 antibody such as rituximab, obinutuzumab or
 15 ofatumumab. Typically, the chemotherapy including an anthracycline and anti-CD20 antibody is R-CHOP (Rituximab, Cyclophosphamide, Hydroxydaunorubicin, Oncovin (vincristine), and Prednisone or Prednisolone), R-CHOP-14, R-CHOP-21, DA-EPOCH-R (dose-adjusted etoposide, doxorubicin, and cyclophosphamide with vincristine, prednisone, and rituximab) or R-ACVBP (rituximab, doxorubicin, cyclophosphamide,
 20 vindesine, bleomycin, and prednisone). More preferably, the chemotherapy is R-CHOP.

In any aspect of the invention described herein, a high likelihood of overall survival is at least 95%, 94%, 93%, 92%, 91%, 90%, 85%, 80%, 75% or 70%.

In any aspect of the invention described herein, a low likelihood of overall survival is less than 60%, 55%, 54%, 53%, 52%, 51%, 50%, 49%, 48%, 47%, 46%, 45% or
 25 44%.

Determining the immune score for a subject may be by any one of the ratios listed in Table 3 or Table 5. Preferably, the ratio is one that has a p-value difference between survival curves as shown in Table 3 or 5 of less than 1E-11 (i.e. 1×10^{-11}), 1E-10, 1E-09, 1E-08, 1E-07, 1E-06, 1E-05, 1E-04, 1E-03, 1E-02 or 0.05. Preferably, the
 30 immune score for a subject is determined using any one of the following ratios:

CD4xCD8xCD137.TNFR9xTNF.AlphaxLMO2.div.M2xSCYA3.CCL3xPD.1xPD.L1

,

CD4xCD8xTNF.AlphaxLMO2.div.M2xSCYA3.CCL3xPD.L2,

CD4xCD8xCD137.TNFR9xTNF.AlphaxLMO2.div.M2xSCYA3..CCL3xPD.1xPD.L

5 2,

CD4xCD8xLMO2.div.M2xSCYA3.CCL3xPD.L2,

CD4xCD8xCD137.TNFR9xLMO2.div.M2xSCYA3.CCL3xPD.L2xTIM3,

CD8xCD137.TNFR9xTNF.AlphaxLMO2.div.M2xPD.1xPD.L1,

CD4xCD8xCD137.TNFR9xTNF.AlphaxLMO2.div.SCYA3.CCL3xPD.L1xPD.L2,

10 CD4xCD8xCD137.TNFR9xTNF.AlphaxLMO2.div.M2xPD.1xPD.L1xPD.L2,

CD4xCD8xTNF.AlphaxLMO2.div.M2xSCYA3.CCL3xLAG3,

CD4xCD8xTNF.AlphaxLMO2.div.M2xSCYA3.CCL3xPD.1xPD.L1,

CD4xCD8xCD137.TNFR9xTNF.AlphaxLMO2.div.M2xSCYA3.CCL3xPD.L1,

CD8xLMO2.div.M2xSCYA3.CCL3xPD.L2,

15 CD4xCD8xTNF.AlphaxLMO2.div.M2xSCYA3.CCL3xPD.L1,

CD4xCD8xCD137.TNFR9xLMO2.div.M2xSCYA3.CCL3xPD.1xPD.L2,

CD4xCD8xTNF.AlphaxLMO2.div.M2xSCYA3.CCL3xPD.1,

CD4xCD8xTNF.AlphaxLMO2.div.M2xPD.1xPD.L2,

CD4xCD8xCD137.TNFR9xLMO2.div.M2xSCYA3.CCL3xPD.L2,

20 CD4xCD8xCD137.TNFR9xLMO2.div.M2xPD.1xPD.L2,

CD4xCD8xCD137.TNFR9xLMO2.div.M2xSCYA3.CCL3xPD.1xPD.L1,

CD4xCD8xCD137.TNFR9xLMO2.div.M2xSCYA3.CCL3xPD.L1,

CD8xTNF.AlphaxLMO2.div.M2xSCYA3.CCL3xPD.L2,

CD4xCD8xTNF.AlphaxLMO2xCD56.div.M2xSCYA3..CCL3xPD.1xPD.L1,

CD8xLMO2.div.M2xPD.L2,

CD4xCD8xCD137.TNFR9xTNF.AlphaxLMO2xCD56.div.M2xSCYA3.CCL3xPD.1
xPD.L2,

5 CD4xCD8xCD137.TNFR9xLMO2.div.SCYA3.CCL3xPD.L2xLAG3,

CD4xCD8xTNF.AlphaxLMO2.div.M2xSCYA3.CCL3xPD.L2xTIM3,

CD4xCD8xTNF.AlphaxLMO2.div.M2xPD.1xPD.L1,

CD4xCD8xCD137.TNFR9xLMO2.div.M2xPD.1xPD.L1,

CD8xCD137.TNFR9xLMO2.div.M2xPD.1xPD.L1,

10 CD4xCD8xCD137.TNFR9xTNF.AlphaxLMO2.div.SCYA3.CCL3xPD.1xPD.L2,

CD4xCD8xCD137.TNFR9xLMO2.div.SCYA3.CCL3xPD.1xPD.L2,

CD8xCD137.TNFR9xTNF.AlphaxLMO2.div.SCYA3.CCL3xPD.1xPD.L2,

CD8xCD137.TNFR9xLMO2.div.M2xPD.1xPD.L2,

CD8xCD137.TNFR9xLMO2.div.M2xSCYA3.CCL3xPD.L2,

15 CD8xCD137.TNFR9xLMO2.div.SCYA3.CCL3xLAG3,

CD4xCD8xCD137.TNFR9xTNF.AlphaxLMO2.div.M2xSCYA3.CCL3xPD.1,

CD8xCD137.TNFR9xTNF.AlphaxLMO2.div.PD.1xPD.L2,

CD4xCD8xTNF.AlphaxLMO2.div.M2xSCYA3.CCL3xPD.L1xTIM3,

CD4xCD8xLMO2.div.M2xSCYA3.CCL3xPD.L2xTIM3,

20 CD8xTNF.AlphaxLMO2xCD56.div.M2xSCYA3.CCL3xPD.1,

CD4xCD8xTNF.AlphaxLMO2.div.M2xSCYA3.CCL3xPD.1xPD.L2,

CD8xLMO2.div.M2xSCYA3.CCL3xPD.L1,

CD4xCD8xLMO2.div.M2xSCYA3.CCL3xLAG3,

CD4xCD8xLMO2.div.M2xSCYA3.CCL3xPD.1xPD.L2,

CD4xCD8xCD137.TNFR9xLMO2xCD56.div.M2xSCYA3.CCL3xPD.1,

CD4xCD8.div.M2xPD-L1,

5 CD4xCD8xCD137.div.M2xPD-1xPD-L1,

CD4xCD8xCD137.div.PD-1xPD-L1,

CD8xCD137.div.M2xPD-1xPD-L1,

CD4xCD8.div.M2xPD-1,

CD4xCD8xCD137.div.M2xPD-L1,

10 CD4xCD8.div.M2xPD-1xPD-L1,

CD56xCD137.div.M2xPD-1xPD-L1,

CD56xCD137.div.M2xPD-1,

CD4xCD56xCD137.div.M2xPD-1,

CD4xCD137.div.PD-1xPD-L1,

15 CD8xCD137.div.PD-1xPD-L1,

CD8xCD137.div.M2xPD-1,

CD8xCD56xCD137.div.M2xPD-1xPD-L1,

CD4xCD137.div.M2xPD-1,

CD4xCD8xCD56xCD137.div.M2xPD-1xPD-L1,

20 CD56xCD137.div.M2xPD-L1, or

wherein M2 is (CD163.div.CD68).

Preferably, the immune score for a subject is determined using the ratio
 $CD4 \times CD8 \div M2 \times PD-L1$, or
 $CD4 \times CD8 \times CD137 \cdot TNFR9 \times TNF \cdot \alpha \times LMO2 \div M2 \times SCYA3 \cdot CCL3 \times PD-1 \times PD-L1$. As
 used herein SCYA3 and CCL3 refer to the same single marker, both terms have been
 5 used as that marker may be referred to by a skilled person as either SCYA3 or CCL3
 (i.e. SCYA3.CCL3 or SCYA3(CCL3) refer only to a single marker). Likewise,
 CD137.TNFR9 refers to the same single marker that may be referred to by the skilled
 person as either CD137 or TNFR9.

In any method of the invention described herein, the reference score and
 10 immune score are determined using the same biomarkers in the same ratios. The
 immune score and reference score are determined using the same statistical method or
 the same ratio described herein. For example, the immune score and reference score
 may be determined using the same ratio being any of those described in Table 3 or 5,
 preferably $CD4 \times CD8 \div M2 \times PD-L1$ or
 15 $CD4 \times CD8 \times CD137 \cdot TNFR9 \times TNF \cdot \alpha \times LMO2 \div M2 \times SCYA3 \cdot CCL3 \times PD-1 \times PD-L1$.
 Thus, in selecting the biomarkers to assay for prognosis of a given subject, the
 prognosis requires that a reference score has previously been determined for those
 specific biomarker ratios, which can be done by the methods described in this
 specification.

20 The level of any one of CD137, CD4, CD8, CD56, TNF α (alpha), LMO2, PD-1,
 PD-L1, CD163, CD68, PD-L2, LAG3, TIM3 and SCYA3(CCL3) may be determined from
 a biological sample derived from the subject or may be determined from information
 obtained from a previously analyzed biological sample.

In any method described herein, determining the level of any one of the
 25 biomarkers described herein in the subject, may be by determining the level in a
 biological sample obtained from the subject or from expression information previously
 obtained from a biological sample. Preferably, the level of a biomarker is determined by
 the amount or relative amount of a nucleic acid encoding, or complementary to a nucleic
 acid encoding, a biomarker as described herein in a biological sample. Preferably, the
 30 nucleic acid is RNA, more preferably mRNA.

In any method described herein, the method further comprises a step of
 obtaining a biological sample from the subject. The biological sample may be selected

from the group consisting of formalin fixed embedded tissue or cells, processed tissue, frozen tissue, and a biopsy.

In a preferred form, more than one immune score is determined using ratios of different biomarkers (such as those in Table 3 or 5), and compared to the corresponding
5 reference score, to improve prognosis accuracy for a subject.

In any method described herein, the method further comprises a step of conducting a COO, R-IPI and/or IPI analysis to further stratify the subject into a group based on likelihood of overall survival, recurrence free survival or response to therapy. The subject may be stratified into one of the following groups R-IPI - 'very good/good'
10 (VG/G) equivalent to IPI 0-2, 'Poor' equivalent to IPI:3-5, COO - germinal centre B-cell (GCB) and non-GCB (activated B-cell and unclassified combined), each group may then have a subgroup based on an immune score that is above the reference score (a high immune score) or an immune score below the reference score (a low immune score).

In one aspect there is provided a kit or article of manufacture for providing a
15 prognosis of a subject having diffuse large B-cell lymphoma responding to a treatment regime including an anthracycline and anti-CD20 antibody including one or more nucleic acid primers or probes for determining the level of any of the biomarkers for determining an immune score as described here using any one or more of the ratios in Table 3 or 5.

In one aspect there is provided a kit comprising one or more nucleic acid primers
20 or probes for determining the level of any of the biomarkers for determining an immune score as described here using any one or more of the ratios in Table 3 or 5.

In another embodiment, the kit or article of manufacture includes written instructions for implementing an above described method. In one particular embodiment, the kit comprises reagents for quantitative amplification of nucleic acid, preferably RNA, of a biomarker that can be used to determine an immune score as
25 described herein. Alternatively, the kit may comprise a microarray. In other embodiments, the kits may contain 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 15, 16, 18, 20, 25, 30, 40, 50, 75, 100, 200, 500 or more probes or primers.

The invention also provides a kit when used in a method of the invention
30 described herein.

Preferably the primer or probe is a nucleic acid having a sequence sharing at least 70% nucleotide sequence identity, preferably 75% identity, preferably 80% identity, preferably 85% identity, preferably 90% identity, preferably 95% identity, preferably 98 or 99 % identity with the sequence of the relevant nucleic acid encoding,
5 or complementary to a nucleic acid encoding, a biomarker as described herein.

The invention also includes a device for determining levels of any one or more of the biomarkers described herein and visual means for reporting (a) an immune score as described herein, and / or (ii) a difference from a reference score.

In addition to the above components, the kit, device or article of manufacture of
10 the invention further include instructions for practicing the methods of the invention.

The current invention provides the clinician or physician caring for a subject with DLBCL information about the likelihood of response to treatment and overall survival. On the basis of the results of the method of the invention, the clinician or physician can (i) avoid treating a subject with a treatment regime that the subject is unlikely to respond
15 to, (ii) avoid treating a subject with a treatment regime that will provide side effects and unlikely to provide any benefit in treating the disease, (iii) enroll the patient in clinical trials for new therapies for DLBCL, (iv) treat the subject with alternative therapies, such as those which target the immune biomarkers, (v) discuss the likely treatment and outcome scenarios with the subject, (vi) provide more regular or extensive post-
20 treatment surveillance for a subject identified as having a low response or survival rate, and / or (vi) proceed to treat a subject identified as likely to respond with added confidence the treatment is likely to provide benefit to the subject.

As used herein, except where the context requires otherwise, the term "comprise" and variations of the term, such as "comprising", "comprises" and
25 "comprised", are not intended to exclude further additives, components, integers or steps. As used herein, "including" and "comprising" are intended to be given the same meaning and are interchangeable.

Further aspects of the present invention and further embodiments of the aspects described in the preceding paragraphs will become apparent from the following
30 description, given by way of example and with reference to the accompanying drawings.

Brief description of the drawings

Figure 1. Kaplan–Meier estimates of survival of the Australian-tissue cohort of DLBCL patients treated with R-CHOP for high and low immune-ratio CD4*CD8:M2*PD-L1. A) All patients; B) VG/G R-IPI; C) Poor R-IPI; D) GCB patients; E) non-GCB; F) Poor-risk (Poor R-IPI-low-ratio and/or non-GCB-low-ratio) versus good-risk (remaining patients).

Figure 2. Paired frozen/paraffin DLBCL gene expression quantified by nanoString™.

Figure 3. Kaplan–Meier estimate of survival of the validation cohort of 233 DLBCL patients treated with R-CHOP-like therapy. Patients stratified by the high and low immune-ratio CD4*CD8:M2*PD-L1 quantified using the Affymetrix platform on frozen tissue.

Detailed description of the embodiments

The instant invention overcomes several major problems with current cancer prognosis in providing methods, devices, kits and compositions using novel combinations of biomarkers identified by expression profiling and survival analysis of diffuse large B-cell lymphoma (DLBCL) patients. In various embodiments, particular biomarkers have been identified that may be used to predict response to chemotherapy and clinical outcome, e.g., overall survival or disease-free survival, in DLBCL patients.

Specifically, the present invention is based on the relationship between immune effectors (beneficial host response to tumour) and inhibitory host-checkpoint response (negative host and/or tumour response to adapt to and evade immune surveillance). The inventors for the first time determine that the net-tumoural immunity, being the relationship of the antagonistic forces of immune effectors and inhibitory checkpoint response, have significant prognostic value. In particular, the net-tumoural immunity as determined by an immune-ratio of immune effectors to inhibitory check-point response can predict a subjects response to a therapy treatment including an anthracycline and a B-cell depleting antibody (e.g. any anti-CD20 antibody), for example R-CHOP treatment.

An advantage of the present invention is that the prognostic value is independent of existing pre-treatment prognosticators such as cell-of-origin (COO), international

prognostic index (IPI) and/or the revised-international prognostic index (R-IPI). However, a further advantage is that the present invention can be used in conjunction with COO, IPI or R-IPI to better predict overall survival and response to initial therapy.

The present invention is a valuable prognosticator as it segregates patients into
5 distinct categories, those categories containing a high proportion of patients and can be used independently of, or in conjunction with to enhance, the discriminatory ability of COO, R-IPI and IPI. Those subjects identified as having a low likelihood to respond to treatment or a low likelihood of survival can then be treated using regimes other than anthracycline and anti-CD20 monoclonal antibody based chemo-immunotherapy (e.g.
10 R-CHOP), such as those which block the PD-1/PD-L1 axis. Further, subjects identified as having a low likelihood to respond to treatment or a low likelihood of survival may still be treated with anthracycline and anti-CD20 monoclonal antibody based chemo-immunotherapy (e.g. R-CHOP) but are instead subject to an increased level of post-therapy surveillance.

15 Further, for a subject who has not yet undergone anthracycline and anti-CD20 monoclonal antibody based chemo-immunotherapy, such as R-CHOP, the methods described herein can be used to determine the likelihood of recurrence-free survival or overall survival if the chemo-immunotherapy was applied. For those patients determined to have a low likelihood of recurrence-free survival or overall survival, in other words
20 stratified by their immune score into a group having a low probability of recurrence-free survival or overall survival, they can be treated by more aggressive or alternative treatments, for example checkpoint-blockage treatments.

'Prognosis' generally refers to a forecast or prediction of the probable course or outcome of the cancer. As used herein, prognosis includes the forecast or prediction of
25 any one or more of the following: duration of survival of a patient susceptible to or diagnosed with diffuse large B-cell lymphoma, duration of recurrence-free survival, duration of progression free survival of a patient susceptible to or diagnosed with diffuse large B-cell lymphoma, response rate in a group of patients susceptible to or diagnosed with diffuse large B-cell lymphoma, and/or duration of response in a patient or a group
30 of patients susceptible to or diagnosed with a diffuse large B-cell lymphoma. Prognosis also includes prediction of favorable responses to cancer treatments, such as a conventional cancer therapy, for example a treatment regime including an anthracycline

and anti-CD20 antibody'. As will be understood by those skilled in the art, the prediction may need not be correct for 100% of the subjects evaluated. The term, however, requires that a statistically significant portion of subjects can be identified as having an increased probability of having a given outcome.

5 'Responding to a treatment regime' refers to a clinically or biochemically favorable detectable response to a treatment, such as a conventional cancer therapy. Typically, a favorable response is survival measured at a later time point after treatment, for example, 1, 2, 3, or 4 years post treatment.

10 'Overall survival' or 'OS' is well known to one of skill in the art and refers to the fate of the patient after an event, preferably after the start or end of a treatment regime, despite the possibility that the cause of death in a patient is not directly due to the effects of the disease (cancer). In other words, it refers to the prognosis that the patient will not die because of DLBCL, preferably within at least 1 year, at least 2 years, at least 3 years, at least 4 years, at least 5 years, at least 10 years or at least 15 years.

15 A 'treatment regime including an anthracycline and anti-CD20 antibody' refers to any treatment administrable to a subject for cancer therapy that includes an anthracycline and anti-CD20 antibody. Exemplary treatments include a chemotherapy or chemo-immunotherapy treatment including an anthracycline and anti-CD20 antibody is R-CHOP (Rituximab, Cyclophosphamide, Hydroxydaunorubicin, Oncovin (vincristine),
20 and Prednisone or Prednisolone), R-CHOP-14, R-CHOP-21, DA-EPOCH-R (dose-adjusted etoposide, doxorubicin, and cyclophosphamide with vincristine, prednisone, and rituximab) or R-ACVBP (rituximab, doxorubicin, cyclophosphamide, vindesine, bleomycin, and prednisone). More preferably, the chemotherapy is R-CHOP.

25 'Anthracycline' as used herein, refers to a class of drugs used in cancer chemotherapy derived from Streptomyces bacteria. The anthracycline may be doxorubicin, daunorubicin, idarubicin or epirubicin.

30 A 'B-cell depleting antibody' is any antibody that reduces the number of clinically or biochemically detectable B-cells in a subject, preferably the number of cancerous B-cells. A non-limiting example of a B-cell depleting antibody is inotuzumab or an anti-CD20 antibody.

'Anti-CD20 antibody', as used herein, refers to antibodies or antigen-binding fragments thereof that specifically target CD20. Exemplary anti-CD20 antibodies include rituximab, obinutuzumab and ofatumumab. In a particular embodiment, the anti-CD20 antibody is rituximab.

5 As used herein 'reference score', 'cut-off value', 'survival cut-off' or 'tree cut-off' are used interchangeably. Preferably, the reference score has been predetermined or is determined from a cohort of patients with known DLBCL outcome, preferably survival (e.g. overall survival, 1 year, 2 years, 3 years or 4 years survival) after treatment with a regime that includes an anthracycline and anti-CD20 antibody. The reference score
10 stratifies the subject into one of two subgroups with the following rule: if the immune score is less than the reference score, then the patient is assigned to the group with low likelihood of response (e.g. overall survival); if the immune score is greater than or equal to the reference score, then the patient is assigned to the group with a high likelihood of response (e.g. overall survival). Preferably, the reference score stratifies
15 subjects into a group with a high likelihood of overall survival of at least 95%, 94%, 93%, 92%, 91%, 90%, 85%, 80%, 75% or 70%, and into a group with a low likelihood of overall survival of less than 60%, 55%, 54%, 53%, 52%, 51%, 50%, 49%, 48%, 47%, 46%, 45% or 44%. The reference score can be determined using statistical methods known in the art, for example, a tree-structure recursive partitioning statistical model or
20 the median value. Survival analysis may be performed using Kaplan–Meier method and the two survival curves from the two subgroups can be compared using the log-rank test, such as that described in the Examples.

'Diffuse large B-cell lymphoma (DLBCL)' as used herein, refers to a type of aggressive non-Hodgkin lymphoma, and includes any lymphoma diagnosed as a
25 DLBCL by any clinical or biochemical measure. DLBCL includes all four major subtypes of DLBCL: activated (ABC-DLBCL), germinal center (GCB-DLBCL), Type III (unclassified) DLBCL and primary mediastinal B-cell lymphoma (PMBL).

The term 'International Prognostic Index (IPI)' refers to the clinical tool developed by oncologists to aid in predicting the prognosis of patients with aggressive non-
30 Hodgkin's lymphoma. The IPI score was developed through a retrospective analysis performed on 2031 patients with aggressive non-Hodgkin's lymphoma, of all ages, treated with a doxorubicin-based chemotherapy regimen such as CHOP

(Cyclophosphamide, Hydroxydaunorubicin, Oncovin (vincristine), and Prednisone or Prednisolone), between 1982 and 1987. Several patient characteristics were analyzed to determine whether they were associated with differences in survival, and the factors that emerged as significant were age, elevated serum lactate dehydrogenase (LDH),
5 performance status, and number of extranodal sites of disease. The term may also include derived scores such as FLIPI, MIPI and age-adjusted IPI. A 'revised International Prognostic Index (IPI)' is a revised version of the IPI as described in Blood. 2007 Mar 1;109(5):1857-61 and *Nature* 2000; 403(6769): 503-11.

The biomarkers or immune markers as used herein may be related to prognosis,
10 for example, prediction of survival, recurrence, or therapy response. The ratio of differential patterns of expression of a plurality of these biomarkers may be used to predict the survival outcome of a subject with diffuse large B-cell lymphoma. The unique pattern of expression of a plurality of biomarkers in a subject may be used to generate an immune score or immuno-biological score of survival using an immune ratio describe
15 herein. Any reference herein to immune score may be a reference to immuno-biological score when any aspect of the invention includes one or more of $TNF\alpha$, PD-L2, LAG3, TIM3, LMO2 or SCYA3.CCL3. An immuno-biological score is explained further in Example 6. Subjects with a high immune score or immuno-biological score relative to a reference score of cut-off value may have, or are more likely to have, a long survival
20 time (e.g., more than about 4 years) after a treatment regime. Subjects with a low immune score or immuno-biological score relative to a reference score or cut-off value may have a short survival time (e.g., less than about 4 years) after a treatment regime. Unless stated otherwise, reference to biomarker or immune marker refers to any one or more of CD137, CD4, CD8, CD56, $TNF\alpha$ (alpha), LMO2, PD-1, PD-L1, CD163, CD68,
25 PD-L2, LAG3, TIM3 and SCYA3(CCL3). The nucleotide sequence of the gene and mRNA, and the polypeptide sequence of the gene product that is translated, can be identified by a person skilled in the art by accessing any one of a number of publicly accessible databases such as GenBank or EMBL or as shown in Table 6 herein.

Regardless of the technique used to measure the level of a plurality of
30 biomarkers, the level of each biomarker may be converted into an immune score using a ratio as outlined in Table 3 or 5.

A 'ratio' or 'immune ratio' as used herein refers to the relationship between two or more biomarkers. Preferably, the ratio or immune ratio is the relationship between one or more of CD137, CD4, CD8, CD56, TNF α (alpha) and LMO2 to one or more of PD-1, PD-L1, CD163, CD68, PD-L2, LAG3, TIM3 and SCYA3(CCL3). Alternatively, the ratio
5 or immune ratio is the relationship between one or more of CD137, CD4, CD4 and CD56 to one or more of PD-1, PD-L1, CD163 and CD68. Preferably, the ratio or immune ratio is one of those listed in Table 3 or 5. The ratio or immune ratio may also be expressed herein as an immune-effector:checkpoint ratio.

The immune scores generated may be used to classify patients into high or low
10 immune score based on a comparison with a survival cut-off value. An immune score that is higher than the survival cut-off is associated with a good prognosis, such as a long survival time or a better survival or more positive response to a treatment regime, and an immune score that is lower than the survival cut-off is associated with a poor prognosis, such as a short survival time or a poor survival or less positive response to a
15 treatment regime. A survival cut-off value may be derived from a control group of patients of known outcome after treatment with a particular regime, for example a treatment regime including an anthracycline and anti-CD20 antibody, using standard statistical techniques, for example, using a permutation test.

In any method described herein, the method further comprises the step of
20 treating the DLBCL by administering one or more therapeutic agents. The therapeutic agents may be an anthracycline and anti-CD20 antibody, or any regime including anthracycline and anti-CD20 antibody as described herein. Further, the method may include treating the subjects identified with a high immune score with a treatment regime including an anthracycline and anti-CD20 antibody, such as R-CHOP or other
25 regime described herein.

In any method described herein, the method further comprises the step of enrolling the subject identified as having a low immune score in a clinical trial for treatment of DLBCL. The method may further comprise the step of administering a compound that binds to and inhibits PD-1 to a subject identified as having a low immune
30 score.

A biological sample, such as a tissue or cell sample, may be collected from a subject with a lymphoma, for example diffuse large B-cell lymphoma. The collection

step may comprise surgical resection. The sample of tissue may be stored in RNAlater or flash frozen, such that RNA may be isolated at a later date. RNA may be isolated from the tissue and used to generate labeled probes for a nucleic acid microarray analysis. The RNA may also be used as a template for a method described herein in
5 which the expression or level of a plurality of biomarkers is analyzed (e.g. microarray, qRT-PCR). The expression data generated may be used to derive an immune score, e.g., using a ratio as described in Table 3 or 5.

In any method described herein, the method further comprises a step of providing a biological sample from the subject or obtaining a biological sample from the
10 subject. In one embodiment, the methods comprise surgically removing a biological sample, preferably a tumour sample, from the subject. The biological sample may be further treated before analysis. The biological sample may be selected from the group consisting of formalin fixed embedded tissue or cells, processed tissue, frozen tissue, and a biopsy. Preferably, at least 40%, 50%, 60%, 70%, 80% or 90% of the cells in the
15 sample are tumour cells.

In certain aspects, the invention described herein involves measuring expression of one or more biomarkers in a biological sample of cells or tissue from a subject with diffuse large B-cell lymphoma. The expression information may be obtained by testing biological samples by a lab, a technician, a device, or a clinician.

20 In one embodiment, determining the level (or obtaining an expression level or profile) of one or more biomarkers in a biological sample from the subject comprises extracting nucleic acids from the sample from the subject. In certain embodiments, the nucleic acid sample is an RNA sample, preferably an mRNA sample. In one embodiment, the expression level of the nucleic acid is determined by any method
25 described herein, such as by hybridizing the nucleic acid, or amplification products thereof, to a DNA microarray or by using RNA-sequencing. Amplification products may be generated, for example, with reverse transcription, optionally followed by PCR amplification of the products.

The pattern or signature of expression in each biological sample may then be
30 used to generate an immune score for prognosis or classification, such as predicting overall survival, disease recurrence or response to therapy. The expression of one or more biomarkers may be measured by a variety of techniques that are well known in the

art. Quantifying the levels of the RNA (e.g. mRNA) of a biomarker may be used to measure the level or expression of the biomarker. Alternatively, quantifying the levels of the protein product of a biomarker may be used to measure the expression of the biomarker. Additional information regarding the methods discussed below may be found
5 in Ausubel et al. (2003) or Sambrook et al. (1989). As would be recognized by one of skill in the art, various parameters may be manipulated to optimize detection of the mRNA or protein of interest.

A nucleic acid microarray may be used to quantify the differential expression of a plurality of biomarkers. Microarray analysis may be performed using commercially
10 available equipment, following manufacturer's protocols, such as by using the Affymetrix GeneChip® technology (Santa Clara, CA) or the Microarray System from Incyte (Fremont, CA). For example, single-stranded nucleic acids (e.g., cDNAs or oligonucleotides) may be plated, or arrayed, on a microchip substrate. The arrayed sequences are then hybridized with specific nucleic acid probes from the cells of
15 interest. Fluorescently labeled cDNA probes may be generated through incorporation of fluorescently labeled deoxynucleotides by reverse transcription of RNA extracted from the cells of interest. Alternatively, the RNA may be amplified by in vitro transcription and labeled with a marker, such as biotin. The labeled probes are then hybridized to the immobilized nucleic acids on the microchip under highly stringent conditions. After
20 stringent washing to remove the non-specifically bound probes, the chip is scanned by confocal laser microscopy or by another detection method, such as a CCD camera. The raw fluorescence intensity data in the hybridization files are generally preprocessed with the robust multichip average (RMA) algorithm to generate expression values.

Quantitative real-time PCR (qRT-PCR) may also be used to measure the
25 differential expression of a plurality of biomarkers. In qRT-PCR, the RNA template is generally reverse transcribed into cDNA, which is then amplified via a PCR reaction. The amount of PCR product is followed cycle-by-cycle in real time, which allows for determination of the initial concentrations of mRNA. To measure the amount of PCR product, the reaction may be performed in the presence of a fluorescent dye, such as
30 SYBR Green, which binds to double-stranded DNA. The reaction may also be performed with a fluorescent reporter probe that is specific for the DNA being amplified. A non-limiting example of a fluorescent reporter probe is a TaqMan® probe (Applied Biosystems, Foster City, CA). The fluorescent reporter probe fluoresces when the

quencher is removed during the PCR extension cycle. Multiplex qRT-PCR may be performed by using multiple gene-specific reporter probes, each of which contains a different fluorophore. Fluorescence values are recorded during each cycle and represent the amount of product amplified to that point in the amplification reaction. To
5 minimize errors and reduce any sample-to-sample variation, qRT-PCR may be performed using a reference standard. The ideal reference standard is expressed at a constant level among different tissues, and is unaffected by the experimental treatment.

Suitable reference standards include, but are not limited to, mRNAs for the housekeeping genes glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) and β -
10 actin. The level of mRNA in the original sample or the fold change in expression of each biomarker may be determined using calculations well known in the art.

Further, RNA quantification may be by the NanoString Gene Expression Assay as described herein, specifically in Example 1.

The level of a biomarker may be detected using Northern blot analysis. For
15 example, total cellular RNA can be purified from cells by homogenization in the presence of nucleic acid extraction buffer, followed by centrifugation. Nucleic acids are precipitated, and DNA is removed by treatment with DNase and precipitation. The RNA molecules are then separated by gel electrophoresis on agarose gels according to standard techniques, and transferred to nitrocellulose filters. The RNA is then
20 immobilized on the filters by heating. Detection and quantification of specific RNA is accomplished using appropriately labelled DNA or RNA probes complementary to the RNA in question.

Methods for preparation of labelled DNA and RNA probes, and the conditions for hybridization thereof to target nucleotide sequences, are described in Molecular
25 Cloning: A Laboratory Manual, J. Sambrook et al, eds., 2nd edition, Cold Spring Harbor Laboratory Press, 1989, Chapters 10 and 11.

In one example, the nucleic acid probe can be labelled with, e.g., a radionuclide, such as ^3H , ^{32}P , ^{33}P , ^{14}C , or ^{35}S ; a heavy metal; or a ligand capable of functioning as a specific binding pair member for a labelled ligand (e.g., biotin, avidin or an antibody), a
30 fluorescent molecule, a chemiluminescent molecule, or an enzyme. Probes can be labelled to high specific activity by nick translation, random priming, or other methods

known to one of skill in the art. For example, by replacing pre-existing nucleotides with highly radioactive nucleotides according to the nick translation method, it is known to prepare ^{32}P -labeled nucleic acid probes with a specific activity well in excess of 108 cpm/microgram. Autoradiographic detection of hybridization can then be performed by
5 exposing hybridized filters to photographic film. Densitometric scanning of the photographic films exposed by the hybridized filters provides an accurate measurement of RNA transcript levels. In another embodiment, RNA gene transcript levels can be quantified by computerized imaging systems, such the Molecular P-71519-PC Dynamics 400-B 2D Phosphorimager available from Amersham Biosciences,
10 Piscataway, NJ.

In another embodiment, determining the levels of an RNA expression can be accomplished using the technique of in situ hybridization. This technique requires fewer cells than the Northern blotting technique, and involves depositing whole cells onto a microscope cover slip and probing the nucleic acid content of the cell with a solution
15 containing radioactive or otherwise labelled nucleic acid (e.g., cDNA or RNA) probes. This technique is particularly well-suited for analyzing tissue biopsy samples from subjects. The practice of the in situ hybridization technique is described in more detail in U.S. Pat. No. 5,427,916, the disclosure of which is incorporated herein by reference.

The Kaplan-Meier method (also known as the product limit estimator) estimates
20 the survival function from life-time data. In medical research, it can be used to measure the fraction of patients living for a certain amount of time after treatment.

A plot of the Kaplan-Meier method of the survival function is a series of horizontal steps of declining magnitude which, when a large enough sample is taken, approaches the true survival function for that population. The value of the survival function between
25 successive distinct sampled observations ("clicks") is assumed to be constant.

An important advantage of the Kaplan-Meier curve is that the method can take into account "censored" data— losses from the sample before the final outcome is observed (for instance, if a patient withdraws from a study). On the plot, small vertical tick-marks indicate losses, where patient data has been censored. When no truncation
30 or censoring occurs, the Kaplan-Meier curve is equivalent to the empirical distribution.

Survival analysis can be performed using the Kaplan-Meier method (as described in the Examples herein and shown in Figure 1).

In statistics, the log-rank test (also known as the Mantel-Cox test) is a hypothesis test to compare the survival distributions of two groups of patients. It is a nonparametric test and appropriate to use when the data are right censored. It is widely used in clinical trials to establish the efficacy of new drugs compared to a control group when the measurement is the time to event. The log-rank test statistic compares estimates of the hazard functions of the two groups at each observed event time. It is constructed by computing the observed and expected number of events in one of the groups at each observed event time and then adding these to obtain an overall summary across all time points where there is an event. The log-rank statistic can be derived as the score test for the Cox proportional hazards model comparing two groups. It is therefore asymptotically equivalent to the likelihood ratio test statistic based from that model.

The invention also provides a device, system, kit or article of manufacture for use in implementing the above described methods.

The device, system, kit or article of manufacture may include a nucleic acid for hybridising to any one or more of the biomarkers useful to determine an immune score by Watson-Crick base pairing. The nucleic acid may be an oligonucleotide or like probe having from about 8 to 100 or more nucleotides. Preferably the nucleic acid has a sequence sharing at least 70% nucleotide sequence identity, preferably 75% identity, preferably 80% identity, preferably 85% identity, preferably 90% identity, preferably 95% identity, preferably 98 or 99 % identity with the sequence of the relevant nucleic acid encoding, or complementary to a nucleic acid encoding, a biomarker as described herein.

Percent sequence identity is determined by conventional methods, by means of computer programs known in the art such as GAP provided in the GCG program package (Program Manual for the Wisconsin Package, Version 8, August 1994, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711) as disclosed in Needleman, S.B. and Wunsch, C.D., (1970), Journal of Molecular Biology, 48, 443-453, which is hereby incorporated by reference in its entirety. GAP is used with the following settings for DNA sequence comparison: GAP creation penalty of 5.0 and GAP extension penalty of 0.3.

The device, system, kit or article of manufacture may include reagents for use in the amplification or digestion or other manipulation of nucleic acids including an enzyme, such as a polymerase or a restriction endonuclease.

5 The device, system, kit or article of manufacture may include reagents for quantitative Northern and Southern blotting, and microarray and PCR, preferably real-time or quantitative PCR.

Arrays and microarrays which contain probes specific for the biomarkers, and determining an immune or immuno-biological score, as disclosed herein are also encompassed within the scope of this invention. Methods of making arrays are well-
10 known in the art and as such do not need to be described in detail. Arrays of the invention can contain the profiles of one or more genes as disclosed in Table 3 or 5. Accordingly, arrays for detection of recurrence can be customized for determining response to therapy or overall survival of a subject with diffuse large B-cell lymphoma. The array can be packaged as part of kit comprising the customized array itself and a
15 set of instructions for how to use the array to determine an subject's likelihood of responding to a treatment regime.

In addition to the above components, the kits of the invention further include instructions for practicing the methods of the invention. These instructions may be present in the kits in a variety of forms, one or more of which may be present in the kit.
20 One form in which these instructions may be present is as printed information on a suitable medium or substrate, e.g., a piece or pieces of paper on which the information is printed, in the packaging of the kit, in a package insert, etc. Yet another means would be a computer readable medium, e.g., diskette, CD, etc., on which the information has been recorded. Yet another means that may be present is a website address which may
25 be used via the internet to access the information at a remote site. Any convenient means of conveying instructions may be present in the kits.

One aspect of the invention provides a kit comprising: (a) any of the gene chips described herein; and (b) one of the computer-readable mediums described herein.

The invention also includes computer readable media that comprises reference
30 gene expression profiles. Such media can contain all or part of the gene expression profiles of the biomarkers described herein that can be used to determine an immune

score. The media can be a list of the genes or contain the raw data for running a user's own statistical calculation, such as the methods disclosed herein.

Another aspect of the invention provides a program product (i.e., software product) for use in a computer device that executes program instructions recorded in a computer-readable medium to perform one or more steps of the methods described herein, such as for estimating the likelihood of a subject with diffuse large B-cell lymphoma responding to a treatment regime including an anthracycline and anti-CD20 antibody.

The invention will now be further described with reference to the following non-limiting examples.

Example 1

Patients

The study was approved by Ethics Committees at participating sites. The initial tissue cohort comprised 252 patients with histologically confirmed DLBCL. All patients received R-CHOP, and were otherwise selected solely on the basis of FFPE tissue availability. Only *de-novo* cases of DLBCL were included. Grade IIIB or transformed follicular lymphoma, HIV-positive and post-transplant patients were excluded. Survival data was available in 158 patients. For validation of the generated model's findings we utilized a publicly available data-set in which gene expression was quantified on fresh-frozen samples using an Affymetix platform (Lenz G, et al. *N Engl J Med* 2008; **359**(22): 2313-23).

RNA quantification

RNA was extracted from FFPE tumour-biopsies using RecoverAll (Ambion, Carlsbad, CA, USA) and stored at -80°C. Genes were quantified (as per below) using the nanoString™ platform (Seattle, WA, USA).

Genes were chosen to permit COO categorization (Wright et al. *Proc Natl Acad Sci U S A* 2003; 100(17): 9991-6.), and analysis of immune effectors and immune-checkpoints. Hybridizations were carried out according to the NanoString Gene Expression Assay Manual. Five microliter of each RNA sample (100ng) was mixed with 20µl of nCounter Reporter probes in hybridization buffer and 5µl of nCounter Capture

probes for a total reaction volume of 30µl. The hybridizations incubated at 65°C for approximately 16-20 hours. Three separate runs were performed requiring the production of three identical codesets. Expression counts were normalized between codesets based on relative differences between duplicate samples in both runs which
 5 allowing us to develop a correction factor for each individual gene between the runs. Raw data was imported and analysed in the NanoString® data analysis tool nSolver. For normalization, gene expression data was internally controlled to the mean of the positive control probes to account for interassay variability. Gene normalization was then performed using the geometric mean of four housekeeper gene to account for
 10 factors that affect RNA quality and quantity (PGK1, GAPDH, PGAM1, OAZ1). Housekeeping genes were selected as previously described and as per manufacturer recommendation (de JHJ, Fehrmann RS, de BES, et al. . PloS one 2007; 2(9): e898).

Statistical analysis

Values between groups of data were tested for statistical significance using the
 15 2-tailed paired (e.g. between time-points) or, where appropriate unpaired T-tests. Categorical data were compared using Fisher's exact test or Chi-squared test as appropriate. R-IPI was categorized as 'very good/good' (VG/G) equivalent to IPI 0-2, and 'Poor' equivalent to IPI:3-5. COO was classified as germinal centre B-cell (GCB) and non-GCB (activated B-cell and unclassified combined). Overall survival (OS) was
 20 measured from diagnosis to date of last follow-up or death.

To assess the prognostic value of each single marker and each ratio, survival analysis was performed using tree-structured recursive partitioning statistical models. This was followed by a two-sample test to test the difference between survival curves, adjusted for multiple testing. The model determines an optimal cutoff for each ratio (or
 25 single marker) to stratify patients into two survival subgroups. Survival analysis was performed using Kaplan–Meier method and compared using the log-rank test. Multivariate analysis was performed using Cox regression. All *P*-values are two-sided, except those in the validation groups which are one-sided in the direction of the observed training group effect, as per Lenz *et al.* (Lenz G, et al. *N Engl J Med* 2008;
 30 **359**(22): 2313-23).

Tree-based recursive portioning analysis

A survival analysis based on the tree-structured recursive partitioning model was applied to stratify patients into two survival subgroups (low or high-risk) based on a single marker or ratio (Hothorn T, Hornik K, Zeileis A. Unbiased recursive partitioning: A conditional inference framework. *Journal of Computational and Graphical Statistics* 2006; 15(3): 651-74.) . The survival tree determines the best optimal cutoff for each single marker or ratio based on a permutation test. On the Nanostring data, one tree was built on each single marker or ratio with only one node split in order to stratify the patients into two subgroups with the following rule: if the ratio value (or the single marker value) is less than or equal to the tree cut-off, then the patient is assigned to the 'low-risk' group; if the ratio value (or the single marker value) is strictly greater than the tree cut-off, then the patient is assigned to the 'high-risk' group. To assess the prognostic value of each marker or ratio given the determined cutoff we applied the log-rank test. We ran the survival analysis on a total of 65 single markers or ratios and all P-values were adjusted using the Bonferroni multiple correction procedure (Table 3 or 5). The tree-structure models were performed using the R statistical software using the packages 'party', 'partykit' and 'survival' (R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.Rproject.org/>).

Example 2 - Correlation of immune-effectors with checkpoints

In keeping with previous reports using nanoString™, we observed strong Spearman correlation between gene expression in three paired frozen/FFPE samples across a range of genes (Figure 2), indicating the validity of this approach for RNA quantification on FFPE biopsies. We then tested correlations between immune-effector and checkpoints quantified by nanoString™ in 252 FFPE tissues. Immune-effectors were CD137 (for T and NK-cell activation), CD4, CD8 and CD56. TAM and PD-1/PD-L1-axis checkpoints were PD-1, PD-L1, CD163 and CD68. Correction for multiple testing was applied (Benjamini Y, Hochberg Y. *Journal of the Royal Statistical Society Series B* 1995; 57: 289-300). Consistent with a dynamic interplay between the inhibitory checkpoint response (initiated by the host to evade destruction) and immune-effector activation, highly significant correlations of all immune-effectors was observed for virtually all checkpoints ranging from up to 10^{-31} (CD4), 10^{-15} (CD8), 10^{-5} (CD56), and 10^{-12} (CD137). The strength of correlations were modest and variable, reflecting the variation between patients in mounting an effective anti-lymphoma immune response (Table 1). **Table 1.** Correlations between immune molecules within the tissue

microenvironment. Spearman correlation co-efficients are listed for immune-effectors (CD4/CD8/CD56/CD137) with checkpoints (CD68/CD163/PD-1/PD-L1). P-values, shown as shaded, were adjusted for multiple testing.

	CD8	CD56	CD137	CD68	CD163	PD-1	PD-L1
CD4	0.5238	0.3603	0.6256	0.7653	0.4397	0.4691	0.5856
	4.03E-12	5.07E-06	6.06E-18	7.50E-31	1.33E-08	9.59E-10	2.26E-15
CD8		0.329	0.5424	0.5811	0.3403	0.5127	0.4183
		3.38E-05	5.31E-13	3.85E-15	1.77E-05	1.21E-11	7.23E-08
CD56			0.3087	0.2937	0.2313	0.3234	0.1362
			9.43E-05	0.000206	0.00381	4.55E-05	ns
CD137				0.4746	0.0081	0.519	0.5256
				5.94E-10	ns	6.47E-12	3.56E-12
CD68					0.6975	0.3127	0.6833
					1.27E-23	7.81E-05	2.07E-22
CD163						0.2108	0.4281
						0.008357	3.38E-08
PD-1							0.3181
							5.94E-05

5 Example 3 - Immune-effector:checkpoint ratios as a measure of net anti-tumoural immunity within the TME.

The cohort of 158 patients with outcome data were utilized (Table 2). Median age was 62 years, range 27-86. Median follow-up was 3.9 years.

Table 2. Characteristics of the Australian-tissue cohort. *Age, sex and R-IPI were unavailable in 5, 2 and 6 patients respectively. **R-IPI VG/G is equivalent to IPI 0-2 and Poor is equivalent to IPI 3-5.

Characteristic	Australian Tissue cohort (n=158)*
Median age (range)	62 years (27-87)
Female	F: 41%
Interim-PET/CT ^{+ve}	N/A
R-IPI**	VG/G: 58%

Characteristic	Australian Tissue cohort (n=158)*
	Poor: 42%

The correlations of immune-effector and checkpoint suggest that identification of elevated immune-effector molecules *per se*, might not be associated with improved outcome if accompanied by a checkpoint response of sufficient magnitude to counter anti-lymphoma immunity. The inventors hypothesized that the ratios of TME immune-effectors to checkpoints would better predict outcome than quantification of any single immune marker in isolation. A survival analysis was run using a tree-structured survival model testing all 65 combinations of single markers and ratios (Table 3). Correction for multiple testing using the Bonferroni procedure was applied. Immune-effectors were numerators of the ratios. The denominators were the checkpoints PD-1, PD-L1, CD163, CD68 and 'M2' (defined as the ratio of CD163.div.CD68 checkpoint molecules). The rationale for including M2 was that the combination of CD68 and CD163 was likely to be more informative than either checkpoint alone. By multiple testing M2 was prognostic ($P=0.0056$) whereas CD163 and CD68 alone were not.

The tree-structured model identified a number of prognostic immune molecule combinations (Table 3 or 5). Within the top ten combinations in Table 3 (based on the tree-based survival curve tests), CD4, CD8, CD137, CD56 as numerators, and M2, PD-1 and PD-L1 as denominators were all present, with all except CD56 occurring frequently ($\geq$ seven times). The highest ranked combination in Table 3 was the products of the immune-effectors CD4 and CD8, in a ratio with the products of M2 and PD-L1 (the $CD4*CD8:M2*PD-L1$ immune-ratio, also shown herein as $CD4*CD8:[CD163.div.CD68]*PD-L1$, $CD4*CD8:[CD163:CD68]*PD-L1$ or $CD4xCD8.div.M2xPD-L1$). This was selected for further testing. Using the log-rank test, the $CD4*CD8:M2*PD-L1$ ratio separated patients into disparate high and low-ratio 4 year survivals that differed by 45% (Figure 1A, $P<0.0001$). The proportion of patients in high and low-ratio groups were 59% and 41%. * or x refers to the product or multiplication of, and 'div' refers to a division or ratio.

Table 3. Results of tree-structured survival analysis. The cutoff for each single marker or ratio was determined by the tree-structured survival model to divide the

patients into two subgroups. 'NA' indicates that the model is unable to stratify the patients into low and high risk based on the marker or ratio. Adjusted P-values (Bonferroni multiple correction procedure) of the survival curve difference test was used to assess the prognostic value of each marker/ratio together with the 4-year survival proportion in each survival group. Other statistical models can be used to calculate the cutoff, for example the median value (e.g. 0.016945008 for CD4xCD8.div.M2xPD-L1). The results are shown to 9 decimal places however the cutoff values still apply when only 2 decimal places are used.

Immune Ratio/Markers	Tree survival cutoff
CD4xCD8.div.M2xPD-L1	-0.278958829
CD4xCD8xCD137.div.M2xPD-1xPD-L1	-0.408504736
CD4xCD8xCD137.div.PD-1xPD-L1	-0.330383033
CD8xCD137.div.M2xPD-1xPD-L1	-0.861352302
CD4xCD8.div.M2xPD-1	-0.862784566
CD4xCD8xCD137.div.M2xPD-L1	-0.550676238
CD4xCD8.div.M2xPD-1xPD-L1	-0.314192504
CD56xCD137.div.M2xPD-1xPD-L1	-1.133721306
CD56xCD137.div.M2xPD-1	-0.917559615
CD4xCD56xCD137.div.M2xPD-1	-0.934333648
CD4xCD137.div.PD-1xPD-L1	-1.318581845
CD8xCD137.div.PD-1xPD-L1	-0.505976594
CD8xCD137.div.M2xPD-1	-0.471063886
CD8xCD56xCD137.div.M2xPD-1xPD-L1	-0.924574211
CD4xCD137.div.M2xPD-1	-0.355210582
CD4xCD8xCD56xCD137.div.M2xPD-1xPD-L1	-0.663232616
CD56xCD137.div.M2xPD-L1	-0.972213657
CD8xCD137.div.M2xPD-L1	-0.147128081
CD4xCD8xCD56.div.M2xPD-1xPD-L1	-0.85331042
CD4xCD137.div.M2xPD-1xPD-L1	-0.44833144
CD4xCD8xCD56xCD137.div.M2xPD-1	-0.610670264
CD4xCD56xCD137.div.M2xPD-L1	-0.908907444
CD8xCD56.div.M2xPD-1xPD-L1	-0.930619828
CD4xCD8xCD56.div.M2xPD-1	-0.57053548
CD8.div.M2	-0.208486041
CD4xCD56xCD137.div.M2xPD-1xPD-L1	-0.508811002
CD4xCD56.div.M2xPD-1	-0.625957198
CD4xCD137.div.M2xPD-L1	-0.554010003
CD4xCD8xCD137.div.M2xPD-1	-0.515666121
CD4xCD8.div.PD-1xPD-L1	-0.932957763
CD4xCD8xCD56xCD137.div.PD-1xPD-L1	-0.475721216
CD56xCD137.div.PD-1xPD-L1	-1.058842763
CD4xCD56xCD137.div.PD-1xPD-L1	-1.014461648
CD8xCD56xCD137.div.M2xPD-1	-0.737190585
CD4xCD8xCD56xCD137.div.M2xPD-L1	-0.216956503
CD137.div.M2	-0.403903226
CD4xCD8xCD56.div.PD-1xPD-L1	-0.603617394
CD8xCD56.div.M2xPD-1	0.085617826
CD8xCD56.div.PD-1xPD-L1	-1.187944961
CD8xCD56xCD137.div.M2xPD-L1	-0.293161856

CD4.div.PD-L1	-0.520161388
CD137.div.PD-1	-0.925500632
CD8xCD56.div.M2xPD-L1	-0.656390695
CD56.div.M2	-0.813187867
M2	0.398692767
CD4xCD8xCD56.div.M2xPD-L1	-0.049414484
CD4.div.M2	-0.311085471
CD4xCD56.div.M2xPD-1xPD-L1	-0.393948393
CD8xCD56xCD137.div.PD-1xPD-L1	0.164498026
CD137.div.PD-L1	-0.704796691
CD4xCD56.div.M2xPD-L1	-0.927424798
CD8.div.PD-L1	-0.615723398
CD137	-0.050398174
CD8.div.PD-1	0.086337219
CD4xCD56.div.PD-1xPD-L1	-0.840675406
CD8	-1.005866186
CD4	NA
CD56	NA
CD68	NA
CD163	NA
PD-1	NA
PD-L1	NA
CD4.div.PD-1	NA
CD56.div.PD-1	NA
CD56.div.PD-L1	NA

The cutoff for each single marker or ratio was determined by the tree-structured survival model to divide the patients into two subgroups. A immune score that is above the cut-off stratifies the patient into a high immune score or high ratio group with a high likelihood of response or overall survival, whereas an immune score that is below the cut-off stratifies the patient into a low immune score or low ratio group with a low likelihood of response or survival. The likelihood of survival for the high and low groups are shown below. As an example, subject with an immune score above -0.278958829 when using the immune ratio CD4xCD8.div.M2xPD-L1 would be stratified into the high immune score group. As an example a subject from the cohort with an immune score of 0.541762908 what stratified into the high immune score group. That subject had a 4.13 year survival time. Another subject from the cohort had an immune score of -1.186672016 that stratified that subject into the low immune score group. That subject had a 0.56 year survival time.

Immune Ratio/Markers	Difference between survival curves (p-value)
CD4xCD8.div.M2xPD-L1	8.16E-08
CD4xCD8xCD137.div.M2xPD-1xPD-L1	9.19E-08
CD4xCD8xCD137.div.PD-1xPD-L1	1.02E-07
CD8xCD137.div.M2xPD-1xPD-L1	2.96E-07
CD4xCD8.div.M2xPD-1	4.30E-07
CD4xCD8xCD137.div.M2xPD-L1	6.23E-07
CD4xCD8.div.M2xPD-1xPD-L1	1.06E-06
CD56xCD137.div.M2xPD-1xPD-L1	1.30E-06
CD56xCD137.div.M2xPD-1	1.78E-06
CD4xCD56xCD137.div.M2xPD-1	1.85E-06
CD4xCD137.div.PD-1xPD-L1	2.52E-06
CD8xCD137.div.PD-1xPD-L1	3.07E-06
CD8xCD137.div.M2xPD-1	3.94E-06
CD8xCD56xCD137.div.M2xPD-1xPD-L1	4.05E-06
CD4xCD137.div.M2xPD-1	5.90E-06
CD4xCD8xCD56xCD137.div.M2xPD-1xPD-L1	7.37E-06
CD56xCD137.div.M2xPD-L1	8.01E-06
CD8xCD137.div.M2xPD-L1	1.53E-05
CD4xCD8xCD56.div.M2xPD-1xPD-L1	2.11E-05
CD4xCD137.div.M2xPD-1xPD-L1	2.38E-05
CD4xCD8xCD56xCD137.div.M2xPD-1	3.40E-05
CD4xCD56xCD137.div.M2xPD-L1	3.63E-05
CD8xCD56.div.M2xPD-1xPD-L1	4.12E-05
CD4xCD8xCD56.div.M2xPD-1	4.41E-05
CD8.div.M2	5.33E-05
CD4xCD56xCD137.div.M2xPD-1xPD-L1	5.70E-05
CD4xCD56.div.M2xPD-1	5.77E-05
CD4xCD137.div.M2xPD-L1	8.09E-05
CD4xCD8xCD137.div.M2xPD-1	8.52E-05
CD4xCD8.div.PD-1xPD-L1	8.53E-05
CD4xCD8xCD56xCD137.div.PD-1xPD-L1	0.000156513
CD56xCD137.div.PD-1xPD-L1	0.000220052
CD4xCD56xCD137.div.PD-1xPD-L1	0.000249729
CD8xCD56xCD137.div.M2xPD-1	0.00025859
CD4xCD8xCD56xCD137.div.M2xPD-L1	0.000401345
CD137.div.M2	0.000425575
CD4xCD8xCD56.div.PD-1xPD-L1	0.000794506
CD8xCD56.div.M2xPD-1	0.001001503
CD8xCD56.div.PD-1xPD-L1	0.001070362
CD8xCD56xCD137.div.M2xPD-L1	0.001115939

CD4.div.PD-L1	0.001550632
CD137.div.PD-1	0.002286803
CD8xCD56.div.M2xPD-L1	0.003156578
CD56.div.M2	0.004006882
M2	0.005617191
CD4xCD8xCD56.div.M2xPD-L1	0.005643973
CD4.div.M2	0.006247426
CD4xCD56.div.M2xPD-1xPD-L1	0.007853043
CD8xCD56xCD137.div.PD-1xPD-L1	0.008847154
CD137.div.PD-L1	0.00891506
CD4xCD56.div.M2xPD-L1	0.01009469
CD8.div.PD-L1	0.021793304
CD137	0.02438736
CD8.div.PD-1	0.048893747
CD4xCD56.div.PD-1xPD-L1	0.051932413
CD8	0.080251345
CD4	NA
CD56	NA
CD68	NA
CD163	NA
PD-1	NA
PD-L1	NA
CD4.div.PD-1	NA
CD56.div.PD-1	NA
CD56.div.PD-L1	NA

The difference between survival curves as shown here as adjusted P-values (Bonferroni multiple correction procedure) is a measure of the prognostic value of each marker/ratio to separate the two subgroups based on their survival. The greater the difference between the survival curves for a ratio (i.e. the lower the P value) the greater the prognostic value.

5

Immune Ratio/Markers	Proportion of patients in low-ratio 4 year survival group
CD4xCD8.div.M2xPD-L1	0.470010087
CD4xCD8xCD137.div.M2xPD-1xPD-L1	0.425886299
CD4xCD8xCD137.div.PD-1xPD-L1	0.440343866
CD8xCD137.div.M2xPD-1xPD-L1	0.282352941
CD4xCD8.div.M2xPD-1	0.342928251
CD4xCD8xCD137.div.M2xPD-L1	0.421753358
CD4xCD8.div.M2xPD-1xPD-L1	0.498806948
CD56xCD137.div.M2xPD-1xPD-L1	0.790011914
CD56xCD137.div.M2xPD-1	0.140092078
CD4xCD56xCD137.div.M2xPD-1	0.227847549
CD4xCD137.div.PD-1xPD-L1	0.787042501
CD8xCD137.div.PD-1xPD-L1	0.414070275
CD8xCD137.div.M2xPD-1	0.462472118
CD8xCD56xCD137.div.M2xPD-1xPD-L1	0.287119471
CD4xCD137.div.M2xPD-1	0.48745026
CD4xCD8xCD56xCD137.div.M2xPD-1xPD-L1	0.36834245
CD56xCD137.div.M2xPD-L1	0.203174603
CD8xCD137.div.M2xPD-L1	0.531735787
CD4xCD8xCD56.div.M2xPD-1xPD-L1	0.3569529
CD4xCD137.div.M2xPD-1xPD-L1	0.463647277
CD4xCD8xCD56xCD137.div.M2xPD-1	0.433277481
CD4xCD56xCD137.div.M2xPD-L1	0.284716901
CD8xCD56.div.M2xPD-1xPD-L1	0.312514378
CD4xCD8xCD56.div.M2xPD-1	0.504818536
CD8.div.M2	0.549449002
CD4xCD56xCD137.div.M2xPD-1xPD-L1	0.457439304
CD4xCD56.div.M2xPD-1	0.452161953
CD4xCD137.div.M2xPD-L1	0.499466318
CD4xCD8xCD137.div.M2xPD-1	0.46381868
CD4xCD8.div.PD-1xPD-L1	0.364596153
CD4xCD8xCD56xCD137.div.PD-1xPD-L1	0.480185393
CD56xCD137.div.PD-1xPD-L1	0.228571429
CD4xCD56xCD137.div.PD-1xPD-L1	0.281481481
CD8xCD56xCD137.div.M2xPD-1	0.372995705
CD4xCD8xCD56xCD137.div.M2xPD-L1	0.571771014
CD137.div.M2	0.532016232
CD4xCD8xCD56.div.PD-1xPD-L1	0.485353182
CD8xCD56.div.M2xPD-1	0.608808112
CD8xCD56.div.PD-1xPD-L1	0.25
CD8xCD56xCD137.div.M2xPD-L1	0.554564719

CD4.div.PD-L1	0.542940342
CD137.div.PD-1	0.38277512
CD8xCD56.div.M2xPD-L1	0.464747069
CD56.div.M2	0.478071922
M2	0.845148212
CD4xCD8xCD56.div.M2xPD-L1	0.626739475
CD4.div.M2	0.607867478
CD4xCD56.div.M2xPD-1xPD-L1	0.599432519
CD8xCD56xCD137.div.PD-1xPD-L1	0.63197277
CD137.div.PD-L1	0.468369632
CD4xCD56.div.M2xPD-L1	0.445824707
CD8.div.PD-L1	0.524692375
CD137	0.607421442
CD8.div.PD-1	0.626418704
CD4xCD56.div.PD-1xPD-L1	0.479679051
CD8	0.53047931
CD4	NA
CD56	NA
CD68	NA
CD163	NA
PD-1	NA
PD-L1	NA
CD4.div.PD-1	NA
CD56.div.PD-1	NA
CD56.div.PD-L1	NA

The values above correlate with the likelihood of survival of a patient with an immune score that, when compared with the cut-off value, stratifies them into a low immune score or low ratio group. For example, a patient that has a low immune score or low ratio when using the immune ratio CD4xCD8.div.M2xPD-L1, has a 47% likelihood of survival after 4 years.

5

Immune Ratio/Markers	Proportion of patients in high-ratio 4 year survival group
CD4xCD8.div.M2xPD-L1	0.921149903
CD4xCD8xCD137.div.M2xPD-1xPD-L1	0.893406931
CD4xCD8xCD137.div.PD-1xPD-L1	0.893584461
CD8xCD137.div.M2xPD-1xPD-L1	0.82909251
CD4xCD8.div.M2xPD-1	0.829645083
CD4xCD8xCD137.div.M2xPD-L1	0.887975918
CD4xCD8.div.M2xPD-1xPD-L1	0.889107535
CD56xCD137.div.M2xPD-1xPD-L1	0.790011914
CD56xCD137.div.M2xPD-1	0.819007127
CD4xCD56xCD137.div.M2xPD-1	0.823093622
CD4xCD137.div.PD-1xPD-L1	0.787042501
CD8xCD137.div.PD-1xPD-L1	0.881948619
CD8xCD137.div.M2xPD-1	0.89243648
CD8xCD56xCD137.div.M2xPD-1xPD-L1	0.835507876
CD4xCD137.div.M2xPD-1	0.878303186
CD4xCD8xCD56xCD137.div.M2xPD-1xPD-L1	0.850242507
CD56xCD137.div.M2xPD-L1	0.819778292
CD8xCD137.div.M2xPD-L1	0.913374899
CD4xCD8xCD56.div.M2xPD-1xPD-L1	0.829697149
CD4xCD137.div.M2xPD-1xPD-L1	0.850514933
CD4xCD8xCD56xCD137.div.M2xPD-1	0.862547186
CD4xCD56xCD137.div.M2xPD-L1	0.827155572
CD8xCD56.div.M2xPD-1xPD-L1	0.812512183
CD4xCD8xCD56.div.M2xPD-1	0.84476117
CD8.div.M2	0.896990297
CD4xCD56xCD137.div.M2xPD-1xPD-L1	0.849486372
CD4xCD56.div.M2xPD-1	0.829685193
CD4xCD137.div.M2xPD-L1	0.852081381
CD4xCD8xCD137.div.M2xPD-1	0.867348682
CD4xCD8.div.PD-1xPD-L1	0.824154572
CD4xCD8xCD56xCD137.div.PD-1xPD-L1	0.859118737
CD56xCD137.div.PD-1xPD-L1	0.801636041
CD4xCD56xCD137.div.PD-1xPD-L1	0.80209873
CD8xCD56xCD137.div.M2xPD-1	0.828395525
CD4xCD8xCD56xCD137.div.M2xPD-L1	0.886890949
CD137.div.M2	0.856581684
CD4xCD8xCD56.div.PD-1xPD-L1	0.8350305
CD8xCD56.div.M2xPD-1	0.927915915
CD8xCD56.div.PD-1xPD-L1	0.786090167
CD8xCD56xCD137.div.M2xPD-L1	0.882030345
CD4.div.PD-L1	0.826314567

CD137.div.PD-1	0.806790601
CD8xCD56.div.M2xPD-L1	0.820930715
CD56.div.M2	0.802915713
M2	0.556598881
CD4xCD8xCD56.div.M2xPD-L1	0.877081306
CD4.div.M2	0.846266485
CD4xCD56.div.M2xPD-1xPD-L1	0.834904752
CD8xCD56xCD137.div.PD-1xPD-L1	0.923601752
CD137.div.PD-L1	0.831824926
CD4xCD56.div.M2xPD-L1	0.799138066
CD8.div.PD-L1	0.82356553
CD137	0.856555673
CD8.div.PD-1	0.899783468
CD4xCD56.div.PD-1xPD-L1	0.798267522
CD8	0.793334218
CD4	NA
CD56	NA
CD68	NA
CD163	NA
PD-1	NA
PD-L1	NA
CD4.div.PD-1	NA
CD56.div.PD-1	NA
CD56.div.PD-L1	NA

The values above correlate with the likelihood of survival of a patient with an immune score that, when compared with the cut-off value, stratifies them into a high immune score or high ratio group. For example, a patient that has a high immune score or high ratio when using the immune ratio CD4xCD8.div.M2xPD-L1, has a 92% likelihood of survival after 4 years.

Example 4 - Immune-effector:checkpoint ratios adds to conventional prognosticators.

Both R-IPI and COO separated patients into prognostic groupings ($P=0.002$ and $P=0.018$ and 4-year survivals of 83% versus 61%, and 82% versus 60% respectively).

- 5 Application of the immune-ratio $CD4^+CD8:M2^+PD-L1$ into low and high-ratio groupings, sub-stratified R-IPI and COO (Table 4 and Figure 1B-E). For R-IPI, 37% of patients were in the best survival groupings (VG/G R-IPI and high-ratio immune score), with ~20% in each of the other categories (Table 4). Similarly for COO, for GCB and high-immune score there were 43% of patients and ~20% in each of the other
- 10 categories. Combining the immune-ratio with R-IPI and COO created two clearly separate groupings (Figure 1F): Poor R-IPI-low-ratio and/or non-GCB-low-ratio (4-year OS=40%, termed poor-risk), versus all other groupings (4-year OS=89%, good-risk). Thirty percent of patients had a poor-risk combined R-IPI-COO-immune-score. Importantly, in Cox Regression multivariate analysis the significance remained, with the
- 15 immune-ratio independent of COO and R-IPI ($P<0.0001$, with COO $P=0.048$ and R-IPI $P=0.014$).

Table 4. 4-year survival stratified by high vs low immune-ratios in the Australian-tissue cohort.

- *There were 152 patients with R-IPI data. R-IPI with the immune-ratio gave the
- 20 following proportions: VG/G-high 37%; VG/G-low 21%; poor-high 22%; poor-low 21%. For COO, proportions were GCB-high 43%; GCB-low 23%; non-GCB-high 16%; non-GCB-low 18%.

Immune Ratio $CD4^+CD8/M2^+PDL1$	4-year survival stratified by high vs low immune ratios (n=158)*	P Value
All Patients	92% (n=93) vs 47% (n=65)	<0.0001
VG/G R-IPI	98% (n=56) vs 52% (n=32)	<0.0001
Poor R-IPI	80% (n=33) vs 44% (n=31)	0.008

Immune Ratio CD4*CD8/M2*PDL1	4-year survival stratified by high vs low immune ratios (n=158)*	P Value
GCB	97% (n=68) vs 54% (n=36)	<0.0001
Non-GCB	81% (n=25) vs 38% (n=29)	0.0016

Example 5 - External validation of immune-ratios using the Affymetrix platform on frozen tissue.

The immune-ratio CD4*CD8:M2*PD-L1 was then applied to the independent cohort of R-CHOP-like treated patients in which gene expression was quantified on fresh-frozen samples using an Affymetrix platform.⁷ The immune-ratio segregated patients into groupings with significantly different survivals (Figure 3). 60% of patients had a high-ratio, almost identical to that seen for the Australian-tissue cohort. By multivariate analysis the ratio was independent of COO and R-IPI (immune-ratio $P < 0.017$, COO $P = 0.011$ and R-IPI $P < 0.0001$ respectively).

In DLBCL, the relationship between immune-effectors and checkpoints is not well-described. The data shown herein demonstrates in tissue that immune-effectors correlate with checkpoints, in keeping with a dynamic (not constitutive) immune response in which checkpoints are activated in response to host anti-lymphoma immunity. This implies that the products of immune-effector molecules in a ratio with the products of immune-checkpoints will reflect the balance of net anti-tumoural immunity and might have prognostic value.

The immune-ratio (CD4*CD8:[CD163.div.CD68]*PD-L1) was identified for further evaluation. Notably, the denominator itself also included a ratio (CD163.div.CD68). The explanation is that TAMs are either 'M1' (CD68^{hi}CD163^{lo}) or 'M2' (CD68^{hi}CD163^{hi}), but only M2 TAMs that are tumour promoting. In keeping with this, the CD163.div.CD68 ratio (as a measure of M2-TAMs) was more informative than either marker alone. The CD4*CD8:M2*PD-L1 immune-ratio high and low survival curves were distinct, and the ratio was independent of COO and R-IPI. Findings were validated in the 'Lenz' R-CHOP-like cohort using an alternative platform on frozen tissues (Lenz G, et al. *N Engl J Med* 2008; **359**(22): 2313-23). Here the separation between high and low survival

curves remained distinct but was less pronounced, perhaps reflecting the heterogeneity of the 'R-CHOP-like' treatment regimens, compared to uniform treatment with R-CHOP in the Australian-tissue cohort.

For practical application, a new prognosticator must segregate patients into
5 distinct categories containing a high proportion of patients, and enhance the discriminatory ability of R-IPI and COO. R-IPI is influenced by patient fitness, age and tumour burden, whereas COO reflects B-cell differentiation. The combination of net anti-tumoural immunity using the immune-ratio with either COO or R-IPI better distinguished patients than R-IPI or COO alone. For example the application of immune-ratio to R-IPI
10 segregated patients into groupings with four distinct, well-spaced 4-year survival outcomes of 98%, 80%, 52% and 44%. Strikingly, 37% of patients were in the 98% survival grouping, with ~20% in each of the other categories. Similar results were seen for COO combined with the immune-ratio.

Immune-effector:checkpoint ratios were prognostic, demonstrating the
15 robustness of this simple approach to capture the relative balance of immune-effectors to checkpoints within the TME as a measure of net anti-tumoural immunity. Based on its superior discrimination of survival curves, the CD4*CD8:M2*PD-L1 ratio was evaluated as a prognosticator.

The data described herein emphasizes the utility of capturing net anti-tumoural
20 immunity by a simple ratio. The findings should be seen within the context that immune-based strategies are gaining ground in DLBCL (Elstrom et al. *Hematol Oncol Clin North Am* 2008; **22**(5): 1037-49, x-xi). Blockade of the PD-1/PD-L1-axis is promising (Armand P et al. *J Clin Oncol* 2013; **31**(33): 4199-206), and strategies that target CD163 may be similarly beneficial (Graversen JH, et al. *Mol Ther* 2012; **20**(8): 1550-8). In solid-organ
25 tumours, therapeutic PD-1 blockade is more effective in cancers enriched with immune-effectors that are negatively regulated by PD-1/PD-L1-axis mediated inhibition (Tumeh PC et al. *Nature* 2014; **515**(7528): 568-71). In recent clinical trials of solid-organ and haematological cancers, it appears that levels of PD-1 on tumour-infiltrating lymphocytes is less predictive of response to PD-1 blockade than PD-L1 expression
30 (Ansell SM et al. *N Engl J Med* 2015; **372**(4): 311-9; Taube JM et al. *Clin Cancer Res* 2014; **20**(19): 5064-74). Immune-ratios measured by digital hybridization technologies

applicable to FFPE, may permit rational selection of patients in whom checkpoint-blockade should be tested.

Example 6 – development of ratios with additional markers

The following are the 2 aims of the experiments exemplifying the invention
5 described in this example:

1. To produce an extended immune score, again based on ratios of immune effectors (numerators) and immune checkpoints (denominators) present within the tumour microenvironment, that has additional prognostic ability to the current markers within the score (current markers immune effectors CD4, CD8, CD56, CD137; immune
10 checkpoints PD-1, PD-L1, CD163 and CD68) in DLBCL. CD163 and CD68 were combined (CD163 div by CD68) as 'M2'. These additional markers were:

immune effectors: TNF.alpha

immune checkpoints: PD-L2, LAG3, TIM3

2. To combine immune effectors and immune checkpoints with 'good outcome'
15 (numerators) and 'poor outcome' (denominators) markers of DLBCL. These were:

'good outcome': LM02

'poor outcome' SCYA3.CCL3.

This would result in a combined 'immuno-biological score'.

Methods: Gene expression of markers in 157 de novo DLBCL biopsies treated
20 with R-CHOP were tested by nanoString. A tree-based survival analysis was run. 13 markers (using 'M2') were tested.

Results: Based on p values of the survival tree after Bonferroni multiple testing correction, 410 combinations were declared as significant, involving all 13 markers (Table 5). Of these, the most significant using the p value of the survival curve of was
25 CD4xCD8xCD137.TNFR9xTNF.Alpha x LMO2.div.M2xSCYA3xCCL3xPD.1xPD.L1, where the p value of the survival curve was 9.96E-12. Here the proportion of the 4-year survival patient identified in the high risk subgroup was 0.255, and the proportion in the low risk was 0.90 (a patient with a high immune score is identified as a low risk (i.e. long

survival time)). The values in the far right column in Table 5 correlate with the likelihood of survival of a patient with an immune score that, when compared with the cut-off value, stratifies them into a high immune score or high ratio group. For example, a patient that has a high immune score or high ratio when using the immune ratio

5 CD4xCD8xCD137.TNFR9xTNF.Alpha x LMO2.div.M2xSCYA3..CCL3xPD.1xPD.L1, has a 90% likelihood of survival after 4 years. Whereas a patient with an immune score that, when compared with the cut-off value, stratifies them into a low immune score or low ratio group (column with heading 'Proportion of patients in low-ratio 4 year survival group') when using the immune ratio

10 CD4xCD8xCD137.TNFR9xTNF.Alpha x LMO2.div.M2xSCYA3..CCL3xPD.1xPD.L1, has a 25.5% likelihood of survival after 4 years.

Interpretation: The immune score could be extended to include combinations that include the additional markers TNF α (alpha) (numerator) and PD-L2, LAG3, TIM3 (denominators), and this combined with the biological markers LM02 (numerator) and

15 SCYA3.CCL3 (denominator), as an 'immuno-biological score' (any reference herein to immune score may be a reference to immuno-biological score when any aspect of the invention includes one or more of TNF α , PD-L2, LAG3, TIM3, LM02 or SCYA3.CCL3).

Table 5 (below): Results of tree-structured survival analysis described in Example 6. The cutoff for each single marker or ratio was determined by the tree-structured survival model to divide the patients into two subgroups. Adjusted P-values

20 (Bonferroni multiple correction procedure) of the survival curve difference test was used to assess the prognostic value of each marker/ratio together with the 4-year survival proportion in each survival group. Other statistical models can be used to calculate the cutoff, for example the median value. The results are shown to 9 decimal places

25 however the cutoff values still apply when only 2 decimal places are used.

Immune Ratios	PvalSurvTree	Tree survival cutoff	Difference between survival curves (p-value)	Proportion of patients in low-ratio 4 year survival group	Proportion of patients in high-ratio 4 year survival group
CD4xCD8xCD137.TNFR9xTNF.AlphaXLMO2.div.M2xSCYA3..CCL3xPD.1xPD.L1	0.022477529	-0.731791973	9.96E-12	0.255237486	0.900487654
CD4xCD8xTNF.AlphaXLMO2.div.M2xSCYA3..CCL3xPD.L2	0.008910849	-0.669357264	3.70E-11	0.28069893	0.909245166
CD4xCD8xCD137.TNFR9xTNF.AlphaXLMO2.div.M2xSCYA3..CCL3xPD.1xPD.L2	0.02187084	-0.797454587	3.86E-10	0.212183812	0.872351065
CD4xCD8xLMO2.div.M2xSCYA3..CCL3xPD.L2	0.003825989	-0.752989112	6.67E-10	0.291783088	0.893200828
CD4xCD8xCD137.TNFR9xLMO2.div.M2xSCYA3..CCL3xPD.L2xTI M3	0.018367985	-0.950200562	1.34E-09	0.158201058	0.848779555
CD8xCD137.TNFR9xTNF.AlphaXLMO2.div.M2xPD.1xPD.L1	0.04100532	-0.439214202	1.71E-09	0.399075917	0.949787521
CD4xCD8xCD137.TNFR9xTNF.AlphaXLMO2.div.SCYA3..CCL3xPD.L1xPD.L2	0.035667528	-0.767539975	1.91E-09	0.248035915	0.877718747
CD4xCD8xTNF.AlphaXLMO2.div.M2xSCYA3..CCL3xLAG3	0.037984713	-0.732935094	3.22E-09	0.299405535	0.882565604
CD4xCD8xTNF.AlphaXLMO2.div.M2xSCYA3..CCL3xPD.1xPD.L1	0.039655795	-0.711282968	3.30E-09	0.281877149	0.883469555
CD4xCD8xCD137.TNFR9xTNF.AlphaXLMO2.div.M2xSCYA3..CCL3xPD.L1	0.03439013	-0.68660595	3.42E-09	0.360696652	0.899764513
CD8xLMO2.div.M2xSCYA3..CCL3xPD.L2	0.034161621	-0.751780699	3.45E-09	0.246641249	0.874340619
CD4xCD8xTNF.AlphaXLMO2.div.M2xSCYA3..CCL3xPD.L1	0.016545032	-0.960403999	4.07E-09	0.198267451	0.873507804
CD4xCD8xCD137.TNFR9xLMO2.div.M2xSCYA3..CCL3xPD.1xPD.L2	0.016433416	-0.6919107	4.16E-09	0.312948565	0.890304102
CD4xCD8xTNF.AlphaXLMO2.div.M2xSCYA3..CCL3xPD.1	0.013439072	-0.735176492	7.62E-09	0.272439182	0.899625383
CD4xCD8xTNF.AlphaXLMO2.div.M2xPD.1xPD.L2	0.025303574	-0.840876997	7.68E-09	0.270531401	0.852528535
CD4xCD8xCD137.TNFR9xLMO2.div.M2xSCYA3..CCL3xPD.L2	0.014348929	-0.468494152	1.15E-08	0.375582358	0.931409194
CD4xCD8xCD137.TNFR9xLMO2.div.M2xPD.1xPD.L2	0.013021291	-0.89769545	1.24E-08	0.195628547	0.851568456
CD4xCD8xCD137.TNFR9xLMO2.div.M2xSCYA3..CCL3xPD.1xPD.L1	0.01078732	-0.555113277	1.37E-08	0.356920919	0.913668228
CD4xCD8xCD137.TNFR9xLMO2.div.M2xSCYA3..CCL3xPD.L1	0.017984354	-0.764081118	1.42E-08	0.28048303	0.882800901
CD8xTNF.AlphaXLMO2.div.M2xSCYA3..CCL3xPD.L2	0.018562806	-0.831397353	1.53E-08	0.268378353	0.855346513
CD4xCD8xTNF.AlphaXLMO2xCD56.div.M2xSCYA3..CCL3xPD.1xPD.L1	0.020601641	-0.792631272	1.59E-08	0.26576376	0.877150392
CD8xLMO2.div.M2xPD.L2	0.006980157	-0.753279123	1.80E-08	0.312951281	0.886426254
CD4xCD8xCD137.TNFR9xTNF.AlphaXLMO2xCD56.div.M2xSCYA	0.01708162	-0.813227511	2.02E-08	0.235442598	0.873686989
	0.012998766	-0.770267023	2.20E-08	0.236108108	0.875691049

Immune Ratios	PvalSurvTree	Tree survival cutoff	Difference between survival curves (p-value)	Proportion of patients in low-ratio 4 year survival group	Proportion of patients in high-ratio 4 year survival group
3..CCL3xPD.1xPD.L2					
CD4xCD8xCD137.TNFR9xLMO2.div.SCYA3..CCL3xPD.L2xLAG3	0.014037597	-0.858287852	2.54E-08	0.200223214	0.882274304
CD4xCD8xTNF.AlphaxLMO2.div.M2xSCYA3..CCL3xPD.L2xTIM3	0.025276115	-0.87188792	2.83E-08	0.257871663	0.864100511
CD4xCD8xTNF.AlphaxLMO2.div.M2xPD.1xPD.L1	0.01428728	-0.457029457	2.90E-08	0.400840023	0.927030217
CD4xCD8xCD137.TNFR9xLMO2.div.M2xPD.1xPD.L1	0.010264053	-0.598859776	3.21E-08	0.354034034	0.906025941
CD8xCD137.TNFR9xLMO2.div.M2xPD.1xPD.L1	0.048866543	-0.43242632	3.67E-08	0.398962576	0.929771852
CD4xCD8xCD137.TNFR9xTNF.AlphaxLMO2.div.SCYA3..CCL3xPD.1xPD.L2	0.004080109	-0.828089476	4.55E-08	0.242366522	0.875053026
CD4xCD8xCD137.TNFR9xLMO2.div.SCYA3..CCL3xPD.1xPD.L2	0.001370373	-0.849929059	5.56E-08	0.245178704	0.870824637
CD8xCD137.TNFR9xTNF.AlphaxLMO2.div.SCYA3..CCL3xPD.1xPD.L2	0.016548562	-0.462319847	5.57E-08	0.404857744	0.929237787
CD8xCD137.TNFR9xLMO2.div.M2xPD.1xPD.L2	0.0466851	-0.593679121	5.65E-08	0.340945584	0.915210447
CD8xCD137.TNFR9xLMO2.div.M2xSCYA3..CCL3xPD.L2	0.014998167	-1.022751194	6.13E-08	0.221484375	0.830935521
CD8xCD137.TNFR9xLMO2.div.SCYA3..CCL3xLAG3	0.01488599	-1.17953812	6.16E-08	0.126488095	0.821194198
CD4xCD8xCD137.TNFR9xTNF.AlphaxLMO2.div.M2xSCYA3..CCL3xPD.1	0.03009975	-0.814947086	6.18E-08	0.297263451	0.867538518
CD8xCD137.TNFR9xTNF.AlphaxLMO2.div.PD.1xPD.L2	0.013902232	-0.658671348	6.27E-08	0.343735055	0.888434927
CD4xCD8xTNF.AlphaxLMO2.div.M2xSCYA3..CCL3xPD.L1xTIM3	0.047805175	-0.832915545	6.31E-08	0.288459882	0.860732555
CD4xCD8xLMO2.div.M2xSCYA3..CCL3xPD.L2xTIM3	0.024292329	-0.916819084	6.93E-08	0.245978014	0.867435146
CD8xTNF.AlphaxLMO2xCD56.div.M2xSCYA3..CCL3xPD.1	0.01352991	-0.562964197	6.98E-08	0.36441351	0.913114917
CD4xCD8xTNF.AlphaxLMO2.div.M2xSCYA3..CCL3xPD.1xPD.L2	0.027243923	-0.513205615	7.46E-08	0.409615166	0.928866155
CD8xLMO2.div.M2xSCYA3..CCL3xPD.L1	0.039106277	-0.799138968	7.56E-08	0.310615406	0.8822284
CD4xCD8xLMO2.div.M2xSCYA3..CCL3xLAG3	0.03919348	-0.871710016	8.10E-08	0.309065934	0.83899997
CD4xCD8xLMO2.div.M2xSCYA3..CCL3xPD.1xPD.L2	0.03216277	-0.660127902	8.39E-08	0.342126428	0.901117276
CD4xCD8xCD137.TNFR9xLMO2xCD56.div.M2xSCYA3..CCL3xPD.1					
CD8xLMO2.div.M2xSCYA3..CCL3	0.035318891	-0.674264611	9.99E-08	0.332534562	0.875519436
CD4xCD8xLMO2.div.M2xPD.L2xLAG3	0.006815901	-0.794306414	1.00E-07	0.297017797	0.875930615
CD4xCD8xCD137.TNFR9xTNF.AlphaxLMO2.div.PD.1xPD.L1xPD.L2	0.039888422	-0.965910009	1.04E-07	0.186098787	0.844013833
CD4xCD8xLMO2xCD56.div.M2xSCYA3..CCL3xPD.1xPD.L1	0.010474949	-0.681112466	1.06E-07	0.329655997	0.891524743
CD8xCD137.TNFR9xLMO2xCD56.div.M2xSCYA3..CCL3xPD.1xPD.1xPD	0.008401068	-0.763661267	1.22E-07	0.352738631	0.872328247
	0.004566203	-0.506981251	1.37E-07	0.386434637	0.921200669

Immune Ratios	PvalSurvTree	Tree survival cutoff	Difference between survival curves (p-value)	Proportion of patients in low-ratio 4 year survival group	Proportion of patients in high-ratio 4 year survival group
D.L2					
CD4xCD8xLMO2.div.M2xSCYA3..CCL3xPD.L1	0.00981332	-0.700773461	1.39E-07	0.363168506	0.897417337
CD4xCD8xTNF.Alpha.xLMO2xCD56.div.M2xSCYA3..CCL3xPD.1xPD.L2	0.004652261	-0.731101932	1.42E-07	0.346002474	0.885711441
CD4xCD8xTNF.Alpha.xLMO2xCD56.div.M2xSCYA3..CCL3xPD.1	0.023814286	-0.655860013	1.48E-07	0.341990728	0.884283307
CD4xCD8xTNF.Alpha.xLMO2.div.SCYA3..CCL3xPD.1xPD.L2	0.008883263	-0.60677883	1.51E-07	0.399334778	0.898433665
CD8xCD137.TNFR9xTNF.Alpha.xLMO2xCD56.div.M2xSCYA3..CCL3xPD.1xPD.L1	0.009715806	-0.569045227	1.57E-07	0.370221193	0.912637746
CD4xCD8xCD137.TNFR9xCD56.div.M2xSCYA3..CCL3xPD.1xPD.L2	0.032943591	-0.961448377	1.68E-07	0.219623132	0.845510904
CD4xCD8xTNF.Alpha.xLMO2.div.SCYA3..CCL3xLAG3	0.017571927	-0.731509802	1.79E-07	0.330255702	0.863750364
CD8xCD137.TNFR9xLMO2xCD56.div.M2xSCYA3..CCL3xPD.1xPD.L1	0.007208412	-0.596213141	1.93E-07	0.375992556	0.913758797
CD4xCD8xLMO2.div.M2xPD.L1	0.011525253	-0.628653594	2.76E-07	0.383420536	0.905033996
CD8xCD137.TNFR9xLMO2.div.M2xSCYA3..CCL3xPD.L1	0.024362908	-0.672835108	2.84E-07	0.3331173	0.888375966
CD4xCD8xCD137.TNFR9xLMO2xCD56.div.M2xPD.1xPD.L2xTIM3	0.014701677	-0.764259665	2.84E-07	0.301711368	0.881862408
CD4xCD8xCD137.TNFR9xTNF.Alpha.xLMO2.div.SCYA3..CCL3xPD.L1xLAG3	0.031323673	-0.863759052	2.86E-07	0.29399682	0.85314911
CD4xCD8xCD137.TNFR9xLMO2xCD56.div.M2xPD.1xPD.L2	0.017432274	-0.582567925	2.90E-07	0.320650949	0.896174749
CD4xCD8xCD137.TNFR9xLMO2xCD56.div.M2xSCYA3..CCL3xPD.1xPD.L1xTIM3	0.015709732	-0.602409316	2.92E-07	0.379576653	0.912993869
CD4xCD8xTNF.Alpha.xLMO2.div.M2xPD.L2	0.020339613	-0.926229361	3.06E-07	0.276087446	0.843558097
CD4xCD8xTNF.Alpha.div.M2xSCYA3..CCL3xPD.1	0.043406598	-0.636984536	3.10E-07	0.410852656	0.867286436
CD4xCD8xCD137.TNFR9xdiv.SCYA3..CCL3xPD.1xPD.L1	0.005221525	-0.916671494	3.38E-07	0.269418321	0.852401707
CD4xCD8xCD137.TNFR9xTNF.Alpha.xLMO2.div.M2xSCYA3..CCL3xPD.1xTIM3	0.042821135	-0.83200977	3.57E-07	0.235594621	0.84694181
CD8xCD137.TNFR9xTNF.Alpha.xLMO2.div.M2xSCYA3..CCL3xPD.L2	0.029893211	-0.737693683	3.59E-07	0.304354242	0.874956753
CD4xCD8xTNF.Alpha.xLMO2xCD56.div.M2xSCYA3..CCL3xPD.1xTIM3	0.022853257	-0.454482749	3.75E-07	0.381826382	0.911641018
CD4xCD8xCD137.TNFR9xTNF.Alpha.div.M2xSCYA3..CCL3xPD.1xPD.L1	0.033592781	-0.64864529	4.01E-07	0.380760761	0.855630816
CD4xCD8xCD137.TNFR9xLMO2.div.PD.1xPD.L1xPD.L2	0.016442945	-0.710090549	4.07E-07	0.304744323	0.894989624

Immune Ratios	PvalSurvTree	Tree survival cutoff	Difference between survival curves (p-value)	Proportion of patients in low-ratio 4 year survival group	Proportion of patients in high-ratio 4 year survival group
CD4xCD8xLMO2xCD56.div.M2xSCYA3..CCL3xPD.L1	0.018184495	-0.675029249	4.12E-07	0.360205509	0.864330409
CD4xCD8xCD137.TNFR9xTNF.AlphaXLMO2.div.SCYA3..CCL3xPD.L1xPD.L1	0.004050546	-0.60181693	4.12E-07	0.368654899	0.893307076
CD4xCD8xCD137.TNFR9xTNF.AlphaXLMO2.div.PD.1xPD.L1xTIM3	0.010191523	-0.699311415	4.67E-07	0.363046846	0.875754022
CD4xCD8xLMO2.div.SCYA3..CCL3xPD.L1	0.016684079	-0.576684442	4.74E-07	0.420099649	0.897222812
CD4xCD8xLMO2.div.SCYA3..CCL3xPD.L1	0.011214272	-0.697489567	4.82E-07	0.321250624	0.890371329
CD4xCD8xCD137.TNFR9xTNF.AlphaXLMO2xCD56.div.SCYA3..CCL3xPD.L1xPD.L2	0.02976472	-0.656498624	4.92E-07	0.350617895	0.879212673
CD4xCD8xCD137.TNFR9xLMO2.div.SCYA3..CCL3xPD.L1xLAG3	0.031024283	-0.712167219	5.01E-07	0.301889464	0.891997451
CD4xCD8xLMO2.div.M2xPD.1xPD.L2	0.015721394	-0.403986581	5.01E-07	0.435346226	0.937991186
CD4xCD8xLMO2.div.SCYA3..CCL3xPD.L2	0.007845719	-0.501258932	5.28E-07	0.433894201	0.92653196
CD4xCD8xCD137.TNFR9xTNF.AlphaXLMO2.div.SCYA3..CCL3xLAG3xTIM3	0.040852679	-1.070430132	5.39E-07	0.119124973	0.830271206
CD4xCD8xTNF.AlphaXLMO2xCD56.div.M2xSCYA3..CCL3xPD.L1xPD.L2	0.012653997	-0.447873253	5.53E-07	0.446932634	0.92238506
CD4xCD8xCD137.TNFR9xTNF.AlphaXLMO2.div.SCYA3..CCL3xPD.L2xLAG3	0.020009795	-0.734938314	5.54E-07	0.342907235	0.889957608
CD4xCD8xCD137.TNFR9xLMO2.div.M2xPD.1xPD.L1xPD.L2	0.043752321	-0.459595393	5.58E-07	0.413529739	0.919269366
CD4xCD8xLMO2xCD56.div.M2xSCYA3..CCL3xPD.1xPD.L2	0.00377717	-0.759301352	5.86E-07	0.323180651	0.877470783
CD4xCD8xCD137.TNFR9xTNF.AlphaXLMO2.div.M2xSCYA3..CCL3xPD.L2	0.027929285	-0.899175639	6.18E-07	0.290480401	0.841082565
CD8xTNF.AlphaXLMO2xCD56.div.M2xSCYA3..CCL3xPD.1xPD.L1	0.008728319	-0.486690809	6.34E-07	0.43519253	0.926502048
CD8xCD137.TNFR9xTNF.AlphaXLMO2xCD56.div.M2xSCYA3..CCL3xPD.1xPD.L1xPD.L2	0.023257972	-0.486004798	6.38E-07	0.426537257	0.9168425
CD8xCD137.TNFR9xLMO2xCD56.div.M2xSCYA3..CCL3xPD.1xTIM3	0.027970422	-0.560029075	6.43E-07	0.358522461	0.904634536
CD4xCD8xLMO2.div.M2xSCYA3..CCL3	0.011348317	-0.758349977	6.44E-07	0.367394176	0.865910136
CD4xCD8xLMO2.div.M2xPD.1xPD.L1	0.021637796	-0.3646541	6.59E-07	0.446138664	0.935309853
CD8xCD137.TNFR9xLMO2xCD56.div.M2xPD.1xPD.L2	0.007637429	-0.699962811	6.68E-07	0.283285289	0.891186497
CD8xCD137.TNFR9xTNF.AlphaXLMO2.div.SCYA3..CCL3xPD.L1	0.0153194	-0.681823688	6.96E-07	0.357455378	0.88057482
CD4xCD8xCD137.TNFR9xLMO2.div.M2xSCYA3..CCL3xPD.L1xPD.L2	0.036788898	-0.631695768	7.13E-07	0.386538447	0.90574808

Immune Ratios	PvalSurvTree	Tree survival cutoff	Difference between survival curves (p-value)	Proportion of patients in low-ratio 4 year survival group	Proportion of patients in high-ratio 4 year survival group
CD8xCD137.TNFR9xTNF.AlphaxLMO2.div.PD.1xPD.L1	0.008321047	-0.564332913	7.22E-07	0.402948901	0.891862098
CD4xCD8xCD137.TNFR9xLMO2.div.M2xSCYA3..CCL3xPD.1	0.017559915	-0.675131362	7.27E-07	0.314248598	0.880428725
CD4xCD8xCD137.TNFR9xLMO2xCD56.div.M2xSCYA3..CCL3xPD.1xPD.L1	0.007970583	-0.512368652	7.50E-07	0.403452186	0.920118983
CD4xCD8xCD137.TNFR9xLMO2.div.PD.1xPD.L2	0.003233294	-0.736217621	7.71E-07	0.330234036	0.888639278
CD4xCD8xLMO2.div.M2xPD.L2xTIM3	0.014664104	-0.764893873	7.76E-07	0.334480215	0.87463944
CD4xCD8xCD137.TNFR9xTNF.AlphaxLMO2.div.M2xPD.1xPD.L1	0.013730627	-0.567705281	7.79E-07	0.367497471	0.894560049
CD4xCD8xCD137.TNFR9.div.SCYA3..CCL3xPD.1xPD.L2	0.00501247	-0.272356123	8.46E-07	0.432623773	0.933680728
CD4xCD8xLMO2.div.M2xPD.L1xTIM3	0.022183686	-0.545695454	8.90E-07	0.404312608	0.910568255
CD4xCD8xCD137.TNFR9xLMO2.div.SCYA3..CCL3xPD.L1	0.015835421	-0.759392858	8.93E-07	0.337306003	0.875690302
CD4xCD8xCD137.TNFR9xTNF.AlphaxCD56.div.M2xSCYA3..CCL3xPD.1xPD.L2	0.015311791	-1.005961336	8.96E-07	0.206708075	0.8385673
CD4xCD8xCD137.TNFR9xTNF.AlphaxLMO2.div.PD.1xPD.L1	0.004940845	-0.518782229	9.11E-07	0.390558694	0.900687789
CD4xCD8xTNF.AlphaxLMO2.div.SCYA3..CCL3xPD.L1	0.017014626	-0.629528069	9.40E-07	0.403503573	0.882433153
CD4xCD8xTNF.AlphaxLMO2.div.SCYA3..CCL3xPD.L2xTIM3	0.023338363	-0.859670504	9.46E-07	0.304732053	0.861146448
CD4xCD8xCD137.TNFR9xTNF.AlphaxLMO2.div.M2xSCYA3..CCL3xPD.L1xPD.L2	0.04473885	-0.571735052	9.71E-07	0.399168123	0.912404644
CD4xCD8xTNF.AlphaxLMO2.div.SCYA3..CCL3xPD.1xPD.L1	0.012912038	-0.582315663	9.87E-07	0.412507565	0.909257561
CD4xCD8xTNF.AlphaxLMO2xCD56.div.M2xPD.1xPD.L2	0.008912657	-0.905608532	1.01E-06	0.276510387	0.858274129
CD4xCD8xTNF.AlphaxLMO2.div.PD.1xPD.L2	0.009475404	-0.479636154	1.01E-06	0.410321949	0.889015446
CD8xTNF.AlphaxLMO2.div.M2xSCYA3..CCL3	0.01979638	-0.684211399	1.04E-06	0.334239565	0.873588359
CD8xCD137.TNFR9xLMO2.div.PD.1xPD.L2	0.011465312	-0.54138315	1.04E-06	0.369857636	0.91408571
CD8xCD137.TNFR9xLMO2.div.M2xSCYA3..CCL3xPD.1	0.038412628	-0.55581996	1.04E-06	0.385966629	0.912812168
CD4xCD8xLMO2xCD56.div.M2xSCYA3..CCL3xPD.1xTIM3	0.028609194	-0.683385256	1.10E-06	0.358950151	0.883907223
CD4xCD8xTNF.AlphaxLMO2.div.SCYA3..CCL3xPD.L2	0.012513174	-0.587217076	1.11E-06	0.374416826	0.904848899
CD4xCD8xTNF.AlphaxLMO2.div.M2xPD.L1	0.021270551	-0.609226975	1.22E-06	0.386135862	0.892962471
CD4xCD8xCD137.TNFR9xTNF.AlphaxLMO2.div.M2xPD.1xPD.L2	0.017659705	-0.743985595	1.22E-06	0.349191167	0.880475842
CD8xLMO2.div.M2xPD.L1	0.023812602	-0.478304585	1.22E-06	0.444716933	0.925949249
CD8xTNF.AlphaxLMO2.div.M2xPD.L2	0.026461933	-0.684514394	1.28E-06	0.335271076	0.882474173
CD4xCD8xCD137.TNFR9xTNF.AlphaxLMO2.div.SCYA3..CCL3xPD.D.1xTIM3	0.019130209	-0.805380181	1.29E-06	0.324470961	0.864961082
CD4xCD8xCD137.TNFR9xLMO2xCD56.div.M2xSCYA3..CCL3xPD	0.011815061	-0.514084624	1.30E-06	0.384011761	0.910826077

Immune Ratios	PvalSurvTree	Tree survival cutoff	Difference between survival curves (p-value)	Proportion of patients in low-ratio 4 year survival group	Proportion of patients in high-ratio 4 year survival group
1xPD.L2xTIM3					
CD8xCD137.TNFR9xTNF.AlphaxLMO2xCD56.div.M2xSCYA3..CC	0.030966705	-0.470164637	1.33E-06	0.420286846	0.918098712
L3xPD.L1xTIM3	0.016566159	-0.674529713	1.38E-06	0.381342561	0.876151744
CD8xCD137.TNFR9xTNF.AlphaxLMO2.div.SCYA3..CCL3xPD.1	0.035358057	-0.768206219	1.42E-06	0.334561052	0.855660816
CD4xCD8xTNF.AlphaxLMO2.div.M2xPD.1					
CD4xCD8xCD137.TNFR9xCD56.div.M2xSCYA3..CCL3xPD.1xPD.L1	0.037822419	-0.550857466	1.46E-06	0.374370296	0.870339893
CD4xCD8xCD137.TNFR9xLMO2.div.M2xSCYA3..CCL3xPD.L1xTIM3	0.029736107	-0.851859759	1.49E-06	0.293933852	0.848556603
CD4xCD8xCD137.TNFR9xTNF.AlphaxLMO2xCD56.div.M2xPD.1xPD.L2	0.032433503	-0.848936308	1.51E-06	0.237203972	0.86153585
CD4xCD8xCD137.TNFR9xLMO2xCD56.div.M2xSCYA3..CCL3xPD.1xPD.L2	0.005867139	-0.413098376	1.59E-06	0.429012532	0.927762644
CD4xCD8xCD137.TNFR9xTNF.AlphaxLMO2xCD56.div.M2xPD.1xPD.L1xPD.L2	0.01516855	-0.432710963	1.66E-06	0.427707741	0.928038784
CD8xCD137.TNFR9xTNF.AlphaxLMO2.div.SCYA3..CCL3xLAG3	0.026896039	-1.032197965	1.67E-06	0.187400177	0.835876204
CD4xCD8xLMO2.div.M2xPD.L2	0.009595332	-0.298590383	1.68E-06	0.457076509	0.962316672
CD8xCD137.TNFR9xLMO2xCD56.div.M2xSCYA3..CCL3xPD.1	0.01352351	-0.540740736	1.69E-06	0.380126897	0.903015488
CD4xCD8xCD137.TNFR9xLMO2.div.PD.1xTIM3	0.027629433	-0.715766561	1.75E-06	0.334121214	0.880367701
CD4xCD8xCD137.TNFR9xLMO2.div.SCYA3..CCL3xPD.1xPD.L1	0.002323097	-0.421272764	1.76E-06	0.424060372	0.92978703
CD8xTNF.AlphaxLMO2.div.M2xSCYA3..CCL3xPD.L1	0.037236695	-0.569825069	1.79E-06	0.426436808	0.908742577
CD8xCD137.TNFR9xTNF.AlphaxLMO2xCD56.div.M2xSCYA3..CC					
L3xPD.1xPD.L2	0.008800393	-0.372358886	1.84E-06	0.432205468	0.928077442
CD4xCD8xCD137.TNFR9xTNF.Alpha.div.SCYA3..CCL3xPD.1xPD.L1	0.00328446	-0.481735895	1.92E-06	0.423832149	0.909811829
CD8xCD137.TNFR9xLMO2xCD56.div.M2xPD.1	0.047420653	-1.004744729	1.97E-06	0.192043896	0.838187049
CD4xCD8xCD137.TNFR9xTNF.AlphaxLMO2xCD56.div.M2xSCYA3..CCL3xPD.1xPD.L1	0.013310194	-0.544280435	2.09E-06	0.390937317	0.909974776
CD4xCD8xCD137.TNFR9xTNF.AlphaxLMO2xCD56.div.M2xSCYA3..CCL3xPD.1xPD.L2xTIM3	0.017322128	-0.366590167	2.47E-06	0.427929008	0.927440139
CD8xLMO2.div.M2xSCYA3..CCL3xTIM3	0.046696168	-0.683527154	2.47E-06	0.400159325	0.89333876
CD8xCD137.TNFR9xLMO2.div.SCYA3..CCL3xPD.1	0.008021092	-0.77833891	2.49E-06	0.304339043	0.867972289
CD4xCD8xCD137.TNFR9xTNF.Alpha.div.PD.1xPD.L1xTIM3	0.025377246	-0.253789421	2.59E-06	0.455571116	0.935932467

Immune Ratios	PvalSurvTree	Tree survival cutoff	Difference between survival curves (p-value)	Proportion of patients in low-ratio 4 year survival group	Proportion of patients in high-ratio 4 year survival group
CD8xCD137.TNFR9xTNF.AlphaLMO2.div.M2xSCYA3..CCL3xPD.L1	0.039478633	-0.570684192	2.60E-06	0.406709616	0.891930211
CD8xTNF.AlphaLMO2xCD56.div.M2xPD.1xPD.L2	0.006399369	-0.502465396	2.60E-06	0.396428799	0.902164653
CD4xCD8xTNF.AlphaLMO2.div.M2xSCYA3..CCL3xTIM3	0.019931169	-0.914481945	2.61E-06	0.249333333	0.841141548
CD4xCD8xCD137.TNFR9xLMO2.div.M2xPD.L1xPD.L2	0.04365364	-0.923453582	2.85E-06	0.244869565	0.837431817
CD4xCD8xCD137.TNFR9xTNF.AlphaLMO2.div.PD.1xPD.L2xTIM3	0.013556222	-0.560750441	2.92E-06	0.408819204	0.899923305
CD8xCD137.TNFR9xLMO2.div.SCYA3..CCL3xPD.1xPD.L2	0.015376377	-0.358226241	2.98E-06	0.4378331	0.950094759
CD4xCD8xCD137.TNFR9xLMO2.div.SCYA3..CCL3xPD.L2xTIM3	0.012109597	-0.811036435	3.16E-06	0.299335038	0.879753626
CD8xCD137.TNFR9xTNF.AlphaLMO2xCD56.div.M2xPD.1xPD.L2	0.014213521	-0.474607311	3.18E-06	0.410286818	0.91993056
CD8xCD137.TNFR9xLMO2xCD56.div.M2xPD.1xPD.L2xTIM3	0.025089689	-0.577960348	3.25E-06	0.357824971	0.895412348
CD4xCD8xCD137.TNFR9xTNF.AlphaLMO2xCD56.div.M2xSCYA3..CCL3xPD.1xPD.L2	0.014805411	-0.303311624	3.37E-06	0.456474974	0.949797412
CD4xCD8xCD137.TNFR9xTNF.AlphaLMO2.div.PD.1xPD.L2	0.007715598	-0.497256026	3.50E-06	0.402995665	0.899559818
CD4xCD8xCD137.TNFR9xTNF.AlphaLMO2xCD56.div.SCYA3..CCL3xPD.1xPD.L2	0.022282134	-0.81888988	3.53E-06	0.292075163	0.858800803
CD4xCD8xTNF.AlphaLMO2.div.M2xPD.L1xTIM3	0.026250044	-0.610098307	3.66E-06	0.415507374	0.900823836
CD8xTNF.AlphaLMO2xCD56.div.M2xPD.1xPD.L1	0.007377077	-0.685374056	3.68E-06	0.388531945	0.874237407
CD4xCD8xTNF.AlphaLMO2xCD56.div.M2xPD.1xPD.L1xTIM3	0.012007241	-0.196995073	3.70E-06	0.474421231	0.944124792
CD8xCD137.TNFR9xTNF.AlphaLMO2.div.SCYA3..CCL3xTIM3	0.038331482	-0.961698468	3.72E-06	0.227246377	0.842899226
CD8xTNF.AlphaLMO2xCD56.div.M2xSCYA3..CCL3xPD.1xPD.L2	0.005715938	-0.442333084	3.78E-06	0.431517306	0.919349706
CD4xCD8xCD137.TNFR9xTNF.AlphaLMO2.div.M2xSCYA3..CCL3xPD.L2xTIM3	0.031615443	-0.902001669	3.87E-06	0.251034483	0.848376096
CD4xCD8xTNF.AlphaLMO2.div.SCYA3..CCL3xPD.L1xTIM3	0.03740179	-0.738894161	3.88E-06	0.380778032	0.867954663
CD8xCD137.TNFR9xTNF.AlphaLMO2xCD56.div.M2xSCYA3..CCL3xPD.1	0.026392967	-0.52533507	4.01E-06	0.384932836	0.900523444
CD8xCD137.TNFR9xLMO2.div.M2xSCYA3..CCL3xTIM3	0.035412819	-1.123129504	4.01E-06	0.192638459	0.82060982
CD4xCD8xCD137.TNFR9xLMO2xCD56.div.M2xPD.1xPD.L1xTIM3	0.013318232	-0.767591786	4.07E-06	0.324859315	0.870844637
CD4xCD8xCD137.TNFR9xTNF.AlphaLMO2.div.PD.L2xLAG3	0.032099674	-0.681956182	4.21E-06	0.325814536	0.871003055
CD4xCD8xLMO2.div.PD.L2xLAG3	0.033236796	-0.88990287	4.21E-06	0.225464052	0.851930889
CD4xCD8xCD137.TNFR9xLMO2.div.PD.1xPD.L2xTIM3	0.011276052	-0.311885999	4.30E-06	0.439581318	0.925694956
CD4xCD8xLMO2xCD56.div.M2xPD.1xPD.L2xTIM3	0.018917719	-0.662986705	4.31E-06	0.376416629	0.883573862

Immune Ratios	PvalSurvTree	Tree survival cutoff	Difference between survival curves (p-value)	Proportion of patients in low-ratio 4 year survival group	Proportion of patients in high-ratio 4 year survival group
CD8xTNF.AlphaxLMO2xCD56.div.M2xSCYA3..CCL3xPD.1xTIM3	0.036063121	-0.526613672	4.54E-06	0.391616802	0.901731876
CD4xCD8xCD137.TNFR9xLMO2.div.SCYA3..CCL3xLAG3xTIM3	0.033338214	-0.786023017	4.60E-06	0.307264763	0.875168491
CD4xCD8xCD137.TNFR9xLMO2xCD56.div.M2xPD.1xPD.L1	0.01410525	-0.627174643	4.67E-06	0.384789329	0.893641686
CD8xCD137.TNFR9xLMO2xCD56.div.M2xSCYA3..CCL3xPD.1xPD.L1xTIM3	0.04233341	-0.551413995	4.70E-06	0.421977403	0.909128146
CD8xCD137.TNFR9xTNF.AlphaxCD56.div.M2xSCYA3..CCL3xPD.1	0.02794037	-1.0522220492	4.72E-06	0.225869809	0.83003052
CD4xCD8xCD137.TNFR9xLMO2.div.M2xPD.L2xTIM3	0.027239177	-0.772976519	5.23E-06	0.252203061	0.867751095
CD8xCD137.TNFR9xTNF.AlphaxLMO2.div.SCYA3..CCL3xPD.L2	0.019976135	-0.589572491	5.48E-06	0.394887005	0.902532638
CD4xCD8xTNF.AlphaxLMO2xCD56.div.SCYA3..CCL3xPD.1xPD.L2	0.013863603	-0.45879394	5.49E-06	0.421350793	0.908862429
CD4xCD8xLMO2.div.SCYA3..CCL3xPD.1xPD.L2	0.017600328	-0.362431962	5.53E-06	0.472659144	0.932806169
CD4xCD8xCD137.TNFR9xTNF.AlphaxLMO2.div.PD.L1xTIM3	0.045374756	-0.767658027	5.60E-06	0.33927923	0.853838416
CD4xCD8xCD137.TNFR9xTNF.AlphaxLMO2xCD56.div.M2xSCYA3..CCL3xPD.1xPD.L1xTIM3	0.017731458	-0.378230531	5.62E-06	0.442476321	0.926933341
CD4xCD8xTNF.AlphaxLMO2xCD56.div.M2xPD.1xPD.L2xLAG3	0.037876755	-0.434914404	5.77E-06	0.402731418	0.890352358
CD4xCD8xPD.L1xPD.L2xTIM3	0.029226196	-0.242109394	6.00E-06	0.474613161	0.946162984
CD4xCD8xTNF.AlphaxLMO2.div.M2xPD.L2xLAG3	0.030337969	-0.739631816	6.07E-06	0.289797009	0.852750611
CD4xCD8xCD137.TNFR9xLMO2xCD56.div.M2xSCYA3..CCL3xPD.1xTIM3	0.024572994	-0.55777951	6.15E-06	0.365086438	0.886101339
CD4xCD8xCD137.TNFR9xTNF.Alpha.div.SCYA3..CCL3xPD.1xPD.L2	0.005115871	-0.267010274	6.37E-06	0.465718963	0.936690182
CD4xCD8xTNF.Alpha.div.M2xSCYA3..CCL3xPD.L1	0.042948289	-0.936149786	6.46E-06	0.389863548	0.831398242
CD8xCD137.TNFR9xTNF.Alpha.div.PD.1xPD.L1	0.035020525	-0.246932279	6.55E-06	0.467690655	0.935859821
CD8xCD137.TNFR9xLMO2.div.SCYA3..CCL3xPD.1xPD.L1	0.021131497	-0.25763503	6.66E-06	0.448790341	0.950094759
CD4xCD8xCD137.TNFR9xLMO2xCD56.div.M2xSCYA3..CCL3xPD.1xPD.L1xPD.L2	0.010674407	-0.408653329	6.78E-06	0.437998532	0.93001567
CD4xCD8xLMO2.div.M2xLAG3	0.038174922	-0.508976546	6.81E-06	0.417819984	0.900876853
CD4xCD8xTNF.AlphaxLMO2xCD56.div.M2xPD.1xPD.L2xTIM3	0.011642906	-0.373311061	7.07E-06	0.408980323	0.909642041
CD4xCD8xTNF.AlphaxLMO2.div.SCYA3..CCL3xTIM3	0.02674484	-0.61602609	7.18E-06	0.386015478	0.8687078
CD4xCD8xTNF.AlphaxLMO2.div.M2xTIM3	0.044486436	-0.663828732	7.45E-06	0.370251262	0.877226092

Immune Ratios	PvalSurvTree	Tree survival cutoff	Difference between survival curves (p-value)	Proportion of patients in low-ratio 4 year survival group	Proportion of patients in high-ratio 4 year survival group
CD8xCD137.TNFR9xTNF.AlphaLMO2xCD56.div.M2xSCYA3..CC L3xPD.1xPD.L2xTIM3	0.030015916	-0.461971127	7.55E-06	0.419040307	0.909968664
CD4xCD8xTNF.AlphaLMO2xCD56.div.M2xPD.1xTIM3	0.0404141	-0.389442316	7.72E-06	0.421587408	0.908536507
CD4xCD8xTNF.Alpha.div.SCYA3..CCL3xPD.1	0.040914375	-0.401491683	7.72E-06	0.453696119	0.915011551
CD4xCD8xCD137.TNFR9xTNF.AlphaLMO2.div.SCYA3..CCL3xPD.L2	0.039739556	-0.702876119	8.25E-06	0.359096228	0.889044038
CD4xCD8xCD137.TNFR9xTNF.AlphaLMO2xCD56.div.SCYA3..CCL3xPD.1xPD.L2xTIM3	0.019446816	-0.832992165	8.25E-06	0.314004156	0.865554397
CD4xCD8xTNF.AlphaLMO2.div.M2xLAG3	0.046656975	-0.992026813	8.48E-06	0.28686166	0.831341866
CD4xCD8xCD137.TNFR9xTNF.AlphaLMO2.div.SCYA3..CCL3xPD.1xPD.L2xTIM3	0.023713015	-0.466984403	8.55E-06	0.461322241	0.925127141
CD8xCD137.TNFR9xTNF.AlphaLMO2xCD56.div.M2xSCYA3..CC L3xPD.1xTIM3	0.033004112	-0.547029168	8.63E-06	0.396135146	0.900987883
CD4xCD8xCD137.TNFR9xLMO2.div.M2xSCYA3..CCL3xTIM3	0.02598489	-1.01733874	8.73E-06	0.195441595	0.825684613
CD8xLMO2xCD56.div.M2xSCYA3..CCL3xPD.1	0.012789593	-0.675348544	8.78E-06	0.398703484	0.8819467
CD4xCD8xTNF.AlphaLMO2.div.SCYA3..CCL3xPD.1	0.021113003	-0.782750598	9.16E-06	0.372940869	0.853337779
CD8xCD137.TNFR9xTNF.AlphaLMO2xCD56.div.M2xPD.1xPD.L1	0.010700935	-0.341115212	9.55E-06	0.441391168	0.928087343
CD4xCD8xCD137.TNFR9xTNF.AlphaLMO2.div.M2xPD.L2xTIM3	0.047619211	-0.858987523	1.00E-05	0.255078646	0.853143872
CD4xCD8xCD137.TNFR9xLMO2.div.SCYA3..CCL3xPD.L1xPD.L2	0.035579917	-0.752551116	1.02E-05	0.329662996	0.876965959
CD8xCD137.TNFR9xTNF.AlphaLMO2.div.SCYA3..CCL3xPD.1xPD.L1	0.014345478	-0.41615193	1.02E-05	0.481101693	0.93342643
CD4xCD8xCD137.TNFR9xTNF.Alpha.div.M2xPD.1xPD.L1	0.028145691	-0.47066381	1.03E-05	0.44293582	0.90470437
CD4xCD8xCD137.TNFR9xTNF.AlphaLMO2xCD56.div.SCYA3..CCL3xPD.1xPD.L2xLAG3	0.048942614	-0.784487192	1.03E-05	0.279040404	0.860316045
CD4xCD8xCD137.TNFR9.div.M2xPD.1xPD.L1	0.048833805	-0.408504736	1.05E-05	0.425886299	0.893406931
CD4xCD8xCD137.TNFR9xTNF.AlphaLMO2xCD56.div.M2xSCYA3..CCL3xPD.1xPD.L1xPD.L2xTIM3	0.048473526	-0.394761839	1.08E-05	0.45816694	0.925418857
CD4xCD8xCD137.TNFR9xTNF.AlphaLMO2.div.SCYA3..CCL3xPD.L1	0.019876504	-0.860482642	1.08E-05	0.280505952	0.861592019
CD4xCD8xCD137.TNFR9.div.PD.1xPD.L2	0.026606706	-0.529868045	1.09E-05	0.355691057	0.878143117
CD4xCD8xCD137.TNFR9.div.PD.1xPD.L1	0.017596796	-0.330383033	1.17E-05	0.440343866	0.893584461
CD8xCD137.TNFR9xTNF.Alpha.div.SCYA3..CCL3xPD.1xPD.L1	0.047146075	-0.712244006	1.27E-05	0.329440248	0.857296528
CD4xCD8xCD137.TNFR9xTNF.AlphaLMO2xCD56.div.M2xPD.1x	0.021435783	-0.327057525	1.28E-05	0.449922835	0.927456555

Immune Ratios	PvalSurvTree	Tree survival cutoff	Difference between survival curves (p-value)	Proportion of patients in low-ratio 4 year survival group	Proportion of patients in high-ratio 4 year survival group
PD.L2xTIM3					
CD4xCD8xCD137.TNFR9xTNF.Alpha.xLMO2.div.SCYA3..CCL3xPD.L1xPD.L2	0.022311668	-0.47617878	1.29E-05	0.461051774	0.926971861
CD4xCD8xLMO2.div.M2xSCYA3..CCL3xPD.1	0.021371962	-0.449588893	1.32E-05	0.462205008	0.924489738
CD4xCD8xTNF.Alpha.xCD56.div.M2xSCYA3..CCL3xPD.1xPD.L1	0.014433328	-0.672594047	1.32E-05	0.434690868	0.85813208
CD4xCD8xCD137.TNFR9xTNF.Alpha.xCD56.div.M2xSCYA3..CCL3xPD.1xPD.L1	0.013385449	-0.735231275	1.37E-05	0.379918972	0.856752751
CD4xCD8xLMO2xCD56.div.M2xPD.1xPD.L2	0.007686465	-0.798213126	1.41E-05	0.336050605	0.861883587
CD8xCD137.TNFR9xTNF.Alpha.xLMO2xCD56.div.M2xPD.1xPD.L1xTIM3	0.019584139	-0.237425834	1.45E-05	0.485707661	0.945281099
CD4xCD8xTNF.Alpha.xLMO2.div.SCYA3..CCL3xLAG3xTIM3	0.043876564	-0.89970851	1.47E-05	0.319734247	0.829848313
CD4xCD8xLMO2.div.SCYA3..CCL3xPD.1xPD.L1	0.04190029	-0.399081121	1.47E-05	0.451021861	0.915651417
CD4xCD8xTNF.Alpha.xLMO2.div.SCYA3..CCL3xPD.L2xLAG3	0.023523741	-0.94985971	1.49E-05	0.28784219	0.825229692
CD4xCD8xCD137.TNFR9xLMO2.div.PD.1xPD.L1	0.00311426	-0.389407284	1.51E-05	0.441779839	0.927559178
CD4xCD8xTNF.Alpha.xLMO2xCD56.div.M2xSCYA3..CCL3xPD.1xPD.L2xTIM3	0.013548843	-0.424848035	1.51E-05	0.423062376	0.909762237
CD8xCD137.TNFR9xTNF.Alpha.div.SCYA3..CCL3xPD.1	0.019878707	-0.391486926	1.56E-05	0.470033639	0.913218508
CD8xCD137.TNFR9xTNF.Alpha.xLMO2xCD56.div.M2xPD.1xPD.L1xPD.L2	0.01686246	-0.384749046	1.57E-05	0.453948675	0.916224929
CD4xCD8xTNF.Alpha.xLMO2.div.M2xPD.L2xTIM3	0.02104393	-0.570930898	1.57E-05	0.405954468	0.890957855
CD4xCD8xCD137.TNFR9xLMO2.div.SCYA3..CCL3xPD.1xPD.L1xTIM3	0.037780982	-0.38105906	1.59E-05	0.455968852	0.92860095
CD4xCD8xLMO2.div.M2xSCYA3..CCL3xTIM3	0.009243036	-0.74924939	1.60E-05	0.386968566	0.869465662
CD8xCD137.TNFR9xLMO2xCD56.div.M2xPD.1xTIM3	0.044582166	-0.736272578	1.61E-05	0.316730967	0.880723933
CD4xCD8xCD137.TNFR9xTNF.Alpha.div.PD.1xPD.L2xTIM3	0.042186249	-0.275656831	1.63E-05	0.472065771	0.934196414
CD8xCD137.TNFR9xTNF.Alpha.xLMO2xCD56.div.SCYA3..CCL3xPD.1xPD.L1xPD.L2	0.014473893	-0.386932047	1.64E-05	0.443719855	0.919273546
CD4xCD8xCD137.TNFR9xTNF.Alpha.xLMO2.div.SCYA3..CCL3xPD.L1xPD.L1xTIM3	0.025006762	-0.481341814	1.65E-05	0.455980396	0.914834499
CD4xCD8xLMO2.div.M2xPD.1	0.031709404	-0.439630618	1.68E-05	0.427575935	0.897014778
CD8xTNF.Alpha.xLMO2.div.SCYA3..CCL3xLAG3	0.027184554	-0.787089959	1.68E-05	0.321716776	0.852591557
CD4xCD8xCD137.TNFR9xTNF.Alpha.xLMO2.div.SCYA3..CCL3xPD.L1xTIM3	0.024519671	-0.714150594	1.70E-05	0.352967441	0.865540242

Immune Ratios	PvalSurvTree	Tree survival cutoff	Difference between survival curves (p-value)	Proportion of patients in low-ratio 4 year survival group	Proportion of patients in high-ratio 4 year survival group
CD8xLMO2.div.M2xTIM3	0.04674458	-0.289621422	1.78E-05	0.480592767	0.933754544
CD4xCD8xCD137.TNFR9xLMO2.div.PD.1xPD.L1xTIM3	0.013790197	-0.117757486	1.78E-05	0.485352056	0.958518233
CD4xCD8xCD137.TNFR9xTNF.Alpha.xLMO2.div.M2xPD.L1xTIM3	0.042668369	-0.716251706	1.81E-05	0.350993292	0.861242536
CD4xCD8xCD137.TNFR9xTNF.Alpha.xLMO2xCD56.div.M2xSCYA3.CCL3xPD.1xPD.L1xTIM3	0.036776806	-0.535805873	1.86E-05	0.418800658	0.899161558
CD4xCD8xTNF.Alpha.xLMO2.div.PD.L2xTIM3	0.041043272	-0.542030332	1.91E-05	0.410698808	0.903409957
CD4xCD8xCD137.TNFR9xTNF.Alpha.xCD56.div.M2xSCYA3..CCL3xPD.1xPD.L1xTIM3	0.033443679	-0.867495066	1.91E-05	0.328454849	0.838743949
CD4xCD8xCD137.TNFR9xTNF.Alpha.div.M2xPD.1xPD.L2	0.038876123	-0.613254616	1.94E-05	0.372043743	0.877103167
CD4xCD8xCD137.TNFR9xTNF.Alpha.xLMO2xCD56.div.M2xPD.1xPD.L1	0.022047063	-0.515800149	1.94E-05	0.413514005	0.899161558
CD4xCD8xCD137.TNFR9xLMO2.div.SCYA3..CCL3xPD.L2	0.016897453	-0.577245703	1.95E-05	0.404142522	0.91404103
CD8xTNF.Alpha.xLMO2xCD56.div.M2xPD.1	0.031848664	-0.336292375	1.95E-05	0.469106639	0.924333389
CD8xCD137.TNFR9xLMO2xCD56.div.M2xSCYA3..CCL3xPD.1xPD.L2xTIM3	0.032324775	-0.60206197	1.96E-05	0.397393164	0.893365537
CD4xCD8xTNF.Alpha.xLMO2.div.M2xSCYA3..CCL3	0.028165695	-0.646346612	2.02E-05	0.368637771	0.87156715
CD4xCD8xTNF.Alpha.xCD56.div.M2xSCYA3..CCL3xPD.1xPD.L2	0.012212749	-0.613135968	2.18E-05	0.427505633	0.852716016
CD4xCD8xCD137.TNFR9xLMO2.div.PD.L1xLAG3	0.028302978	-0.759246268	2.20E-05	0.296995793	0.870466297
CD4xCD8xCD137.TNFR9xLMO2xCD56.div.M2xPD.1xPD.L1xPD.L2	0.011538879	-0.200099769	2.26E-05	0.474805396	0.951381256
CD4xCD8xLMO2.div.SCYA3..CCL3xPD.L2xTIM3	0.040508758	-0.7893202	2.35E-05	0.361718475	0.880707138
CD8xCD137.TNFR9xLMO2xCD56.div.M2xPD.1xPD.L1	0.007805985	-0.268355726	2.46E-05	0.472498122	0.936146273
CD8xTNF.Alpha.xLMO2xCD56.div.M2xPD.1xTIM3	0.043161738	-0.253999641	2.48E-05	0.477525477	0.935177054
CD4xCD8xTNF.Alpha.xLMO2xCD56.div.SCYA3..CCL3xPD.1xPD.L1	0.01854793	-0.552259869	2.65E-05	0.421774395	0.899878909
CD4xCD8xCD137.TNFR9xTNF.Alpha.xLMO2.div.SCYA3..CCL3xPD.L1	0.032914287	-0.616898338	2.66E-05	0.424227551	0.890906118
CD8xCD137.TNFR9xTNF.Alpha.xLMO2xCD56.div.SCYA3..CCL3xPD.1xPD.L2xTIM3	0.024662396	-0.453749369	2.66E-05	0.415742852	0.901146381
CD4xCD8xCD137.TNFR9xTNF.Alpha.div.PD.1xPD.L2	0.020542606	-0.593356095	2.67E-05	0.396608614	0.893463355
CD8xCD137.TNFR9xTNF.Alpha.xLMO2xCD56.div.SCYA3..CCL3xPD.1xPD.L2	0.008834115	-0.250555622	2.67E-05	0.4678614	0.93835407
CD4xCD8xLMO2.div.SCYA3..CCL3xLAG3	0.014616438	-0.638487059	2.71E-05	0.378927689	0.877311464

Immune Ratios	PvalSurvTree	Tree survival cutoff	Difference between survival curves (p-value)	Proportion of patients in low-ratio 4 year survival group	Proportion of patients in high-ratio 4 year survival group
CD4xCD8xCD137.TNFR9xTNF.AlphaxCD56.div.M2xSCYA3..CCL3xPD.1xPD.L1xPD.L2	0.030384008	-0.489059628	2.94E-05	0.442954722	0.892900768
CD4xCD8xLMO2.div.PD.1xPD.L2	0.018691989	-0.31374955	2.98E-05	0.467690045	0.924820276
CD4xCD8xLMO2.div.PD.1xPD.L1	0.034728467	-0.164650084	2.99E-05	0.487725835	0.931404999
CD4xCD8xCD137.TNFR9xTNF.AlphaxCD56.div.PD.L1xLAG3	0.032817055	-0.812425736	3.17E-05	0.330851944	0.85030482
CD4xCD8xLMO2xCD56.div.M2xPD.1xPD.L1xTIM3	0.026497619	-0.343748185	3.32E-05	0.456852432	0.902282001
CD4xCD8xLMO2.div.M2xTIM3	0.020836461	-0.544951702	3.44E-05	0.42150997	0.890876871
CD8xTNF.AlphaxCD137.TNFR9xTNF.AlphaxCD56.div.M2xSCYA3..CCL3xPD.L2	0.017422259	-0.7664692	3.57E-05	0.353918428	0.874933331
CD4xCD8xCD137.TNFR9xTNF.AlphaxCD56.div.M2xSCYA3..CCL3xPD.1xPD.L2	0.035607784	-0.27909485	3.65E-05	0.489959481	0.933964737
CD4xCD8xLMO2xCD56.div.M2xPD.1xPD.L1	0.010973347	-0.568875385	3.67E-05	0.427902593	0.887444008
CD8xCD137.TNFR9xLMO2.div.M2xSCYA3..CCL3	0.027323481	-0.969634828	3.77E-05	0.239051383	0.829739415
CD4xCD8xCD137.TNFR9xTNF.AlphaxCD56.div.PD.1xPD.L1xPD.L2xTIM3	0.036898617	-0.125840456	3.99E-05	0.49676101	0.960169861
CD4xCD8xCD137.TNFR9xLMO2xCD56.div.SCYA3..CCL3xPD.1xPD.L2	0.015778942	-0.354523409	4.03E-05	0.455423312	0.930132247
CD4xCD8xCD137.TNFR9xTNF.AlphaxCD56.div.M2xSCYA3..CCL3xPD.1xTIM3	0.043923268	-0.696544599	4.17E-05	0.369864072	0.849726658
CD8xCD137.TNFR9xLMO2.div.PD.1xPD.L1	0.011354897	-0.135795277	4.44E-05	0.496880085	0.957936508
CD4xCD8xCD137.TNFR9xTNF.AlphaxCD56.div.PD.1xTIM3	0.038952126	-0.676223676	4.51E-05	0.41054283	0.874213154
CD4xCD8xLMO2xCD56.div.SCYA3..CCL3xPD.1xPD.L2	0.026658199	-0.611858252	4.68E-05	0.398744543	0.884783838
CD8xCD137.TNFR9xTNF.AlphaxCD56.div.SCYA3..CCL3xPD.1xPD.L1	0.007558994	-0.211958042	4.74E-05	0.487959268	0.948241827
CD4xCD8xCD137.TNFR9xTNF.AlphaxCD56.div.SCYA3..CCL3xPD.1xPD.L1xTIM3	0.01683307	-0.184422251	4.88E-05	0.484366533	0.947764145
CD8xCD137.TNFR9xLMO2xCD56.div.SCYA3..CCL3xPD.1xPD.L1	0.009924419	-0.345794663	5.06E-05	0.44023972	0.921180408
CD8xTNF.AlphaxCD56.div.M2xSCYA3..CCL3xPD.1	0.028614685	-0.481116172	5.09E-05	0.496124689	0.885120658
CD4xCD8xCD137.TNFR9xLMO2.div.M2xPD.1	0.04223601	-0.450897393	5.34E-05	0.449129001	0.907894067
CD8xCD137.TNFR9xLMO2.div.SCYA3..CCL3xPD.L1	0.007094428	-0.796207607	5.76E-05	0.353722908	0.867013293
CD8xCD137.TNFR9xLMO2.div.M2xPD.L2	0.034999812	-0.83205909	5.80E-05	0.237683172	0.855024336
CD4xCD8xLMO2xCD56.div.M2xSCYA3..CCL3xPD.1xPD.L1xTIM3	0.0394909	-0.582890153	5.85E-05	0.43779187	0.88561956
CD8xCD137.TNFR9xLMO2xCD56.div.PD.1xPD.L1	0.030878029	-0.979152146	6.39E-05	0.263768116	0.82917884

Immune Ratios	PvalSurvTree	Tree survival cutoff	Difference between survival curves (p-value)	Proportion of patients in low-ratio 4 year survival group	Proportion of patients in high-ratio 4 year survival group
CD4xCD8xCD137.TNFR9xTNF.AlphaxLMO2xCD56.div.M2xPD.1xPD.L1xTIM3	0.015554052	-0.182735559	6.46E-05	0.503688998	0.94389256
CD4xCD8xTNF.AlphaxLMO2.div.M2xPD.L1xPD.L2	0.038225155	-0.330493019	6.98E-05	0.506211969	0.957729342
CD4xCD8xCD137.TNFR9xLMO2.div.SCYA3..CCL3xPD.L1xTIM3	0.014920278	-0.734812212	7.17E-05	0.363521053	0.87240754
CD8xCD137.TNFR9xTNF.AlphaxLMO2xCD56.div.SCYA3..CCL3xPD.1xPD.L1xTIM3	0.021065988	-0.329829961	7.35E-05	0.462341117	0.915765092
CD4xCD8xCD137.TNFR9xLMO2.div.SCYA3..CCL3xPD.1xPD.L1xPD.L2	0.033661507	-0.289431315	7.69E-05	0.495812613	0.945882353
CD8xCD137.TNFR9xTNF.AlphaxLMO2xCD56.div.PD.1xPD.L1xPD.L2	0.021787462	-0.301620625	8.32E-05	0.473770992	0.926845398
CD8xCD137.TNFR9xTNF.AlphaxLMO2xCD56.div.M2xPD.1xPD.L2xTIM3	0.025165434	-0.627710013	8.37E-05	0.396629951	0.884283338
CD4xCD8xCD137.TNFR9xLMO2xCD56.div.SCYA3..CCL3xPD.1xPD.L2xTIM3	0.023165808	-0.423327951	8.42E-05	0.423577748	0.911990808
CD4xCD8xCD137.TNFR9xTNF.AlphaxLMO2xCD56.div.SCYA3..CCL3xPD.1xPD.L1	0.018370429	-0.42963985	8.85E-05	0.44246487	0.918936759
CD8xCD137.TNFR9xLMO2xCD56.div.M2xSCYA3..CCL3xPD.1xPD.L2	0.024401135	-0.597664591	9.17E-05	0.42163644	0.882318242
CD4xCD8xCD137.TNFR9xTNF.AlphaxCD56.div.M2xSCYA3..CCL3xPD.1xPD.L2xTIM3	0.037268481	-0.808753208	9.30E-05	0.36180828	0.837129777
CD4xCD8xCD137.TNFR9xLMO2xCD56.div.PD.1xPD.L1xPD.L2	0.040279966	-0.810263335	9.45E-05	0.30715947	0.851828887
CD4xCD8xTNF.AlphaxCD56.div.M2xSCYA3..CCL3xPD.1	0.031794559	-0.624201874	9.47E-05	0.427972042	0.860446081
CD4xCD8xCD137.TNFR9xLMO2.div.SCYA3..CCL3xPD.1xPD.L2xTIM3	0.023366071	-0.324376945	9.55E-05	0.483350827	0.934785134
CD8xCD137.TNFR9xTNF.AlphaxLMO2xCD56.div.PD.1xPD.L2xTIM3	0.042543833	-0.3474165	9.71E-05	0.449811593	0.905899325
CD4xCD8xCD137.TNFR9xLMO2xCD56.div.SCYA3..CCL3xPD.1xPD.L1xPD.L2	0.014330959	-0.531586395	9.92E-05	0.431813339	0.901061509
CD8xCD137.TNFR9xLMO2xCD56.div.M2xPD.1xPD.L1xPD.L2	0.017930293	-0.77404292	0.000100317	0.302949134	0.866246022
CD4xCD8xTNF.AlphaxLMO2xCD56.div.M2xPD.1xPD.L1xPD.L2	0.010225341	-0.160285446	0.000103272	0.523866038	0.970798183
CD4xCD8xCD137.TNFR9xLMO2.div.SCYA3..CCL3xLAG3	0.026880172	-0.630282379	0.000103385	0.418030019	0.882806256
CD8xCD137.TNFR9xLMO2xCD56.div.SCYA3..CCL3xPD.1xPD.L2	0.007633035	-0.327048826	0.000103387	0.450008226	0.920271718
CD4xCD8xCD137.TNFR9xLMO2.div.PD.L2xLAG3	0.020380429	-0.614070344	0.000107641	0.401673011	0.888429044

Immune Ratios	PvalSurvTree	Tree survival cutoff	Difference between survival curves (p-value)	Proportion of patients in low-ratio 4 year survival group	Proportion of patients in high-ratio 4 year survival group
CD4xCD8xCD137.TNFR9xTNF.AlphaXLMO2.div.SCYA3..CCL3xPD.L2xTIM3	0.025014212	-0.413737383	0.000109135	0.461840966	0.916608023
CD4xCD8xCD137.TNFR9xTNF.AlphaXLMO2xCD56.div.SCYA3..CCL3xPD.L1xPD.L2xTIM3	0.032133035	-0.418699828	0.000109387	0.433733587	0.90075452
CD4xCD8xLMO2xCD56.div.M2xSCYA3..CCL3xPD.L1xPD.L2	0.021874621	-0.481525734	0.000112865	0.474545444	0.913307038
CD4xCD8xCD137.TNFR9xLMO2xCD56.div.M2xPD.L1xPD.L2xTIM3	0.032326128	-0.349469986	0.000116755	0.471178089	0.913990428
CD8xCD137.TNFR9xTNF.AlphaXLMO2.div.SCYA3..CCL3xPD.L1xTIM3	0.046342489	-0.64718931	0.000119545	0.427971111	0.872216018
CD4xCD8xCD137.TNFR9xLMO2xCD56.div.SCYA3..CCL3xPD.L1xPD.L2	0.017618618	-0.230215859	0.000125541	0.476272347	0.937790095
CD8xLMO2xCD56.div.M2xSCYA3..CCL3xPD.L1xPD.L2	0.017678038	-0.547216844	0.000131624	0.460682202	0.893954993
CD8xLMO2xCD56.div.M2xSCYA3..CCL3xPD.L1xPD.L2	0.008276197	-0.398387132	0.000150663	0.491143411	0.922844053
CD8xCD137.TNFR9xTNF.AlphaXLMO2xCD56.div.PD.L1xPD.L2	0.035369903	-0.346568967	0.000163367	0.474707733	0.91345908
CD4xCD8xTNF.AlphaXLMO2xCD56.div.M2xPD.L1xPD.L1	0.008865186	-0.243288593	0.000163379	0.477082749	0.912243537
CD4xCD8xCD137.TNFR9xLMO2.div.M2xPD.L1xTIM3	0.025785013	-0.561698653	0.000167157	0.432868057	0.890401224
CD8xCD137.TNFR9xLMO2xCD56.div.M2xPD.L1xPD.L1xTIM3	0.025317957	-0.350846631	0.000169598	0.473464121	0.913785727
CD8xLMO2xCD56.div.M2xPD.L1xPD.L2	0.008557318	-0.650791939	0.000173416	0.416550141	0.874762355
CD4xCD8xCD137.TNFR9xLMO2.div.SCYA3..CCL3xPD.L1	0.008273513	-0.683543139	0.000177529	0.366485339	0.86962895
CD4xCD8xCD137.TNFR9xTNF.AlphaXLMO2xPD.L1xPD.L1xPD.L2	0.031846116	-0.648712709	0.00018033	0.420802012	0.861590101
CD4xCD8xCD137.TNFR9xTNF.AlphaXLMO2xCD56.div.PD.L1xPD.L2	0.017510011	-0.61952947	0.000183609	0.417438647	0.867242251
CD4xCD8xCD137.TNFR9xTNF.AlphaXLMO2xCD56.div.PD.L1xPD.L2	0.025599314	-0.120709453	0.000185205	0.515614712	0.959078256
CD4xCD8xCD137.TNFR9xLMO2xCD56.div.SCYA3..CCL3xPD.L1xPD.L1xTIM3	0.009316346	-0.401461038	0.000186903	0.444903863	0.899471013
CD8xTNF.AlphaXLMO2xCD56.div.M2xSCYA3..CCL3xPD.L1xPD.L2	0.027091944	-0.461572969	0.000195001	0.435407299	0.911498426
CD4xCD8xCD137.TNFR9xTNF.Alpha.div.SCYA3..CCL3xPD.L1xTIM3	0.036474642	-0.70606927	0.000199359	0.392869294	0.851574239
CD4xCD8xCD137.TNFR9xTNF.Alpha.div.SCYA3..CCL3xPD.L1xTIM3	0.021267285	-0.276216721	0.000214149	0.49039154	0.909774241
CD4xCD8xTNF.AlphaXLMO2xCD56.div.SCYA3..CCL3xPD.L1xPD.L2	0.021314118	-0.809466608	0.000216906	0.347616332	0.855171421

Immune Ratios	PvalSurvTree	Tree survival cutoff	Difference between survival curves (p-value)	Proportion of patients in low-ratio 4 year survival group	Proportion of patients in high-ratio 4 year survival group
1xPD.L2					
CD4xCD8xLMO2.div.SCYA3..CCL3xTIM3	0.014989145	-0.230465681	0.000227994	0.495933058	0.936531879
CD8xCD137.TNFR9xTNF.AlphaxLMO2xCD56.div.PD.1xPD.L1xTI					
M3	0.025584769	-0.525130763	0.00024253	0.430123075	0.879767324
CD4xCD8xLMO2xCD56.div.M2xSCYA3..CCL3xPD.1xPD.L2xTIM3	0.022761087	-0.471193296	0.000255095	0.489464263	0.899504457
CD8xTNF.AlphaxLMO2.div.SCYA3..CCL3xPD.L1	0.021272258	-0.562283232	0.000259398	0.47015411	0.897186852
CD4xCD8xTNF.AlphaxLMO2xCD56.div.SCYA3..CCL3xPD.1xPD.L2xTIM3	0.028786118	-0.665884469	0.000271585	0.413002946	0.864839248
CD4xCD8xTNF.AlphaxLMO2.div.PD.1xPD.L1	0.009934419	-0.413291932	0.000282004	0.475663231	0.902956119
CD8xCD137.TNFR9xLMO2xCD56.div.PD.1xPD.L2	0.039709892	-0.192601496	0.000282817	0.493170375	0.936922688
CD4xCD8xLMO2xCD56.div.M2xPD.1xPD.L1xPD.L2	0.01882055	-0.431432396	0.000299847	0.471451262	0.893296517
CD4xCD8xTNF.Alpha.div.SCYA3..CCL3xLAG3	0.042192296	-0.865262947	0.000319751	0.401187879	0.826875968
CD4xCD8xCD137.TNFR9xLMO2.div.M2xPD.L1	0.039339334	-0.261749365	0.000328793	0.512521897	0.946214561
CD8xCD137.TNFR9xTNF.AlphaxLMO2.div.PD.L2xLAG3	0.043965777	-0.590572765	0.000370505	0.455371249	0.888639972
CD8xTNF.AlphaxLMO2xCD56.div.PD.1xPD.L2	0.036501791	-0.40370693	0.000373759	0.482604247	0.889954222
CD8xLMO2.div.SCYA3..CCL3xPD.L2	0.027560416	-0.653272709	0.000385308	0.399960889	0.888482128
CD4xCD8xTNF.Alpha.div.SCYA3..CCL3xPD.L1	0.046542819	-0.52294203	0.00040735	0.510709944	0.864095306
CD4xCD8xCD137.TNFR9xTNF.AlphaxCD56.div.SCYA3..CCL3xPD.L1xPD.L1	0.0197826	-0.6396912	0.000409941	0.426314358	0.863559703
CD8xCD137.TNFR9xLMO2.div.SCYA3..CCL3xPD.L2	0.007649167	-0.781159791	0.000414973	0.359213151	0.875466081
CD4xCD8xTNF.AlphaxLMO2xCD56.div.PD.1xPD.L1xPD.L2	0.040572656	-0.541577143	0.000424116	0.443096402	0.879499408
CD8xCD137.TNFR9.div.SCYA3..CCL3xPD.1	0.021460196	-0.196233835	0.000429646	0.504065756	0.929194828
CD4xCD8xTNF.AlphaxLMO2xCD56.div.M2xSCYA3..CCL3xPD.1xPD.L1xTIM3	0.018320568	-0.272608906	0.000439683	0.514525347	0.930748473
CD4xCD8xCD137.TNFR9xLMO2.div.SCYA3..CCL3xPD.1xTIM3	0.011454658	-0.295597929	0.000442199	0.48648752	0.910219025
CD4xCD8xCD137.TNFR9xLMO2.div.PD.L1xTIM3	0.031234388	-0.537521131	0.000484553	0.453528824	0.888227244
CD4xCD8xTNF.AlphaxLMO2.div.PD.L1xTIM3	0.035709383	-0.641521434	0.000501603	0.445638422	0.862448656
CD4xCD8xTNF.AlphaxLMO2.div.PD.L1xLAG3	0.034849666	-0.820273957	0.000526515	0.384693878	0.832355408
CD8xCD137.TNFR9xLMO2xCD56.div.SCYA3..CCL3xPD.1xPD.L1xPD.L2	0.031024046	-0.651061603	0.00052792	0.387832133	0.887758945
CD8xTNF.AlphaxLMO2xCD56.div.SCYA3..CCL3xPD.1xPD.L2	0.012205944	-0.211351465	0.000549733	0.508850625	0.935970769
CD4xCD8xLMO2.div.SCYA3..CCL3xPD.1	0.023547908	0.07553268	0.000552871	0.547237565	0.953357753

Immune Ratios	PvalSurvTree	Tree survival cutoff	Difference between survival curves (p-value)	Proportion of patients in low-ratio 4 year survival group	Proportion of patients in high-ratio 4 year survival group
CD8xCD137.TNFR9xLMO2.div.M2xPD.L1	0.03117987	-0.413593466	0.000571491	0.477279524	0.904375677
CD8xLMO2xCD56.div.M2xPD.1	0.03078842	-0.084265227	0.000599321	0.518559184	0.931594625
CD4xCD8xTNF.AlphaxLMO2.div.LAG3xTIM3	0.037947435	-0.96997464	0.000638181	0.341715976	0.822456053
CD8xLMO2.div.SCYA3..CCL3xTIM3	0.040704469	-0.20952504	0.000707384	0.504914743	0.92395999
CD8xTNF.AlphaxLMO2.div.SCYA3..CCL3xTIM3	0.031241848	-0.96674415	0.000713989	0.337301587	0.832076682
CD8xTNF.AlphaxLMO2xCD56.div.M2xPD.1xPD.L2xTIM3	0.029919737	-0.381153085	0.000771072	0.458656581	0.896457825
CD4xCD8xCD137.TNFR9xTNF.Alpha.div.SCYA3..CCL3xPD.L1xTI M3	0.043588749	-0.323927896	0.000771549	0.506769463	0.909975359
CD8xTNF.AlphaxCD56.div.M2xSCYA3..CCL3xPD.1xPD.L1	0.045021078	-0.879479173	0.000772083	0.355555556	0.825073577
CD4xCD8xCD137.TNFR9.div.SCYA3..CCL3xPD.1	0.014020811	0.102691901	0.000797815	0.572171819	0.943605062
CD4xCD8xCD137.TNFR9xTNF.Alpha.div.SCYA3..CCL3xPD.D.1xPD.L1xPD.L2	0.02697283	-0.308107836	0.00092604	0.49697129	0.911215326
CD4xCD8xCD137.TNFR9xTNF.Alpha.div.SCYA3..CCL3xPD.1	0.019105189	-0.191867568	0.000958764	0.514143633	0.933923471
CD4xCD8xTNF.Alpha.div.M2xSCYA3..CCL3xPD.L2	0.030975891	-0.314583282	0.000971482	0.507162846	0.922749552
CD4xCD8xCD137.TNFR9.div.SCYA3..CCL3xPD.1xTIM3	0.023981862	-0.636914077	0.000979557	0.399450607	0.848434033
CD8xTNF.AlphaxLMO2xCD56.div.SCYA3..CCL3xPD.1xPD.L1	0.016790849	-0.337463008	0.00102648	0.505927993	0.90914179
CD4xCD8xCD137.TNFR9xTNF.Alpha.div.SCYA3..CCL3xPD.D.1xPD.L2	0.030117107	-0.758899187	0.00119948	0.385900279	0.844549285
CD4xCD8xTNF.Alpha.div.SCYA3..CCL3xPD.1xPD.L2	0.048385235	-0.547774053	0.001263296	0.458745391	0.85822033
CD4xCD8xCD137.TNFR9.div.M2xSCYA3..CCL3xPD.1	0.039354306	-0.219768066	0.001279454	0.557232419	0.906342843
CD8xTNF.AlphaxLMO2xCD56.div.M2xPD.1xPD.L1xPD.L2	0.022521351	-0.159676986	0.001296426	0.550470099	0.950985975
CD8xCD137.TNFR9xTNF.Alpha.div.SCYA3..CCL3xPD.1xPD.L1	0.017309311	-0.39605219	0.001459834	0.526847257	0.889720045
CD8xLMO2.div.SCYA3..CCL3xPD.L1	0.049631471	-0.348008232	0.001491514	0.510182818	0.911441895
CD4xCD8xCD137.TNFR9xTNF.Alpha.div.SCYA3..CCL3xPD.L1xTIM3	0.030982125	-0.337115042	0.001705894	0.488391308	0.901106182
CD8xCD137.TNFR9xTNF.Alpha.div.SCYA3..CCL3xPD.L1	0.019353163	-0.103555284	0.001767781	0.528634242	0.945530833
CD8xCD137.TNFR9xTNF.Alpha.div.SCYA3..CCL3xPD.L2	0.032515219	-0.536887624	0.001837572	0.487871279	0.871579319
CD8xTNF.AlphaxLMO2xCD56.div.PD.1xPD.L1	0.035241463	-0.655084927	0.00194745	0.436433371	0.855039436
CD8xCD137.TNFR9xLMO2.div.SCYA3..CCL3xTIM3	0.014940074	-0.594579474	0.001967462	0.431962611	0.871808933
CD4xCD8xTNF.Alpha.div.SCYA3..CCL3xPD.1xPD.L1	0.023354103	-0.359796306	0.002049594	0.489753786	0.871227692
CD8xCD137.TNFR9xTNF.Alpha.div.SCYA3..CCL3xPD.L1xLAG3	0.043076295	-0.589389951	0.002102349	0.474088756	0.869445994

Immune Ratios	PvalSurvTree	Tree survival cutoff	Difference between survival curves (p-value)	Proportion of patients in low-ratio 4 year survival group	Proportion of patients in high-ratio 4 year survival group
CD8xTNF.AlphaxLMO2xCD56.div.M2xSCYA3..CCL3xPD.1xPD.L2xTIM3	0.046978225	-0.55769691	0.002369155	0.462595411	0.879271753
CD8xCD137.TNFR9xTNF.AlphaxCD56.div.M2xSCYA3..CCL3xPD.1xPD.L2	0.017147857	-0.225349251	0.002404494	0.556893032	0.951417004
CD4xCD8xTNF.AlphaxLMO2xCD56.div.SCYA3..CCL3xPD.1xPD.L1xTIM3	0.037174047	-0.107906716	0.002438801	0.546812545	0.935701854
CD4xCD8xTNF.AlphaxLMO2xCD56.div.M2xPD.1xPD.L1xPD.L2xTIM3	0.040116668	-0.122767878	0.002481243	0.556506829	0.950125354
CD8xTNF.AlphaxLMO2xCD56.div.M2xPD.1xPD.L1xTIM3	0.031824732	-0.16172694	0.002567517	0.545642597	0.938764072
CD4xCD8xCD137.TNFR9xTNF.AlphaxCD56.div.M2xPD.1xPD.L1	0.030417403	-0.612501042	0.003322505	0.45442211	0.85143122
CD8xCD137.TNFR9xTNF.AlphaxCD56.div.SCYA3..CCL3xPD.1xPD.L1	0.01497944	-0.291465144	0.003640853	0.502953986	0.901401202
CD8xLMO2xCD56.div.SCYA3..CCL3xPD.1xPD.L2	0.042281474	-0.707002007	0.003736365	0.415719279	0.865671553
CD8xCD137.TNFR9xTNF.AlphaxCD56.div.M2xPD.1xPD.L1	0.023400148	-0.365319096	0.003739158	0.500109198	0.890394353
CD8xLMO2xCD56.div.M2xPD.1xPD.L1	0.014911462	-0.579738689	0.004048653	0.457506375	0.870698613
CD8xCD137.TNFR9xTNF.AlphaxCD56.div.SCYA3..CCL3xPD.1xPD.L2	0.020628141	-0.5782472	0.004803919	0.462357556	0.859348359
CD8xTNF.AlphaxCD56.div.M2xSCYA3..CCL3xPD.1xPD.L2	0.035485908	-0.122045912	0.005193588	0.563897528	0.950841955
CD4xCD8xCD137.TNFR9xTNF.AlphaxCD56.div.M2xPD.1xPD.L1xTIM3	0.031663823	-0.519317394	0.005344186	0.467830885	0.856684061
CD8xCD137.TNFR9xTNF.Alpha.div.SCYA3..CCL3xPD.L1	0.039780316	-0.171842645	0.005608161	0.545628245	0.927324002
CD4xCD8xCD137.TNFR9xTNF.AlphaxCD56.div.SCYA3..CCL3xPD.1xPD.L1xTIM3	0.036143195	-0.613049164	0.005910238	0.461931389	0.859333037
CD4xCD8xCD137.TNFR9xTNF.Alpha.div.SCYA3..CCL3xPD.L1	0.049046193	-0.622068042	0.006544385	0.492035414	0.86425576
CD4xCD8xCD137.TNFR9xLMO2.div.SCYA3..CCL3xTIM3	0.036956977	-0.401086288	0.007330046	0.523408223	0.911104664
CD4xCD8xCD137.TNFR9xTNF.AlphaxCD56.div.M2xPD.1xPD.L2xTIM3	0.048132264	-0.488207507	0.008374768	0.440538615	0.862034465
CD8xCD137.TNFR9xTNF.AlphaxLMO2.div.PD.L1xTIM3	0.046948468	-0.296798326	0.008785854	0.526252171	0.909725938
CD4xCD8xCD137.TNFR9xTNF.Alpha.div.M2xSCYA3..CCL3xPD.1	0.03216489	-0.329195597	0.009751623	0.535326284	0.905428743
CD4xCD8xTNF.AlphaxCD56.div.M2xPD.1xPD.L2	0.028443014	-0.356085441	0.012406442	0.524497554	0.886341925
CD8xTNF.AlphaxCD56.div.M2xPD.1xPD.L2	0.040719482	-0.033676196	0.024782326	0.572175914	0.936975084
CD8xTNF.AlphaxCD56.div.M2xPD.1xPD.L1	0.041607807	-0.137874602	0.026000288	0.562673485	0.911307665

Table 6 (below): List of the NCBI reference sequences or accession numbers from the GenBank database including version numbers for each gene sequence referred to herein. Exemplary probes that target the relevant gene sequences are listed (NSID: unique identifier used by NanoString to track and identify probes comprised of the accession number and the nucleotide position immediately prior to the target region, i.e. target region for CD4 probe is 976-1075).

Gene Name	Accession	Probe NSID	Tot Isoforms	Isoforms Hit by Probe
CD4	NM_000616.4	NM_000616.4:975	5	NM_000616;NM_001195017;NM_001195016;NM_001195014;NM_001195015
CD56	NM_000615.5	NM_000615.5:1620	5	NM_000615;NM_001242608;NM_001242607;NM_001076682;NM_181351
TNF alpha	NM_000594.2	NM_000594.2:1010	1	NM_000594
LMO2	NM_005574.3	NM_005574.3:1415	5	NM_005574;NM_001142315;NM_001142316;XM_005252921;XM_005252920
PD-1/PDCCD1	NM_005018.1	NM_005018.1:175	1	NM_005018
PD-L1/CD274	NM_014143.2	NM_014143.2:684	3	NM_014143;NM_001267706
PD-L2/PDCCD1LG2	NM_025239.3	NM_025239.3:235	6	NM_025239;XM_005251604;XM_005251603;XM_005251602;XM_005251601;XM_005251600
CD163	NM_004244.4	NM_004244.4:1630	5	NM_004244;NM_203416;XM_005253529;XM_005253528;XM_005253527
CD68	NM_001251.2	NM_001251.2:1140	2	NM_001251;NM_001040059
LAG3	NM_002286.5	NM_002286.5:1735	1	NM_002286
TIM3/HAVCR2	NM_032782.3	NM_032782.3:955	1	NM_032782
SYCA3(CCL3)	NM_002983.2	NM_002983.2:681	1	NM_002983
CD137/TNFR9	NM_001561.4	NM_001561.4:255	1	NM_001561

It will be understood that the invention disclosed and defined in this specification extends to all alternative combinations of two or more of the individual features mentioned or evident from the text, figures or drawings. All of these different combinations constitute various alternative aspects of the invention.

CLAIMS

1. A method for providing a prognosis of a subject having diffuse large B-cell lymphoma responding to a treatment regime including an anthracycline and a B-cell depleting antibody, the method comprising:

- 5 determining an immune score for the subject based upon the ratio of a level of any one or more of CD137, CD4, CD8, CD56, TNF α (alpha) and LMO2 in the subject to a level of any one or more of PD-1, PD-L1, CD163, CD68, PD-L2, LAG3, TIM3 and SCYA3(CCL3) in the subject,

comparing the immune score to a reference score;

- 10 wherein the immune score in comparison with the reference score is indicative of the subject's prognosis of responding to a treatment regime including an anthracycline and a B-cell depleting antibody.

2. A method according to claim 1, wherein the reference score is a score above which correlates with an increased probability of recurrence free survival or overall
15 survival of the subject at a later time, and below which correlates with a decreased probability of recurrence free survival or overall survival of the subject at the later time, thereby providing a prognosis of the subject for responding to a treatment regime including an anthracycline and a B-cell depleting antibody.

3. A method for predicting the likelihood of overall survival of a subject having
20 diffuse large B-cell lymphoma if treated with a regime including an anthracycline and a B-cell depleting antibody, the method comprising:

determining an immune score for the subject based upon the ratio of a level of any one or more of CD137, CD4, CD8, CD56, TNF α (alpha) and LMO2 in the subject to a level of any one or more of PD-1, PD-L1, CD163, CD68, PD-L2, LAG3, TIM3 and
25 SCYA3(CCL3) in the subject,

comparing the immune score to a reference score;

wherein the reference score is a score above which correlates with an increased probability of recurrence free survival or overall survival of the subject at a later time, and below which correlates with a decreased probability of recurrence free survival or

overall survival of the subject, thereby providing a prognosis of the subject for responding to a treatment regime including an anthracycline and a B-cell depleting antibody

4. A method according to claim 3, further comprising:

5 analysing levels of at least one of CD137, CD4, CD8, CD56, TNF α (alpha) and LMO2, and at least one of PD-1, PD-L1, CD163, CD68, PD-L2, LAG3, TIM3 and SCYA3(CCL3) in a biological sample obtained from the subject

determining an immune score for the subject based upon the ratio of a level of any one or more of CD137, CD4, CD8, CD56, TNF α (alpha) and LMO2 in the subject to a level

10 of any one or more of PD-1, PD-L1, CD163, CD68, PD-L2, LAG3, TIM3 and SCYA3(CCL3) in the subject,

comparing the immune score to a reference score, wherein the reference score has been derived from a patient cohort of known survival outcome treated with a regime including an anthracycline and a B-cell depleting antibody, and

15 assigning the subject to a risk group based on whether the immune score is higher or lower than the reference score, wherein an immune score higher than the reference score indicates a high likelihood of overall survival and an immune score lower than the reference indicates a low likelihood of overall survival,

thereby predicting the likelihood of overall survival of a subject.

20 5. A method according to any one of claims 1 to 4, wherein the immune score for the subject is based upon the ratio of a level of each of CD137, CD4, CD8, CD56, TNF α (alpha) and LMO2 in the subject to a level of each of PD-1, PD-L1, CD163, CD68, PD-L2, LAG3, TIM3 and SCYA3(CCL3) in the subject.

6. A method according to any one of claims 1 to 4, wherein the immune score for
25 the subject is based upon the ratio of a level of each of CD137, CD4, CD8 and CD56 in the subject to a level of each of PD-1, PD-L1, CD163 and CD68 in the subject.

7. A method according to any one of claims 1 to 4, wherein the B-cell depleting antibody is an anti-CD20 antibody.

8. A method according to any one of claims 1 to 5, wherein the chemotherapy including an anthracycline and a B-cell depleting antibody is R-CHOP (Rituximab, Cyclophosphamide, Hydroxydaunorubicin, Oncovin (vincristine), and Prednisone or Prednisolone), R-CHOP-14, R-CHOP-21, DA-EPOCH-R (dose-adjusted etoposide, 5 doxorubicin, and cyclophosphamide with vincristine, prednisone, and rituximab) or R-ACVBP (rituximab, doxorubicin, cyclophosphamide, vindesine, bleomycin, and prednisone).
9. A method according to claim 8, wherein the chemotherapy is R-CHOP.
10. A method according to claim 4, wherein a high likelihood of overall survival is at 10 least 95%, 94%, 93%, 92%, 91%, 90%, 85%, 80%, 75% or 70%.
11. A method according to claim 4, wherein a low likelihood of overall survival is less than 60%, 55%, 54%, 53%, 52%, 51%, 50%, 49%, 48%, 47%, 46%, 45% or 44%.
12. A method according to any one of claims 1 to 11 wherein the immune score for a subject may be by any one of the ratios listed in Table 3 or 5.
- 15 13. A method according to claim 12, wherein the ratio is one that has a p-value difference between survival curves as shown in Table 3 or 5 of less than 0.05.
14. A method according to claim 12, wherein the ratio is one that has a p-value difference between survival curves as shown in Table 3 or 5 of less than 1E-07.
15. A method according to claim 12, wherein the ratio is one that has a p-value 20 difference between survival curves as shown in Table 3 or 5 of less than 1E-08.
16. A method according to claim 12, wherein the ratio is one that has a p-value difference between survival curves as shown in Table 3 or 5 of less than 1E-9.
17. A method according to claim 12, wherein the ratio is one that has a p-value difference between survival curves as shown in Table 3 or 5 of less than 1E-10.
- 25 18. A method according to claim 12, wherein the ratio is one that has a p-value difference between survival curves as shown in Table 3 or 5 of less than 1E-11.
19. A method according to claim 13, wherein the immune score for a subject is determined using any one of the following ratios:

CD4xCD8.div.M2xPD-L1,

CD4xCD8xCD137.div.M2xPD-1xPD-L1,

CD4xCD8xCD137.div.PD-1xPD-L1,

CD8xCD137.div.M2xPD-1xPD-L1,

5 CD4xCD8.div.M2xPD-1,

CD4xCD8xCD137.div.M2xPD-L1,

CD4xCD8.div.M2xPD-1xPD-L1,

CD56xCD137.div.M2xPD-1xPD-L1,

CD56xCD137.div.M2xPD-1,

10 CD4xCD56xCD137.div.M2xPD-1,

CD4xCD137.div.PD-1xPD-L1,

CD8xCD137.div.PD-1xPD-L1,

CD8xCD137.div.M2xPD-1,

CD8xCD56xCD137.div.M2xPD-1xPD-L1,

15 CD4xCD137.div.M2xPD-1,

CD4xCD8xCD56xCD137.div.M2xPD-1xPD-L1,

CD56xCD137.div.M2xPD-L1,

wherein M2 is (CD163.div.CD68).

20. A method according to claim 13, wherein the immune score for a subject is
20 determined using the ratio CD4xCD8.div.M2xPD-L1.

21. A method according to claim 13, wherein the immune score for a subject is
determined using the ratio
CD4xCD8xCD137.TNFR9xTNF.Alpha $\times$ LMO2.div.M2xSCYA3.CCL3xPD.1xPD.L1.

22. A method according to any one claims 1 to 21, wherein the level of any one of CD137, CD4, CD8, CD56, TNF α (alpha), LMO2, PD-1, PD-L1, CD163, CD68, PD-L2, LAG3, TIM3 and SCYA3(CCL3) is determined from a biological sample derived from the subject.

5 23. A method according to any one claims 1 to 21, wherein the level of any one of CD137, CD4, CD8, CD56, TNF α (alpha), LMO2, PD-1, PD-L1, CD163, CD68, PD-L2, LAG3, TIM3 and SCYA3(CCL3) is determined from information obtained from a previously analyzed biological sample.

24. A method according to claim 22 or 23, wherein the biological sample is selected
10 from the group consisting of formalin fixed embedded tissue or cells, processed tissue, frozen tissue, and a biopsy.

25. A method according to any one of claims 1 to 24, wherein the level of any one of CD137, CD4, CD8, CD56, TNF α (alpha), LMO2, PD-1, PD-L1, CD163, CD68, PD-L2, LAG3, TIM3 and SCYA3(CCL3) is determined by the amount or relative amount of a
15 nucleic acid encoding, or complementary to a nucleic acid encoding, any one of CD137, CD4, CD8, CD56, TNF α (alpha), LMO2, PD-1, PD-L1, CD163, CD68, PD-L2, LAG3, TIM3 and SCYA3(CCL3).

26. A method according to claim 25, wherein the nucleic acid is RNA

27. A method according to claim 26, wherein the RNA is mRNA.

20 28. A method according to any one of claims 1 to 27, wherein the method further comprises a step of conducting a COO, R-IPI and/or IPI analysis.

29. A method according to any one of claims 1 to 28, wherein the method further comprises the step of treating the subject by administering one or more therapeutic agents.

25 30. A method according to claim 29, wherein the therapeutic agents are selected from the group consisting of an anthracycline, anti-CD20 antibody, or any regime including anthracycline and anti-CD20 antibody.

31. A method according to claim 30, wherein the regime including anthracycline and anti-CD20 antibody is R-CHOP.

32. A method according to any one of claims 29 to 31, wherein the subjects treated are those identified with a high immune score.

33. A method according to any one of claims 1 to 28, wherein the method further comprises the step of enrolling the subject identified as having a low immune score in a clinical trial for treatment of DLBCL.

34. A method according to any one of claims 1 to 28, wherein the method further comprises the step of administering a compound that binds to and inhibits PD-1 to a subject identified as having a low immune score.

35. A kit or article of manufacture for providing a prognosis of a subject having diffuse large B-cell lymphoma responding to a treatment regime including an anthracycline and anti-CD20 antibody, the kit comprising one or more nucleic acid primers or probes for determining the level of any of CD137, CD4, CD8, CD56, TNF α (alpha), LMO2, PD-1, PD-L1, CD163, CD68, PD-L2, LAG3, TIM3 and SCYA3(CCL3) for determining an immune score using any one or more of the ratios in Table 3 or 5.

36. A kit or article of manufacture according to claim 35, further comprising written instructions for implementing method according to any one of claims 1 to 34.

37. A kit or article of manufacture according to claim 30 or 31, wherein kit further comprises reagents for quantitative amplification of nucleic acid of a biomarker that can be used to determine an immune score according to a method of any one of claims 1 to

34.

38. A kit or article of manufacture according to any one of claims 35 to 37, wherein the kit is in the form of a microarray.

39. A device for determining levels of any one or more of CD137, CD4, CD8, CD56, TNF α (alpha), LMO2, PD-1, PD-L1, CD163, CD68, PD-L2, LAG3, TIM3 and SCYA3(CCL3) and visual means for reporting (a) an immune score for a subject determined using any one of the ratios listed in Table 3 or 5, and / or (ii) a difference from a reference score determined from a cohort of patients of known treatment outcome.

FIGURE 1

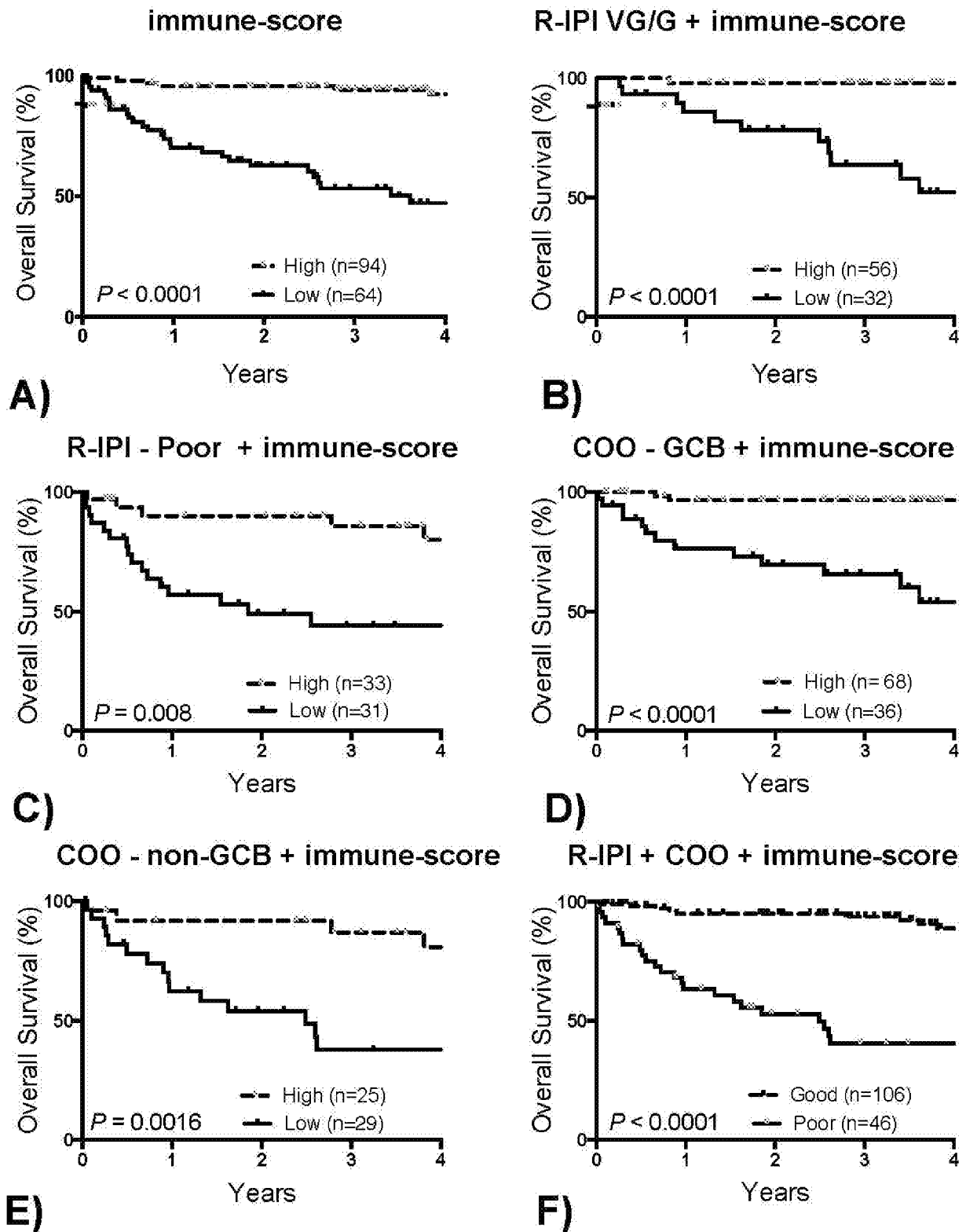


FIGURE 2

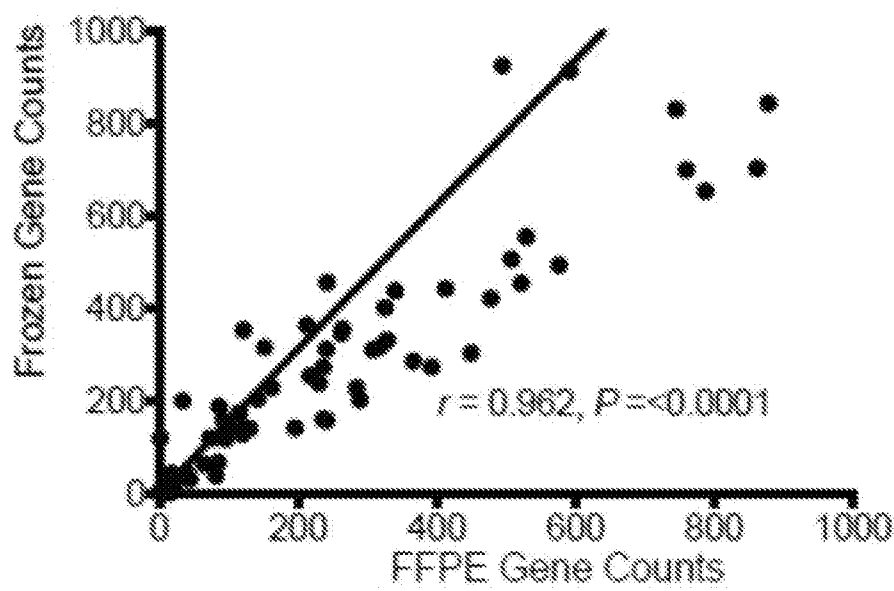
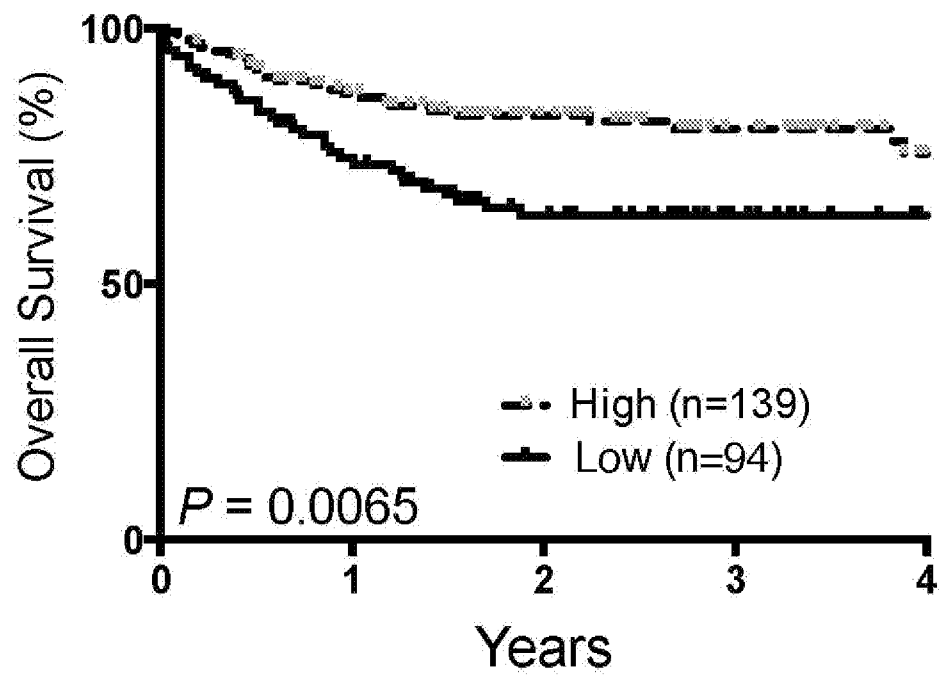


FIGURE 3



INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2016/050118

A. CLASSIFICATION OF SUBJECT MATTER

C12Q 1/68 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPIAP, EPODOC: C12Q, G01N and keywords

CA, Medline, BIOSI: keywords

Keywords: diffuse large B cell lymphoma, anthracycline, rituximab, R-CHOP and similar terms.

PatentScope, PubMed, Internal databases provided by IPAustralia: Applicant/Inventor search.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

☒ Further documents are listed in the continuation of Box C☒ See patent family annex

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
10 May 2016Date of mailing of the international search report
10 May 2016

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INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/AU2016/050118
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014/145843 A2 (COMBIMMUNE, INC) 18 September 2014 pages 1 to 3, examples, whole of document	1-4, 7-11, 22-39
X	Arrayit H25K Whole Human Genome Microarrays [retrieved from internet on 02 May 2016] <URL: https://web.archive.org/web/20150206050840/http://www.arrayit.com/Products/DNA_Microarrays/DNA_Microarray/dna_microarray.html > as captured by the Web Archive on 6 February 2015 whole of document	35-39
A	WO 2013/082105 A1 (ALBERT EINSTEIN COLLEGE OF MEDICINE OF YESHIVA UNIVERSITY) 06 June 2013 whole of document	
A	Malumbres R <i>et al</i> , "Paraffin-based 6-gene model predicts outcome in diffuse large B-cell lymphoma patients treated with R-CHOP", <i>Blood</i> , 2008, 111(12):5509-5514 whole of document	
P,A	Keane C <i>et al</i> , "The T Cell Receptor (TCR) Repertoire Is a Key Determinant of the Tumour Microenvironment (TME) in Diffuse Large B Cell Lymphoma (DLBCL)", 57th Annual Meeting of the American Society of Hematology, 05-08 December 2015, <i>Blood</i> 03 December 2015, 126(23): online abstract [retrieved from internet 04 May 2016] <URL: http://www.bloodjournal.org/content/126/23/3893 > whole of document	
P,X P,A	Keane C <i>et al</i> , "Ratios of T-cell immune effectors and checkpoint molecules as prognostic biomarkers in diffuse large B-cell lymphoma: a population-based study", <i>Lancet Haematology</i> , October 2015, 2(10):e445-455, Epub 1 October 2015 whole of document whole of document	1-34 1-34
Form PCT/ISA/210 (fifth sheet) (July 2009)		

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2016/050118

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
the subject matter listed in Rule 39 on which, under Article 17(2)(a)(i), an international search is not required to be carried out, including
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box for Details

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Supplemental Box**Continuation of: Box III**

This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

This Authority has found that there are different inventions based on the following features that separate the claims into distinct groups:

- Claims 1 to 34 are directed to methods of providing a prognosis or predicting overall survival of a subject having diffuse large B-cell lymphoma responding to a treatment regime including an anthracycline and a B-cell depleting antibody. The methods of prognosis and prediction are specific to this group of claims.
- Claims 35 to 39 are direct to a kit, article of manufacture or a device. While the kit, article of manufacture and device are defined in such a way that they must be suitable for use in the methods of the first invention, they are not limited to the kit, article of manufacture or apparatus only when used in the first invention. In addition, standard prior art items, such as human expression microarrays, fall within the scope of the claims. Claims 35 to 39 are therefore considered to represent additional inventions.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

When there is no special technical feature common to all the claimed inventions there is no unity of invention.

In the above groups of claims, the identified features may have the potential to make a contribution over the prior art but are not common to all the claimed inventions and therefore cannot provide the required technical relationship. Therefore there is no special technical feature common to all the claimed inventions and the requirements for unity of invention are consequently not satisfied *a priori*.

INTERNATIONAL SEARCH REPORT Information on patent family members		International application No. PCT/AU2016/050118	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
WO 2014/145843 A2	18 September 2014	WO 2014145843 A2	18 Sep 2014
		US 2016032398 A1	04 Feb 2016
WO 2013/082105 A1	06 June 2013	WO 2013082105 A1	06 Jun 2013
		US 2014329714 A1	06 Nov 2014
End of Annex			
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001. Form PCT/ISA/210 (Family Annex)(July 2009)			