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(54) **METHOD OF ASSESSING RISK OF PML**

VERFAHREN ZUR BEURTEILUNG EINES PML-RISIKOS

PROCÉDÉ D'ÉVALUATION DU RISQUE DE LEMP

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Description

FIELD OF THE INVENTION

[0001] The invention relates to methods of assessing a patient's risk of developing Progressive multifocal leukoencephalopathy (PML).

BACKGROUND OF INVENTION

[0002] The anti-VLA-4 (Very Late Antigen 4) antibody therapeutic natalizumab is indicated to treat relapsing forms of multiple sclerosis (MS) and moderate-to-severe Crohn's Disease. Natalizumab treatment, however, is associated with an increased risk of progressive multifocal leukoencephalopathy (PML), an opportunistic brain infection caused by the JC virus (JCV). PML occurs primarily in immunocompromised individuals and in patients receiving certain immunomodulatory therapies, including natalizumab. PML is hypothesized to be the result of a complex interaction between host and viral factors, leading to reactivation and mutation of latent archetype JCV to a neurotrophic form which can infect oligodendrocytes in the central nervous system.

[0003] Sandrock et al, 2011, Neurology, 76 (9, suppl 4), A248) relates to the risk stratification for PML in MS patients and the role of prior immunosuppressant use, natalizumab treatment duration and anti-JCV antibody status. Gorelik et al, 2010, Ann Neurol, 68(3), 295-303 relates to anti-JCV antibodies and implications for PML risk stratification. Subramanyam et al, 2011, Neurology, 76 (9, suppl 4), A636-A637 relates to the detection of anti-JCV antibodies prior to and after PML diagnosis in natalizumab treated MS patients. Warnke et al, 2010, March Neurol, (6788), 923-930 relates to the causal factors of natalizumab and PML. Wright et al, 1993, Rev Sci Tech; 12(2):435-50 relates to the standardisation and validation of enzyme-linked immunosorbent assay techniques for the detection of antibody in infectious disease diagnosis. WO 2011/085369 A1 relates to methods and reagents for analyzing samples for the presence of JC virus antibodies, such as a method that includes obtaining a biological sample from a subject, contacting the sample with highly purified viral-like particles (HPVLPs) under conditions suitable for binding of a JCV antibody in the sample to an HPVLP, and detecting the level of JCV antibody binding in the sample to HPVLP. The pathogenesis of JCV infection is described, for example, in the book Polyomaviruses and Human Diseases, edited by Nasimul Ahsan, ISBN: 0-387-29233-0, Chapter 19, pages 266-273.

SUMMARY OF INVENTION

[0004] The invention relates, to an optimized analytically validated, sensitive assay for detecting the presence of JCV antibodies in serum or plasma.

[0005] Accordingly, the invention provides a method of evaluating a patient's risk of developing Progressive Multifocal Leukoencephalopathy (PML), the method comprising:

i) determining, in a serum or plasma sample of the patient, an anti-JC Virus (JCV) antibody titer, wherein the anti-JCV antibody titer is determined by an ELISA assay comprising the following steps:

(a) forming a reaction mixture comprising an aliquot of sample and a substrate on which is disposed Highly Purified Viral-Like Particles (HPVLPs), and

(b) detecting the level of anti-JCV antibody bound to said substrate on which is disposed HPVLPs;

wherein the anti-JCV antibody titer is expressed as an index value, wherein the index value is determined by normalizing an optical density (OD) value of the sample to a cut-off calibrator adjusted to have an nOD of 1, and a positive control is adjusted to have an nOD of 1.3; wherein the cut-off calibrator and positive control comprise a mixture of serum positive for anti-JCV antibodies and serum negative for anti-JCV antibodies, and wherein a negative control comprises anti-JCV antibody negative serum and has an nOD of 0.1;
and

ii) determining the patient to be at high risk of developing PML if the anti-JCV antibody index value is determined to be > 1.5.

[0006] The method may further comprise:

(c) forming a second reaction mixture containing a second aliquot of sample and solution-phase HPVLP, and detecting

the level of unbound anti-JCV antibody in said second reaction mixture, such as by detecting anti-JCV antibody capable of binding with a substrate on which is disposed HPVLP, *e.g.,* a high signal-to-noise HPVLP substrate (as is discussed herein, the method can comprise classifying, or assigning, to the sample, a value indicative of the degree to which incubation with the soluble-phase HPVLP reduces the level of unbound anti-JCV antibody in the second reaction mixture, which value is sometimes referred to herein as inhibition, % inhibition, or the like. This value can be used to evaluate the sample or a patient),
thereby evaluating the level of anti-JCV antibody in a sample.

[0007] In an embodiment the method further comprises:
(d) forming a third reaction mixture containing a third aliquot under conditions where anti-JCV antibodies in the sample are not bound by HPVLP or other antigen, and detecting the level of anti-JCV antibody in the third reaction mixture, such as by detecting anti-JCV antibody capable of binding with a substrate on which is disposed HPVLP, *e.g.,* a high signal-to-noise HPVLP substrate. The inhibition or % inhibition can be calculated as a function of the degree that incubation with soluble-phase HPVLP (step (c)) reduces the amount of unbound anti-JCV antibody, as compared to the result in step (d).

[0008] In an embodiment the method comprises steps (a) and (b), and optionally, providing the results to another entity, *e.g.,* a healthcare provider.

[0009] In an embodiment the method comprises steps (a), (b), and (c), and optionally, providing the results to another entity, *e.g.,* a healthcare provider.

[0010] In an embodiment the method comprises steps (a), (b), (c), and (d), and optionally, providing the results to another entity, *e.g.,* a healthcare provider.

[0011] Methods described herein use optimized levels and amounts of reagents, allowing for improved performance. Thus, in an embodiment, for the first reaction mixture, 20 ngs to 60 ngs, 30 ngs to 50 ngs, 20 ngs to 40 ngs, 35 ngs to 45 ngs of HPVLP are disposed on said substrate. In an embodiment about 20 ngs, 30 ngs, 40 ngs, 50 ngs or 60 ngs of HPVLP are disposed on said substrate. Typically, a multi-substrate device, *e.g.,* a multi-well plate, *e.g.,* a polystyrene multi-well plate, will have an amount of HPVLP specified herein on each of a plurality of substrates. A typical substrate is the interior of a well on a multi-well plate.

[0012] Methods described herein use optimized ratios of reagents and sample, allowing for improved performance. In an embodiment the ratio of μl of sample (this refers to undiluted sample, or the amount of sample in a dilution, so 100 μl of a 1 μl :100 μl dilution would be 1 μl of sample), to ngs of HPVLP disposed on the substrate in the first reaction is: between 1: 100 and 1:20; 1:80 and 1:30; 1:60 and 1:20; 1:20 and 1: 60; 1:30 and 1:50. In an embodiment the ratio of μl of sample, to ngs of HPVLP disposed on the substrate is about: 1:30, 1:40, or 1:50. In an embodiment the ratio of μl of sample, to ngs of HPVLP disposed on the substrate is about: (0.08 to 1.2): 30, (0.08 to 1.2): 40, or (0.08 to 1.2): 50.

[0013] In one embodiment, the sample, *e.g.,* serum, for the first reaction is diluted, such as by about 100-fold, in buffer, for example, prior to contact with the substrate on which is disposed HPVLP, *e.g.,* a high signal-to-noise HPVLP substrate. In one embodiment, detection is with an enzyme labeled antibody, *e.g.,* an enzyme labeled IgG, such as an HRP (Horseradish Peroxidase) labeled IgG. In another embodiment, the detection reagent, *e.g.,* an HRP labeled IgG, is added at a concentration of at least 0.01 μg/mL, 0.02 μg/mL, 0.03 μg/mL, 0.04 μg/mL, 0.05 μg/mL, 0.06 μg/mL, or 0.08 μg/ml. In one embodiment, the detection reagent is provided at 10x to 100x excess over antibody bound to the substrate. In an embodiment the detection reagent is provided, in an amount that gives equal to or more than 10x, 20x, 50x, 75x or 100x) excess as compared to the antibody bound to the substrate.

[0014] In an embodiment the solution-phase HPVLP in (c) is present at 2x to 100x excess particles over anti-JCV antibody in the second reaction mixture or sample. In an embodiment the excess of particles over the anti-JCV antibody in the second reaction mixture or sample is equal to or greater than 2x, 4x, 5x, 10x, 15x, 20x, 40x, 50x, 70x, 80x, 100x or 110x.

[0015] In an embodiment, for the second reaction mixture, 20 ngs to 60 ngs, 30 ngs to 50 ngs, 20 ngs to 40 ngs, 35 ngs to 45 ngs of HPVLP are disposed on said substrate. In an embodiment about 20 ngs, 30 ngs, 40 ngs, 50 ngs or 60 ngs of HPVLP are disposed on said substrate. Typically, a multi-substrate device, *e.g.,* a multi-well plate, *e.g.,* a polystyrene multi-well plate, will have an amount of HPVLP specified herein on each of a plurality of substrates. A typical substrate is the interior of a well on a multi-well plate.

[0016] In an embodiment, for the second reaction mixture, the sample is contacted with the soluble-phase HPVLP and then unbound anti-JVC antibody is allowed to bind to a HPVLP disposed on a substrate. In an embodiment, for the second reaction mixture, the sample is in simultaneous contact with the soluble-phase HPVLP and HPVLP disposed on a substrate.

[0017] In an embodiment the ratio of μl sample (this refers to undiluted sample, or the amount of sample in a dilution, so 100 μl of a 1 μl :100 μl dilution would be 1 μl of sample), to ngs of HPVLP disposed on the substrate is: between 1: 100 and 1:20; 1:80 and 1:30; 1:60 and 1:20; 1:20 and 1: 60; 1:30 and 1:50. In an embodiment the ratio of μl of sample, to ngs of HPVLP disposed on the substrate is about: 1:30, 1:40, or 1:50. In an embodiment the ratio of μl of sample, to

ngs of HPVLP disposed on the substrate is about: (0.08 to 1.2): 30, (0.08 to 1.2): 40, or (0.08 to 1.2): 50.

[0018] In one embodiment, the sample, *e.g.,* serum, is diluted, such as by about 100-fold, in, for example, buffer, prior to contact with the substrate on which is disposed HPVLP, *e.g.,* a high signal-to-noise HPVLP substrate. In one embodiment, detection is with an enzyme labeled antibody, *e.g.,* an enzyme labeled IgG, such as an HRP labeled IgG. In another embodiment, the detection reagent, *e.g.,* an HRP labeled IgG, is added at a concentration of at least 0.01 μg/mL, 0.02 μg/mL, 0.03 μg/mL, 0.04 μg/mL, 0.05 μg/mL, 0.06 μg/mL, or 0.08 μg/ml. In one embodiment, the detection reagent is provided at 10x to 100x excess over antibody bound to the substrate. In an embodiment the detection reagent is provided, in an amount that gives equal to or more than 10x, 20x, 50x, 75x or 100x) excess as compared to the antibody bound to the substrate.

[0019] In one embodiment, responsive to the level of anti-JCV antibodies detected in step (b), steps (c) and/or (d) are performed.

[0020] Responsive to the level of anti-JCV antibodies detected in step (b), *e.g.,* the index level (nOD) is >0.2 and is < 0.4, then steps (c) and (d) can be performed.

[0021] In one embodiment, the serum or plasma sample is diluted, such as by an amount equal to or greater than about 50, 100, or 150 fold, in, *e.g.,* buffer, prior to forming said second reaction mixture. In another embodiment, the sample, is diluted, such as by an amount equal to or greater than about 50-fold, 100-fold, or 150-fold, in, *e.g.,* buffer, prior to forming said third reaction mixture. In another embodiment, detection of one or both of the second and third reaction mixture is with an enzyme labeled antibody, *e.g.,* an enzyme labeled IgG, *e.g.,* an HRP labeled IgG.

[0022] Detection of one or both of the second and third reaction mixtures can be with an HRP labeled IgG, added at a concentration of at least 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, or 0.08 μg/ml. In one embodiment, the detection reagent is provided at 10x to 100x (*e.g.,* 10x, 20x, 50x, 75x or 100x) excess as compared to the antibody bound to the substrate.

[0023] In the invention, evaluating the level of anti-JCV antibody in a sample further includes a cut off calibrator, having an index value of 1.

[0024] In an embodiment the method includes determining the amount that binding to said soluble phase HPVLP particles inhibits or reduces binding to substrate disposed HPVLP particles as compared with binding to substrate disposed HPVLP particles in said first aliquot. The results of the first step of the two-step assay (steps (a) and (b) above), are typically expressed as a normalized OD (nOD, or "index") value. The results of the second step of the two-step assay (steps (c) and optionally (d) above), are typically expressed as "percent inhibition." In an embodiment the nOD is OD_{450}. In an embodiment said inhibition is less than or equal to a predetermined value, *e.g.,* 45 %, and said sample is classified as negative.

[0025] In an embodiment said inhibition is greater than a predetermined value, *e.g.,* 45 % and said sample is classified as positive.

[0026] According to the invention, a cut-off calibrator (CO) is adjusted to have a reactivity index (nOD) of about 1.0, and a positive control (PC) is adjusted to have a reactivity index of about 1.3. The CO and PC solutions are made by mixing a serum positive for JCV antibodies with a serum that is negative for JCV antibodies. For the negative control (NC), which can be, for example, a bottle of anti-JCV antibody-negative sera, the index (nOD) target is about 0.1.

[0027] A HPVLP can be chromatographically purified prior to use in an assay featured in the invention. In one embodiment the sample is a serum sample diluted 1:101 prior to forming the first reaction mixture comprising a first aliquot of the sample and the substrate on which is disposed HPVLPs.

[0028] In another embodiment, the secondary detection reagent *(e.g.,* an anti-human IgG) is conjugated to a detectable agent, such as a peroxidase, such as HRP. In one embodiment, the secondary detection reagent can be anti-human IgG, wherein the anti-human IgG is conjugated to HRP. In another embodiment, the detection reagent solution containing IgG-HRP is used at 0.04 μg/mL. For example, a 0.8 mg/mL stock solution of IgG-HRP is diluted 1:15,000, 1:20,000, 1:30000 or more, prior to use in the assay to detect the level of anti-JCV antibody bound to HPVLP. In another embodiment, the concentration of the secondary detection reagent is adjusted for new lots to match signal to previous lot and the incubation time with the conjugate is only 30 min. In one embodiment, TMB (tetramethylbenzidine) and hydrogen peroxide in buffer are incubated with the reaction mix containing the HRP IgG mixture bound to anti-JCV antibody for 20 minutes, ± 2 minutes.

[0029] A decrease in the detected level in the secondary assay sample compared to the sample that was not preincubated indicates the sample is positive for anti-JCV antibody, and a change in the detected level below a specified percentage indicates that there is no JCV-specific antibody present in the sample.

[0030] The sample can be determined to have an index value (*i.e.,* nOD value) >0.2 and <0.4 (the "indeterminant zone") after the first step of the assay, which is the formation of a first reaction mixture comprising a first aliquot of sample and a substrate on which is disposed HPVLP, *e.g.,* a high signal-to-noise HPVLP substrate, and detecting the level of anti-JCV antibody bound to said substrate on which is disposed HPVLP, *e.g.,* a high signal-to-noise HPVLP substrate. A second aliquot of the sample can then be tested in the second step of the assay, which comprises formation of a second mixture between the second aliquot and a solution-phase HPVLP prior to detecting unbound anti-JCV antibody in the second mixture by contacting the second mixture with a substrate on which is disposed HPVLP, *e.g.,* a high signal-

to-noise HPVLP substrate.

[0031] If the sample is determined to have an index value <0.2 after the first step of the assay, then the sample is determined to be anti-JCV antibody negative. A sample determined to be anti-JCV antibody negative may not be evaluated using the second step of the assay.

[0032] If the sample is determined to have an index value > 0.4 after the first step of the assay, then the sample determined to be anti-JCV antibody positive. A sample determined to be anti-JCV antibody positive may not be evaluated using the second step or the assay.

[0033] The methods disclosed herein are based at least in part on the discovery that anti-JCV antibody titer and other characteristics such as affinity/avidity can be indicators of a patient's risk of developing Progressive Multifocal Leukoencephalopathy (PML).

[0034] Accordingly, the invention features, a method of evaluating a patient's risk of developing PML, comprising acquiring knowledge of a JC Virus (JCV) antibody titer (*e.g.*, determined as described herein and expressed as normalized optical density (nOD) or index) as defined in the claims.

[0035] In one embodiment, an anti-JCV antibody titer or percent inhibition is determined in a biological sample from a patient, such as a blood (serum or plasma), or CSF sample.

[0036] If the titer or/and percent inhibition, or a function of both values is determined to be below a pre-determined level, the patient is determined to be at a lower risk of developing PML, and if the titer and/or percent inhibition, or a function of both values is determined to be at or above the pre-determined level the patient is determined to be at a higher risk of developing PML.

[0037] In one embodiment, the subject has multiple sclerosis, *e.g.*, a multiple sclerosis patient that is already receiving therapy with an anti-VLA-4 antibody, *e.g.*, natalizumab.

[0038] The patient can be determined to be at a higher risk of developing PML, and the patient can be identified as someone who should receive an alternative therapy, *e.g.,* the patient should stop receiving anti-VLA-4 antibody therapy, *e.g.,* natalizumab, and, *e.g.,* receive an alternative therapy, *e.g.,* an alternative approved multiple sclerosis (MS) therapy such as Avonex®. The patient can be determined to be at a higher risk of developing PML, and the patient can be administered an anti-VLA-4 antibody therapy, *e.g.*, natalizumab.

[0039] The patient can be determined to be at a higher risk of developing PML based upon anti-JCV antibody titer or percent inhibition, and the patient can be identified as someone who should receive additional testing to determine risk of developing PML.

[0040] The percent inhibition of anti-JCV antibodies can be measured, for example, by: (i) contacting a biological sample from the subject with HPVLPs in a solution under conditions suitable for binding of an anti-JCV antibody in the sample to an HPVLP; (ii) separating the JCV antibodies bound to HPVLP from the solution to create a secondary sample; (iii) contacting the secondary sample with HPVLP under the same conditions as (i); and (iv) detecting the level of anti-JCV antibody binding to HPVLP in the secondary sample.

[0041] Anti-JCV antibody titer can be measured by a commercial platform, such as a VIDAS® assay (bioMérieux), or another alternative platform, such as a solution-phase method or a lateral flow method.

[0042] If the assay indicates that the biological sample does not contain JCV antibodies, the assay then further includes: (iv) contacting a portion of the biological sample from the subject with HPVLP in a solution prior to step (i) and where the HPVLP of step (i) is attached to a solid substrate, such as to provide a secondary sample; (v) contacting the secondary sample with HPVLP under the same conditions as (i); (vi) detecting the level of anti-JCV antibody binding to HPVLP in the secondary sample; and (vii) comparing the detected level of anti-JCV antibody in the secondary sample to the level of binding in the biological sample when incubated with the solution without HPVLP. A decrease in the detected level in the sample pre-incubated with HPVLP compared to the solution-incubated sample indicates that the sample is positive for an anti-JCV antibody, and no change in the detected level indicates that anti-JCV antibody is not present above background levels in the sample.

[0043] If the assay indicates that the biological sample contains JCV antibodies, the patient can be determined to be at higher risk for PML.

[0044] The patient can be determined to have a lower risk of PML if the anti-JCV antibody titer as indicated by index value or nOD is determined to be < 0.5, the patient can be determined to have a higher risk if the anti-JCV antibody titer as indicated by index value or nOD is determined to be > 0.5 and <1.5. The patient is determined to have an even higher risk if the anti-JCV antibody titer as indicated by index value or nOD is determined to be > 1.5.

[0045] If the assay indicates that the biological sample does not contain JCV antibodies above a background level, the patient can be determined to be at lower risk for PML.

[0046] An anti-JCV antibody assay can be reevaluated for effectiveness at a predetermined interval, such as every 6 months or every year. In one exemplary proficiency assay, a collection of samples, *e.g.*, 30, 40 or 50 serum samples and 30, 40, or 50 plasma samples are provided such as for evaluation by the current optimized method and a preceding earlier-generation method. The concordance between the results is assessed and if the concordance is found to be greater than, *e.g.,* 90% or 95%, the performance of the assay can be determined to be acceptable. A panel of samples,

e.g., containing 90, 100, 150 or more samples, with known anti-JCV antibody status can be utilized to assess consistency of assay performance over time. The concordance between the results is assessed and if the concordance is found to be greater than, for example, 90% or 95%, the performance of the assay can be determined to be acceptable. The panel of samples is patient sera available in sufficient volume to create a sample bank.

[0047] An entity, *e.g.,* a healthcare provider, can acquire information resulting from an anti-JCV antibody assay described herein, and responsive to the information, administer a treatment described herein to the patient, *e.g.,* a MS patient.

[0048] A JCV assay described herein can be performed on a patient, and then the patient can be treated, *e.g.,* the MS patient can be treated, based on the results of the assay.

[0049] The anti-JCV antibody titer or percent inhibition in a patient can be reevaluated at regular intervals, such as every 3 months, every 6 months, or every 12 months or at longer intervals or more frequently. An observed increase in antibody titer or percent inhibition can indicate an increase in the patient's risk of developing PML. For example, an increase of 2 fold or 3 fold in antibody titer (nOD or index) can indicate an increased risk of PML. A patient receiving an anti-VLA-4 therapy, such as a natalizumab, may stop therapy with the anti-VLA-4 therapy, and optionally begin therapy with an alternative agent, *e.g.,* an immunosuppressant other than an anti-VLA-4 therapy, or other than natalizumab. An increase in titer may present differently in patients having a high baseline titer *(e.g.,* at a more narrow range in range of titer) than in patients having a low baseline titer.

[0050] In one embodiment, a patient receiving an anti-VLA-4 antibody, *e.g.*, natalizumab, can be monitored, *e.g.*, every five, six, seven, eight, nine, ten, eleven, twelve, fifteen, twenty, thirty, forty months, for anti-JCV antibody titer and/or percent inhibition.

[0051] In one embodiment, a patient is not re-evaluated for the presence of JCV antibodies, or for anti-JCV antibody titer or percent inhibition within one or two or three weeks after having received plasmapheresis. In another embodiment, a patient is not re-evaluated for the presence of JCV antibodies, or for anti-JCV antibody titer or percent inhibition within one or two or three weeks after having received intravenous immunoglobulin (IVIG) treatment.

[0052] The measure of anti-JCV antibody titer is in terms of nOD or an index value.

[0053] Evaluation of a patient as described herein can be conducted prior to administration of an anti-VLA-4 therapy, or after the patient has begun an anti-VLA-4 therapy.

[0054] The patient can be monitored at regular intervals, *e.g.*, every 3 months, every 6 months, every year, or more or less frequently, for a decrease in anti-JCV antibody titer or a decrease in percent inhibition of JCV antibodies. A decrease in anti-JCV antibody titer or a decrease in percent inhibition of JCV antibodies can indicate that the patient has a lowered risk of developing PML.

[0055] After a patient is determined to be at a higher risk of PML, then the patient may not tested for JCV status again. For example, the patient can stop therapy with an anti-VLA-4 therapy such as natalizumab, and not be tested again for anti-JCV antibody status.

[0056] In one embodiment, a method of evaluating a patient as described herein, such as to determine an anti-JCV antibody titer or percent inhibition, can further include assessing other measures of risk predictors. For example, a method of evaluating a patient can further include: (a) determining if the patient has received extended treatment with an anti-VLA-4 therapy *(e.g.,* longer than 24 months); or (b) determining if the patient has received a specified non-anti-VLA-4 immunosuppressant therapy *(e.g.,* mitoxantrone or other therapies in the last 2, 3, 5 years or ever in the patient's life). The relative risk of PML for a patient who has an anti-JCV antibody titer or percent inhibition above a pre-determined level but has no specified prior immunosuppressant use and has not had an extended treatment with an anti-VLA-4 therapy is less than the relative risk of a patient who has an anti-JCV antibody titer or percent inhibition below a pre-determined level and has specified prior immunosuppressant use or an extended treatment with an anti-VLA-4 therapy, which is less than the relative risk of a patient who has an anti-JCV antibody titer or percent inhibition above a pre-determined level and has specified prior immunosuppressant use and extended treatment with an anti-VLA-4 therapy.

[0057] In some embodiment, factors to be included in the stratification model are the patient's age or gender.

[0058] Method described herein can incorporate one or more factors into the evaluation of the patient.

[0059] The method can include, for example, acquiring or determining a JC Virus (JCV) antibody titer and percent inhibition in a biological sample from the patient, *e.g.*, by a method described herein. If the antibody titer or percent inhibition is determined to be below a pre-determined level, then the patient can be classified as being suitable for treatment with a first category of therapy, such as an anti-VLA-4 therapy, *e.g.,* natalizumab. If the antibody titer or percent inhibition is determined to be at or above the pre-determined level the patient is classified as being suitable for a second category of therapy, *e.g.*, interferon, glatiramer acetate or a corticosteroid. Acquiring an anti-JCV antibody titer and percent inhibition in a sample of a patient may include removing a biological sample from the patient's body or analyzing a sample from the patient. The method of evaluation may also include administering a therapy, such as from the first category (*e.g.,* natalizumab) or the second category (*e.g.,* interferon, glatiramer acetate or a corticosteroid), to the patient.

[0060] The patient is determined to have a higher risk of PML if, (i) the anti-JCV antibody titer as indicated by index value or nOD is determined to be > 1.5 and the percent inhibition value is determined to be > 70%,

[0061] As discussed above, methods of evaluating a patient can incorporate more than one consideration or factor.

Thus, methods of evaluating a patient can further include:

(aa) determining if the patient has received extended treatment with an anti-VLA-4 therapy (*e.g.,* longer than 24 months) and providing a prior anti-VLA-4 therapy exposure classification; or

(bb) determining if the patient has received a specified non-anti-VLA-4 immunosuppressant therapy (*e.g.,* in the last 2, 3, 5 years or ever in the patient's life), and providing a prior immunosuppressive exposure classification.

[0062] Typically, a patient who has an anti-JCV antibody titer or percent inhibition above a pre-determined level but has no specified prior immunosuppressant use and has not had an extended treatment with an anti-VLA-4 therapy is classified as having less risk of developing PML than the relative risk of a patient who has an anti-JCV antibody titer or percent inhibition below a pre-determined level and has specified prior immunosuppressant use or an extended treatment with an anti-VLA-4 therapy, which is less than the relative risk of a patient who has an anti-JCV antibody titer or a percent inhibition above a pre-determined level and has specified prior immunosuppressant use and extended treatment with an anti-VLA-4 therapy.

[0063] In one embodiment, the patient has previously received an anti-VLA-4 therapy. In another embodiment, the method includes administering an anti-VLA-4 therapy, *e.g.,* natalizumab to the patient.

[0064] In one embodiment, the patient is classified as a candidate for anti-VLA-4 therapy, and the patient is further administered the anti-VLA-4 therapy.

[0065] Patients who have received an anti-VLA-4 therapy, such as natalizumab for 24 months or less, who have not previously received an immunosuppressant therapy (other than anti-VLA-4 therapy), and who test negative for exposure to JCV *(e.g.,* negative for JCV antibodies) typically have the lowest risk for developing PML. Conversely, patients who received anti-VLA-4 therapy for longer than 24 months, who have previously received an immunosuppressant therapy (other than an anti-VLA-4 therapy), and who test positive for exposure to JCV (*e.g.*, positive for JCV antibodies) typically have the highest risk for developing PML.

[0066] A patient's risk level for PML can be assessed by evaluating one, or any two or all three of the identified risk factors. For example, a patient, *e.g.,* a patient with multiple sclerosis (MS) who tests negative for anti-JCV antibody titer can be determined to be at a lower risk for PML. A patient at a lower risk for PML can have a risk of less than about 0.2/1000 patients, *e.g.*, $\leq$0.11/1000.

[0067] A patient, *e.g.,* a patient with MS, who has received an anti-VLA-4 therapy, such as natalizumab, for 24 months or less *(e.g.,* for 23 months, 22 months, 20 months, 15 months, 12 months, 6 months, 1 month or less), and who has not previously received an immunosuppressant therapy can be determined to be at a lower risk for PML. For example, the patient can be determined to have a risk of PML of about 0.54/1000 patients. The patient can accordingly be determined to be a candidate to receive further treatment with an anti-VLA-4 therapy, such as natalizumab.

[0068] A patient who has received an anti-VLA-4 therapy, such as natalizumab, for longer than 24 months, such as for about 25 to 48 months or more *(e.g.,* 26, 28, 30, 36, 40, or 48 months or more), and who has not previously received an immunosuppressant therapy can be determined to be at, or classified as having, a higher risk for PML. A patient at a higher risk of PML can have a risk of $\geq$ about 3.7/1000 patients, *e.g.*, about 1.37/1000 patients. The patient can accordingly be determined to be a candidate to receive further treatment with an anti-VLA-4 therapy, such as natalizumab.

[0069] A patient who has received an anti-VLA-4 therapy, such as natalizumab, for 24 months or less *(e.g.,* for 24 months, 22 months, 20 months, 15 months, 12 months, 6 months, 1 month or less), and who is determined to be negative for anti-JCV antibodies, or JCV nucleic acid, can be determined to be at, or classified as having, a lower risk for PML. For example, the patient can be determined to be at a risk of $\leq$0.2/1000 patients. The patient can accordingly be determined to be a candidate to receive further treatment with an anti-VLA-4 therapy, such as natalizumab.

[0070] A patient who has not received prior treatment with an immunosuppressant (other than an anti-VLA-4 therapy), and who is determined to be negative for JCV, can be determined to be at a lower risk for PML, *e.g.,* $\leq$0.2/1000 patients. The patient can accordingly be determined to be a candidate to receive further treatment with an anti-VLA-4 therapy, such as natalizumab.

[0071] A patient who has received an anti-VLA-4 therapy, such as natalizumab, for longer than 24 months (*e.g.*, for 25 months, 26 months, 28 months, 30 months, 35 months, 38 months, 40 months, 48 months or longer), and who has previously received an immunosuppressant therapy other than an anti-VLA-4 therapy can be determined to be at a higher risk for PML. A patient at a higher risk for PML can have a risk of about 0.37/1000 or greater, *e.g.,* about 4.3/1000 patients. The patient can accordingly be determined not to be a candidate to receive further treatment with an anti-VLA-4 therapy, such as natalizumab, or can be determined to be a candidate to receive treatment with an anti-VLA-4 therapy accompanied by more frequent monitoring. For example, a patient at higher risk for PML who receives therapy with an anti-VLA-4 therapy can receive more frequent monitoring for development of PML, then a patient at lower risk of PML.

[0072] A patient who has received an anti-VLA-4 therapy, such as natalizumab, for 24 months or less *(e.g.,* for 24 months, 22 months, 20 months, 15 months, 12 months, 6 months, 1 month or less), and who has previously received an immunosuppressant therapy other than an anti-VLA-4 therapy can be determined to be at a higher risk for PML. For

example, the patient can be determined to have a risk of PML of 0.66/1000 patients. The patient can accordingly be determined not to be a candidate to receive further treatment with an anti-VLA-4 therapy, such as natalizumab, or can be determined to be a candidate to receive treatment with an anti-VLA-4 therapy accompanied by more frequent monitoring. For example, a patient at higher risk for PML who receives therapy with an anti-VLA-4 therapy can receive more frequent monitoring for development of PML, then a patient at lower risk of PML.

[0073] A patient who has received an anti-VLA-4 therapy, such as natalizumab, for longer than 24 months (*e.g.*, for 25 to 48 months, such as 26, 30, 36, 42 or 48 months or longer), and who is determined to be positive for JCV, can be determined to be at a higher risk for PML. The patient can accordingly be determined not to be a candidate to receive further treatment with an anti-VLA-4 therapy, or can be determined to be a candidate to receive treatment with an anti-VLA-4 therapy accompanied by more frequent monitoring.

[0074] In an embodiment, a patient who has received an anti-VLA-4 therapy, such as natalizumab, for longer than 24 months (*e.g.*, for 25 to 48 months, such as 26, 30, 36, 42 or 48 months or longer), and who has not received prior treatment with an immunosuppressant (other than an anti-VLA-4 therapy), and who is determined to be positive for JCV, and is determined to be at a higher risk for PML. For example, the patient can be determined to have a risk of PML of 4/1000 patients. The patient can accordingly be determined not to be a candidate to receive further treatment with an anti-VLA-4 therapy, or can be determined to be a candidate to receive treatment with an anti-VLA-4 therapy accompanied by more frequent monitoring.

[0075] A patient, *e.g.,* an MS patient, who has received prior treatment with an immunosuppressant other than an anti-VLA-4 therapy, and who is determined to be positive for anti-JCV antibodies, or JCV nucleic acid, can be determined to be at a higher risk for PML. The patient can accordingly be determined not to be a candidate to received further treatment with an anti-VLA-4 therapy, or can be determined to be a candidate to receive treatment with an anti-VLA-4 therapy accompanied by more frequent monitoring.

[0076] A patient who has received prior treatment with an immunosuppressant other than an anti-VLA-4 therapy, and who is determined to be positive for anti-JCV antibodies, or JCV nucleic acid, and who has received an anti-VLA-4 therapy, such as natalizumab, for longer than 24 months (*e.g.*, for 25 to 48 months, such as 26, 30, 36, 42 or 48 months or longer) can be determined to be at a higher risk for PML. For example, the patient can be determined to have a risk of 9.8/1000 patients. The patient can accordingly be determined not to be a candidate to received further treatment with an anti-VLA-4 therapy, or can be determined to be a candidate to receive treatment with an anti-VLA-4 therapy accompanied by more frequent monitoring.

[0077] A patient who has received an anti-VLA-4 therapy, such as natalizumab, for 24 months or less *(e.g.,* for 24 months, 22 months, 20 months, 15 months, 12 months, 6 months, 1 month or less), and who has received prior treatment with an immunosuppressant other than an anti-VLA-4 therapy, and who is determined to be positive for JCV, can be determined to be at a higher risk for PML. For example, the patient can be determined to have a risk of PML of 4.5/1000 patients. The patient can accordingly be determined not to be a candidate to receive further treatment with an anti-VLA-4 therapy, or can be determined to be a candidate to receive treatment with an anti-VLA-4 therapy accompanied by more frequent monitoring.

[0078] A patient who has received an anti-VLA-4 therapy, such as natalizumab, for 24 months or less *(e.g.,* for 24 months, 22 months, 20 months, 15 months, 12 months, 6 months, 1 month or less), and who has not received prior treatment with an immunosuppressant (other than an anti-VLA-4 therapy), and who is determined to be positive for JCV, can be determined to be at a higher risk for PML. For example, the patient can be determined to have a risk of PML of 0.35/1000 patients. The patient can accordingly be determined not to be a candidate to receive further treatment with an anti-VLA-4 therapy, or can be determined to be a candidate to receive treatment with an anti-VLA-4 therapy accompanied by more frequent monitoring.

[0079] A patient determined to have a lower risk of developing PML can be determined to have a risk of $\leq$ about 0.54/1000 patients, *e.g.*, $\leq$ 0.25/1000, $\leq$ 0.2/1000, 0.19/1000, $\leq$ 0.15/1000, $\leq$ 0.11/1000, $\leq$ 0.1/1000, *e.g.,* 0.3/1000, 0.25/1000, 0.2/1000, 0.19/1000, 0.15/1000, 0.11/1000, or 0.1/1000 or lower. A patient determined to have a higher risk of PML can be determined to have a risk of about 0.54/1000 or greater, *e.g.,* about 0.55/1000, about 0.60/1000, about 0.66/1000, about 1.2/1000, about 1.37/1000, about 2.0/1000, about 2.5/1000, about 3.0/1000, about 4.3/1000, about 5.0/1000, about 7.8/1000, about 8.0/1000, or higher. For example, a patient determined to have a higher risk of PML can be determined to have a risk of 0.3/1000, 0.35/1000, 0.5/1000, 0.66/1000, 1.2/1000, 1.37/1000, 2.0/1000, 2.5/1000, 3.0/1000, 4.3/1000, 5.0/1000, 7.8/1000, 8.0/1000 or higher.

[0080] A patient who has received prior treatment with an anti-VLA-4 therapy for longer than 24 months, and who has not received prior therapy with an immunosuppressant other than anti-VLA-4 therapy, and who is determined to be JCV negative, can be determined to be at lower risk of developing PML, and therefore a suitable candidate to receive further treatment with an anti-VLA-4 therapy, such as natalizumab. However, due to having received anti-VLA-4 therapy for longer than 24 months, the risk assessment can include a recommendation to monitor the patient more frequently for the development of adverse symptoms, such as symptoms that may indicate the development of PML.

[0081] Enhanced monitoring of patients for the development of PML can include increased frequency of tests to identify

the presence of JCV, *e.g.*, increased testing by anti-JCV antibody assays or nucleic acid-based assays. Enhanced monitoring can also include MRI scans to identify brain lesions due to PML.

[0082] A patient who has anti-JCV antibodies at less than a preselected criterion can have an undetectable level of anti-JCV antibodies. The patient may have previously received an anti-VLA-4 therapy, and the patient may not have previously received an anti-VLA-4 therapy.

[0083] The patient can be classified as a candidate for anti-VLA-4 therapy, and an anti-VLA-4 therapy, *e.g.*, natalizumab, can be administered to the patient.

[0084] If the patient is classified as a candidate for anti-VLA-4 therapy, the patient can be further administered an anti-VLA-4 therapy. A patient classified as a candidate for anti-VLA-4 therapy is determined to have a lower risk for developing PML, *e.g.,* a risk of less than about 0.2/1000 patients, e.g., 0.3/1000 patients, or 0.2/1000 patients or 0.19/1000 patients or 0.11/1000 patients. For example, a patient having a lower risk of PML can have a risk of ≤0.2/1000.

[0085] A patient not classified as a candidate for anti-VLA-4 therapy, or determined to be a candidate for anti-VLA-4 therapy with enhanced monitoring for development of PML, is determined to have a higher risk for developing PML, *e.g.,* a risk of greater than or equal to about 0.3.7/1000 patients. For example, a patient determined to have a higher risk of PML can have a risk of 0.37/1000, 0.35/1000, 0.66/1000, 1.2/1000, 1.37/1000, 2.5/1000, 4.3/1000, or 7.8/1000 patients.

[0086] A prior immunosuppressant exposure classification, if selected, is one of the following:

a positive prior immunosuppressant exposure classification that corresponds to having received a non-anti-VLA-4 immunosuppressant therapy within a preselected time period, *e.g.,* within 1, 3, or 5 years, or in the patient's lifetime; and

a negative prior immunosuppressant exposure classification that corresponds to being free of a non-anti-VLA-4 immunosuppressant therapy for a preselected time period, *e.g.,* within 1, 3, or 5 years, or in the patient's lifetime.

[0087] A prior VLA-4 therapy exposure classification, if selected, is one of the following:

a positive prior VLA-4 therapy exposure classification that corresponds to having received an anti-VLA-4 therapy for more than a preselected period of time, *e.g.,* as much or more than 1, 2, 3, or 5 years; and

a negative prior VLA-4 therapy exposure classification that corresponds to having received an anti-VLA-4 therapy for less than a preselected period of time, *e.g.,* less than 6 months, 1, 2, 3, or 5 years

[0088] A treatment suitability classification can be selected from one of:

a positive treatment suitability classification that is correlated with suitability of the patient for anti-VLA-4 treatment (the positive treatment suitability classification can be further subdivided into positive treatment suitability classifications that are accompanied by various warnings or requirements for monitoring, such as increased monitoring for development of PML); and

a negative treatment suitability classification that is correlated with unsuitability of the patient for anti-VLA-4 treatment, or suitability of the patient for anti-VLA-4 treatment, accompanied by various warnings or requirements for increased monitoring, such for development of PML.

[0089] A positive treatment suitability classification correlates with a lower risk of developing PML, and a negative treatment suitability classification correlates with a higher risk of developing PML. A lower risk of developing PML typically corresponds to a risk less than 0.2/1000 patients, and a higher risk of developing PML corresponds to a risk of ≥0.37/1000.

[0090] If the patient is assigned a low exposure classification, and a negative JCV status classification, the patient is assigned a positive treatment suitability classification, *e.g.,* a modified positive treatment suitability classification that advises or requires monitoring for development of PML.

[0091] If the patient is assigned a negative prior immunosuppressant exposure classification, and a negative anti-JCV antibody status classification, the patient can be assigned a positive treatment suitability classification, *e.g.,* a modified positive treatment suitability classification that advises or requires monitoring for development of PML.

[0092] If the patient is assigned a low exposure classification, a negative prior immunosuppressant exposure classification, and a negative JCV antibody classification, the patient is assigned a positive treatment suitability classification.

[0093] If the patient is assigned a positive treatment suitability classification, the patient can be further administered an anti-VLA-4 therapy, *e.g.,* natalizumab.

[0094] The assay for the presence of anti-JCV antibodies is an ELISA assay. An assay for JCV nucleic acid can be, *e.g.,* a PCR assay or a Next Generation Sequencing (NGS) method.

[0095] A patient determined to be at lower risk for PML can further be administered an anti-VLA-4 therapy, such as natalizumab. A patient determined to be at higher risk for PML can further be administered an alternative to an anti-VLA-4 therapy, such as an interferon, glatiramer acetate, a corticosteroid or a TNF agonist. A patient determined to be at

higher risk for PML can be further administered an anti-VLA-4 therapy, and can be required to receive an increased frequency of testing for PML, and where the patient is initially determined to be JCV negative, can also be required to received an increased frequency of testing for JCV.

[0096] Treatment can include *e.g.*, determining the patient's prior exposure to an anti-VLA-4 therapy, and determining whether the patient previously received treatment with an immunosuppressant. Optionally, the patient's status for JCV can also be determined.

[0097] If the patient is determined to have received the anti-VLA-4 therapy for 24 months or less, and not to have previously received treatment with an immunosuppressant, then the patient is determined to be at lower risk for PML, and the patient is administered the anti-VLA-4 therapy. If the patient is determined to have received natalizumab for longer than 24 months (*e.g.*, 25 months or longer), and not to have previously received treatment with an immunosuppressant, then the patient is determined to be at higher risk for PML, and the patient is administered an alternative to anti-VLA-4 therapy, *e.g.,* an interferon, a corticosteroid, a statin or a TNF antagonist.

[0098] Determining the patient's prior exposure to an anti-VLA-4 therapy or an immunosuppressant can include asking the patient or a caregiver, *e.g.,* a physician, nurse, parent or other caregiver. In some cases, determining the patient's prior exposure can include accessing the information in a database, *e.g.,* a database of medical records.

[0099] A method of determining a patient's risk for PML includes determining the patient's previous exposure to an anti-VLA-4 therapy, and determining whether the patient previously received treatment with an immunosuppressant. Optionally, the patient's anti-JCV antibody status may also be determined. If the patient is determined to have received an anti-VLA-4 therapy for 24 months or less, and not to have previously received treatment with an immunosuppressant, then the patient is determined to be at lower risk for PML. If the patient is determined to have received anti-VLA-4 therapy for longer than 24 months, and not to have previously received treatment with an immunosuppressant, then the patient is determined to be at higher risk for PML. A patient determined to be at lower risk for PML may further be administered an anti-VLA-4 therapy, *e.g.,* natalizumab. Conversely, a patient determined to be at higher risk for PML may further be administered an alternative to anti-VLA-4 therapy, *e.g.,* an interferon, a corticosteroid, a statin or a TNF antagonist.

[0100] The patient's JCV status can be also determined, and if the patient is determined to be JCV negative, then the patient is determined to be at a lower risk for PML than if the patient was determined to be JCV positive.

[0101] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, suitable methods and materials are described below. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

[0102] The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

BRIEF DESCRIPTION OF DRAWINGS

[0103]

FIGs. 1A and 1B are graphs depicting natalizumab-associated PML incidence by cumulative treatment duration (FIG. 1A) and by 12-month treatment interval duration (FIG. 1B).

FIG. 2 is a schematic diagram depicting the approximate incidence of PML stratified by prior immunosuppressant use and natalizumab treatment duration.

FIG. 3 is a schematic diagram depicting the approximate incidence of PML stratified by anti-JCV antibody sero status, prior immunosuppressant use, and natalizumab treatment duration.

FIGs. 4A and 4B are graphs depicting sensitivity analyses of PML incidence estimates in anti-JCV antibody positive patients, stratified by prior immunosuppressant use (yes or no) and natalizumab treatment duration (1-24 months (FIG. 4A) or 25-48 months (FIG. 4B)). Base= Base case scenario.

FIGs. 5A and 5B are graphs depicting nODs and titers, respectively, of patient 1.

FIGs. 6A and 6B are graphs depicting nODs and titers, respectively, of patient 2.

FIGs. 7A and 7B are graphs depicting nODs and titers, respectively, of patient 3.

FIGs. 8A and 8B are graphs depicting nODs and titers, respectively, of patient 4.

FIGs. 9A and 9B are graphs depicting nODs and titers, respectively, of patient 5.

FIGs. 9C and 9D are graphs depicting nODs and titers, respectively, of patient 6.

FIGs. 10A and 10B are graphs depicting nODs and titers, respectively, of patient 7.

FIG. 11 is a scatter plot depicting index (x axis) and percent inhibition (y axis) data collected for a group of MS patients.

FIG. 12 is a scatter plot depicting index (x axis) and percent inhibition (y axis) data collected for a group of MS patients.

FIG. 13 is a scatter plot depicting index values determined for a group of MS patients.

DETAILED DESCRIPTION

[0104] The invention is based, at least in part, on the discovery of new and improved methods of assessing the risk of a patient for PML that include assessing anti-JCV antibody titers or percent inhibition. The invention is based at least in part on the discovery that anti-JCV antibody titer and percent antibody inhibition can be an indicator of a patient's risk of developing Progressive Multifocal Leukoencephalopathy (PML).

[0105] Applicants have also developed an optimized assay for determining anti-JCV antibody titer levels in a biological sample, and a method for assaying the antibodies qualitatively by determining percent inhibition values, and using this information to determine the risk of a patient for developing PML. The assay includes: (a) forming a first reaction mixture comprising a first aliquot of a sample and a substrate on which is disposed HPVLPs, where, the VLP particles are present at an amount of 0.04μg, and a concentration of 0.4 μg/mL; b) detecting the level of anti-JCV antibody bound to HPVLP disposed on the substrate, such as by detecting a labeled secondary detection reagent, *e.g.,* an enzyme labeled anti-IgG antibody, bound to anti-JCV antibody bound to said substrate; (c) forming a second reaction mixture comprising a second aliquot of sample with solution-phase HPVLP provided at a concentration of, *e.g.,* 0.4 μg/mL, and a second aliquot of sample provided at, *e.g.,* a 1:100 or 1:101, dilution; (d) forming a third reaction mixture comprising a negative control solution containing no HPVLP, and a third aliquot of sample diluted, e.g., 1:100 or 1:101, or 1:110 in the negative control solution; (e) detecting the level of unbound anti-JCV antibody in the second and third reaction mixtures, such as by detecting JCV capable of binding a substrate on which is disposed HPVLPs, where said HPVLP is present; (f) providing a first value, which corresponds to the level of anti-JCV antibody binding to HPVLP disposed on substrate in the first aliquot of sample, and a second value, which corresponds to the level of unbound anti-JCV antibody in the second reaction mixture, e.g., the level anti-JCV antibody that binds to HPVLP disposed on a substrate from said second reaction mixture; and (g) optionally, comparing the first and second antibody levels.

[0106] Applicants have also discovered that a patient has a lower risk of developing PML if, (i) the anti-JCV antibody titer as indicated by index value or nOD is determined to be < 0.5, or (ii) the anti-JCV antibody titer as indicated by index value or nOD is determined to be > 0.5 and < 3.0, and the percent inhibition is determined to be less than or equal to 70%. The patient has a higher risk of PML if, (i) the anti-JCV antibody titer as indicated by index value or nOD is determined to be > 3 and the percent inhibition value is determined to be > 70%, or (ii) the patient showed an increase in index, nOD or titer by 2-fold from a previous test.

[0107] A patient can be monitored at regular intervals, such as every 6 months or every 12 months for a change in anti-JCV antibody titer or percent inhibition. If the results of this later assay indicate that the patient still has an anti-JCV antibody titer of nOD less than 0.5, and a percent inhibition of <70%, then the patient can be determined to still be at a lower risk for developing PML. If a later assay indicates that the patient's antibody titer is increased by 2 to 3 fold from the initial assay, then the patient can be determined to be at increased or higher risk for developing PML. Applicants observed patients diagnosed with PML tend to demonstrate an increase in antibody titer and nOD by 2 to 3 fold in the six months prior to diagnosis.

[0108] A patient has a higher risk of PML if, (i) the anti-JCV antibody titer as indicated by index value or nOD is determined to be > 3 and the percent inhibition value is determined to be > 70%, or (ii) the patient showed an increase in index, nOD or titer by 2-fold from a previous test.

[0109] A patient satisfying these criteria can, optionally, be determined not to be a candidate to receive therapy with an anti-VLA-4 therapy, such as an anti-VLA-4 antibody, *e.g.,* natalizumab, or the patient can further be assessed for other risk factors of developing PML. These risk factors include whether or not the patient has previously received an anti-VLA-4 therapy, such as natalizumab, and for how long the patient has received the therapy; and whether and for how long the patient has previously received an immunosuppressant therapy other than an anti-VLA-4 therapy. A patient's risk of PML is a combination of each of these factors.

[0110] Antibody titer is herein measured by "nOD" or "index." "nOD" is the normalized optical density value in a test, such as an ELISA test, for anti-JCV antibody detection.

[0111] Applicants previously discovered that patients who received an anti-VLA-4 therapy, such as natalizumab, for 24 months or less, and who have not previously received an immunosuppressant therapy, are at lower risk for developing PML, than patients who do not meet these two criteria. Further, patients who have the lowest risk are those who meet these two criteria, and who are also JCV negative, *e.g.,* patients who do not test positive for anti-JCV antibodies or JCV nucleic acid, *e.g.,* JCV DNA. It was previously unknown that each of these three risk factors ((i) the amount of time the patient has previously received an anti-VLA-4 therapy; (ii) whether or not a patient has previously received treatment with an immunosuppressant other than an anti-VLA-4 therapy; and (iii) JCV status) independently contribute to a patient's risk of PML. The inventions described herein can be used in general for patients treated with a VLA-4 inhibitor. The ability to identify subpopulations of patients at distinctly different PML risks allows for better characterization of risk than previous methods (*i.e.,* overall PML risk) and should assist healthcare professionals and patients in making more informed benefit-risk treatment decisions. These risk assessment criteria are described in co-owned U.S. provisional applications 61/491,810, filed May 31, 2011, and 61/508584, filed July 15, 2011. The risk criteria described herein directed to anti-

JCV antibody titer (*e.g.,* as measured by nOD or index level) and percent inhibition can be considered in combination with the risk factors described in the prior co-owned provisional applications.

[0112] The methods for determining PML risk can require acquiring one, two or all three of a JCV classification for a patient (*e.g.,* anti-JCV antibody titer, such as measured by nOD or index level and percent inhibition), prior anti-VLA-4 therapy history for the patient, and prior immunosuppressant therapy history (other than anti-VLA-4 therapy) for the patient. Responsive to these classifications, a patient can be assigned a treatment suitability classification. Patients who are determined to have low risk of developing PML can be assigned a positive treatment classification, and patients who are determined to have a higher relative risk of developing PML can be assigned a negative treatment classification. A patient who receives a positive treatment classification can receive a recommendation for further treatment or for initiating treatment with an anti-VLA-4 therapy. A patient receiving a negative treatment classification may receive a recommendation to terminate treatment with an anti-VLA-4, a recommendation to initiate treatment with a non-anti-VLA-4 therapy, a recommendation for continuing or initiating anti-VLA4 therapy with increased surveillance for signs and symptoms of PML.

[0113] A recommendation for further treatment with an anti-VLA-4 therapy may be accompanied with further instructions or requirements that the patient receive additional or enhanced monitoring, such as if one or more factors indicate that the patient may be at an increased risk of PML, *e.g.,* prior treatment with an anti-VLA-4 therapy for longer than 24 months, *e.g.,* 25 months or longer, or prior treatment with an immunosuppressant other than an anti-VLA-4 therapy.

[0114] A patient can be determined to have previously received an anti-VLA-4 therapy or an immunosuppressant therapy other than an anti-VLA-4 therapy through self-reporting by the patient, or through information (verbal or written) provided by a parent, physician, physician's assistant, nurse or other healthcare provider. The information can also be obtained through a database, such as a medical database or a clinical trials database.

[0115] Prior immunosuppressant therapies, other than anti-VLA-4 therapy, that will be indicative of an increased risk of PML can include prior treatment with antineoplastics, immunosuppressants or immunomodulators, such as one or more beta-interferon or glatiramer acetate. Exemplary immunosuppressants include, *e.g.,* mitoxantrone, methotrexate, azathioprine, cyclophosphamide, and mycophenolate, anti-CD20 therapy *(e.g.,* rituximab), an anti-CD11a therapy *(e.g.,* efalizumab), or mycophenolate mofetil. Prior treatment with other immunosuppressant therapies as described below will also be predicted to increase a patient's risk of PML following further administration of an anti-VLA-4 therapy. In general, a determination of prior immunosuppressant use is a specified use which can be any prior use of an immunosuppressant that is not a VLA-4 inhibitor (*e.g.,* an anti-VLA-4 antibody) or prior use within a specified period of time, for example, within the previous 1, 2, 3, 5, or 10 years prior to the evaluation of PML risk.

[0116] JCV antibodies are detected by an ELISA assay. In one embodiment, JCV antibodies can be detected by the method described in International Application Number PCT/US2011/20832, which utilizes HPVLPs under conditions suitable for binding of an anti-JCV antibody for detecting the level of anti-JCV antibody binding in a biological sample. Methods of determining JCV status also include methods of determining anti-JCV antibody titer and percent inhibition. Detection of anti-JCV antibody titer and percent inhibition typically include a two-step antibody detection assay as described in International Application Number PCT/US2011/20832.

[0117] If the presence of JCV is identified in a biological sample from a patient, *e.g.,* JCV antibodies, proteins, peptides, or nucleic acids, the patient is determined to be "JCV positive." A positive JCV classification corresponds to the presence of JCV antibodies in the biological sample, *e.g.,* JCV antibodies that are equal to or greater than a preselected criterion. The preselected criterion is typically a qualitative value, *e.g.,* a "detectable" amount of antibody according to a particular assay, *e.g.,* an immunoassay.

[0118] The methods described herein for determining PML risk can be useful for any human subject, including a subject considering treatment with an immunomodulator, for example an anti-VLA-4 therapy *(e.g.,* natalizumab), an anti-CD20 therapy *(e.g.,* rituximab), an anti-CD11a therapy (*e.g.,* efalizumab), or mycophenolate mofetil; in a subject currently being treated with an immunomodulator; or a subject that has ceased treatment with an immunomodulator. The method may be useful to others who may be susceptible to PML such as individuals having lymphoproliferative disorders, such as multiple myeloma or a lymphoma; individuals infected with human immunodeficiency virus (HIV), or having acquired immune deficiency syndrome (AIDS), hematologic malignancies, or an autoimmune disease such as systemic lupus erythematosus (SLE), an inflammatory bowel disease, such as Crohn's Disease (CD) or ulcerative colitis, multiple sclerosis (MS) or arthritis, *e.g.,* rheumatoid arthritis (RA). The risk-assessment method may also be useful to subjects receiving immunosuppressive or immunomodulatory therapies, such as transplant patients. Exemplary immunosuppressive or immunomodulatory therapies include natalizumab, rituximab, efalizumab, and mycophenolate mofetil. The method can be useful for assessing risk in a subject having a disorder, or being treated with a drug, disclosed in Piccinni et al. "Stronger association of drug-induced progressive multifocal leukoencephalopathy (PML) with biological immunomodulating agents" Eur. J. Clin. Pharmacol. 66:199-206, 2010.

Definitions

[0119] As used herein, an "HPVLP" is a highly purified VLP ("virus-like particle") consisting predominantly of the VP1 protein. An "HPVLP" featured in the invention is composed mainly of the major capsid protein "VP1," which can be a naturally-occurring VP1 or a recombinant VP1, from the polyomavirus, JC Virus (JCV). An HPVLP can be composed of, *e.g.,* at least one pentameric subunit, more than one pentameric subunit, up to seventy-two pentameric subunits or more of VP1. An HPVLP of the invention can bind antibodies against naturally-occurring, intact JC virus. In some embodiments, an HPVLP includes a second, and optionally a third, polypeptide that is a minor capsid protein of JC virus, *e.g.,* at least one VP2 or VP3 polypeptide. The VP2 or VP3 can be recombinant or naturally-occurring or naturally-derived polypeptides.

[0120] Such "highly purified" particles contain more than one VP1 pentamer, *e.g.,* at least 5, 10, 20, 30, 40, 50, 60, 70, 72 VP1 pentamers, or less than 100 VP1 pentamers. Such highly purified particles can be obtained, for example, by a method that involves double filtration. For example, in one embodiment, a highly purified preparation of VLPs is obtained by purifying the particles at least twice by centrifugation, *e.g.,* through a sucrose cushion. In other embodiments, HPVLPs are prepared using chromatographic methods. In general, an HPVLP preparation can be identified by its activity in an ELISA assay using defined control samples. In some cases, such control samples are negative controls and/or control samples containing low levels of JCV antibodies.

[0121] As used herein, a "high signal-to-noise HPVLP substrate" is a substrate on which is disposed HPVLP. It can be used to evaluate the level of free (that is unbound to antigen or other target, *e.g.,* HPVLP, in a sample. The concentration of HPVLP on the substrate is such that, when measuring the amount of anti-JCV antibody present, it provides for a signal-to-noise ratio of 10 to 30, 15 to 30, 15 to 25, 18 to 22. In embodiment the signal-to-noise ratio is at least 10, 15, 18 or 20. In embodiments signal-to-noise ratio is about 10, 15, 18 or 20. The signal-to noise ratio can be determined with a sample, *e.g.,* a calibration control, that gives an optical density of 1.0. In an embodiment the HPVLP is provided on said substrate at a concentration which results from lyophilizing 0.5 ml, 0.8 ml, 1.0 ml, 1.2 ml, or 1.5 ml of 0.4 μg/ml of HPVLP in a well of a 96 well plate. In an embodiment the HPVLP is provided on said substrate at a concentration which results from lyophilizing 1.0 ml of 0.4μg/ml of HPVLP in a well of a 96 well plate, which as used herein, is equivalent to 30 ng to 50 ng *(e.g.,* 40 ng) HPVLP per well. In an embodiment the HPVLP is provided on said substrate at a concentration which results from lyophilizing 0.05 ml to 0.35 mL or 0.1 ml to 0.2 ml of 0.4 μg/ml of HPVLP in a well of a 96 well plate. The amount of HPVLP disposed on the substrate, or the conditions under which deposition is achieved, can vary as long as the desired signal-to-noise ratio is obtained.

[0122] A signal-to-noise ratio is computed by comparing the optical density value of the negative control to the calibrator control to determine the dynamic range of the signal intensity in the assay.

[0123] In an embodiment the sample is diluted about 100 fold and the cut off for negative score is a reduction that is less than or equal to 45 % and, the cutoff for a positive score is greater than 45 %. In embodiments the dilution is other than 100 fold but is less than 200 fold. For example, the dilution is between 50- and 150-fold, 75- and 125-fold, 85- and 115-fold. In embodiments, the dilution is less than 150-fold, 125-fold, 100-fold, or 75-fold. In embodiments where the dilution is other than 100-fold *(e.g.,* 200-fold 400-fold, 500-fold, 800-fold, up to >1,000,000-fold, the cutoff, or other parameters, are adjusted such that a sample would receive the same score (positive or negative) as it would if the dilution was 100-fold and the cut off for negative is less than 45% and the cut off for positive is greater than or equal to 45%.

[0124] *Anti-JCV Antibody Detection Assay.* Assays are conducted by adding a biological sample to a substrate that has been coated with an HPVLP and detected using methods known in the art. In general, a solid base platform is used such as a microtiter plate (for example, a 96 well plate); although other formats known in the art can be used. In some embodiments, the biological sample is diluted prior to use in an assay.

[0125] The assay format is an enzyme-linked immunoassay (ELISA). Broadly, the method typically includes coating the substrate with capture antigen such as HPVLP, incubating sample containing binding antibodies directed to capture reagent, washing to remove non-specifically bound species, and detecting the bound immune complexes, *e.g.,* by a chromogenic or chemiluminescent assay. Chromogenic substrates produce a colored end product, which can be detected and measured visually or with the use of a spectrophotometer. Chemiluminescent substrates produce light, which can be measured using a luminometer.

[0126] Coating a plate with HPVLP generally includes incubating the solid substrate (such as wells of a microtiter plate) with a solution of HPVLP at a suitable concentration *(e.g.,* 0.4 μg/ml), either overnight or for a specified number of hours. The HPVLP can include VP1 as the only JCV viral component, or the HPVLP can be a heterologous particle, that contains at least one of VP2 or VP3 per particle or at least one each of VP2 and VP3 per particle. After coating with the HPVLP, the wells of the plate are washed. The substrate is then "coated" with a nonspecific protein that is antigenically neutral with regard to the samples to be tested. Suitable coating materials are known in the art and include bovine serum albumin (BSA), casein, sugars or solutions of milk powder. Plates may then be dried and stored for a longer period of time, such as 1 days, 1 month or 1 year prior to proceeding to the next step of the assay.

[0127] The sample or reference is incubated on the prepared substrate under conditions effective to permit complex

formation (HPVLP/JCV antibody), thus forming a bound complex. Detection of the bound complex is performed using a labeled antibody that can bind to human antibody. In general, the labeled antibody can detect human IgG or human IgG and IgM. In some cases, the assay can be performed using secondary or tertiary detection methods.

[0128] A reference sample can be of the same biological material (*e.g.*, plasma, serum, urine, or CSF) isolated from an individual known to be infected with JC virus based on the presence of JCV DNA in urine of the individual (uropositive). A reference sample is used to establish the assay cut-point such that the false negative rate of the assay is not greater than 1%-3%.

[0129] "Under conditions effective to permit complex formation" generally means conditions in which the reagents have been diluted to reduce background and provide readouts of results that lie within a specified range. Diluents can include, in non-limiting examples, solutions that include BSA, phosphate buffered saline (PBS), or PBS containing Tween.

[0130] "Suitable" conditions also include conditions that are at a temperature and/or for a period of time sufficient to allow effective binding. Incubations are typically from about one to two hours or one to four hours, at temperatures of approximately 25°C to 27°C, or may be overnight at about 4°C. However, those in the art will understand that other conditions may be suitable.

[0131] In general, one or more washes are conducted between the incubations of the assay. Appropriate wash solutions include diluent buffer *(e.g.,* PBS or PBS/Tween) or borate buffer.

[0132] In general, the detection of antibody bound to HPVLP is performed using methods well known in the art. In general, such methods are based on the detection of a label or marker, such as a radioactive, fluorescent, biological or enzymatic tag. U.S. patents concerning the use of such labels include, for example, U.S. Pat. Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149 and 4,366,241. In general, the detection of anti-JCV antibody binding is detected using a secondary antibody that is labeled. In general, the secondary antibody is specific for detecting human IgG. Quantification is achieved by measuring the degree of color generated, *e.g.,* using a visible spectra spectrophotometer.

[0133] In one embodiment, the assay is performed in a medical office, such as by a healthcare provider, *e.g.,* a doctor, a nurse or a technician, working in a facility where the biological sample is obtained from a patient. In another embodiment, the biological sample obtained from a patient is transported to another facility, *e.g.,* to a third party facility, where the assay is performed. In this latter case, the results of the assay can be reported back to the healthcare provider, such as through a form, which can be submitted by mail or electronically (*e.g.,* through facsimile or e-mail) or through an on-line database. In one embodiment, the results of the assay (including the screening assay and, optionally, a confirmatory assay) can be stored in a database and can be accessed by a healthcare provider, such as through the worldwide web.

[0134] *Secondary Test.* In some cases, for example, when the level of anti-JCV antibody in a sample falls into a designated "equivocal zone" or "indeterminate zone," *e.g.,* where it is determined that there is limited certainty regarding the presence or absence of anti-JCV antibody (such as when the nOD value is determined to be >0.2 and <0.4), a secondary test (also referred to herein as a "confirmatory assay") of the sample is employed. For the secondary test, two aliquots of a biological sample are used. The first is prepared prior to use in the assay by preincubating the sample in the presence of assay buffer in solution for a period of time *(e.g.,* for 30 minutes, one hour, or longer such as overnight at 4°C). The second aliquot is prepared prior to use in the assay by preincubating the sample in the presence of HPVLP in solution for a period of time *(e.g.,* for 30 minutes, or one hour or longer). The two aliquots are then used in the HPVLP assay as described herein, and the assignment of the sample to anti-JCV antibody positive or antibody negative is made. If the assay results for the aliquot incubated with HPVLP indicate a value of <45% inhibition *(i.e.,* the "cut-point"), then the sample is interpreted to be negative for the presence of JCV-specific antibodies. If the assay results indicate a value of ≥45% inhibition, then the sample is interpreted to have JCV-specific antibodies and therefore as antibody positive.

[0135] An assay featured in the invention that utilizes a secondary test is also referred to herein as a "two-step test" or a "two-step assay." An earlier version of the two step assay is described in co-owned International Application No. PCT/US2011/020832.

[0136] *Substrates and Solution Based Methods.* Any suitable solid substrate can be used for the HPVLP assay format. In some embodiments, the substrate is a microtiter plate *(e.g.,* a 96-well plate) a slide, a bead, or a column. The substrate can be suitable for chromogenic or chemiluminescent detection methods, or solution based methods such as proximal ligation.

[0137] *Cut-point.* The invention provides methods of analysis that employ "cut-points" to reduce false negative and false positive rates. The cut-points are established based on data from the HPVLP assays (*e.g.,* to detect JCV antibodies in a biological sample), averaged, for example, between duplicate test samples and multiple replicates (for example, at least two, at least four, or at least eight replicates of control samples). Cut-points can also be determined statistically using large panels of non-PML and PML samples.

[0138] In one version of an assay according to the present invention, results from initial HPVLP screening assays, *e.g.,* ELISA assays, will cause a test sample to be classified as having or not having JCV-specific antibodies, or, if the sample does not fall under one of these two classifications, then the sample will be subjected to a supplemental confirmation assay. For example, samples that produce a result in an HPVLP ELISA assay featured in the invention less than

an established level (*e.g.,* an $nOD_{450} < 0.2$) will be classified as lacking JCV-specific antibodies, and samples that provide a result in the ELISA greater than an established level (*e.g.,* an $nOD_{450} > 0.4$) will be classified as positive for JCV-specific antibodies. Samples that do not clearly fall into one of these classifications (*e.g.,* $0.2 < OD_{450} < 0.4$) can be tested in a confirmatory assay.

[0139] In one embodiment, the confirmatory assay requires a pre-incubation step, where the test sample is pre-incubated with buffer (or other suitable solution) control or with HPVLPs (in buffer or other suitable solution) to pre-adsorb JCV-specific antibodies prior to analysis in an HPVLP ELISA, as described in further detail below. After pre-incubation with HPVLP if the reaction in the primary assay decreases by less than 45% compared to buffer control, then the sample is interpreted to be negative for the presence of JCV-specific antibodies. If the results show a ≥45% reduction in reaction compared to buffer control in the primary assay after pre-incubation with HPVLP then the sample is interpreted to contain JCV specific antibodies. In some embodiments, only the confirmatory assay is performed.

[0140] *VP1.* The use of HPVLPs in an assay for JCV antibodies can improve the accuracy of the assay and is useful in an assay suitable for analytic and diagnostic purposes. VP1 for use in producing HPVLPs can be generated using methods known in the art and can be either naturally-occurring VP1 or recombinantly produced VP1, *e.g.,* a VP1 from a JC virus. In general, the VP1 used is VP1 from a MAD1 strain of JCV. In some embodiments, the VP1 used in the assay comprises VP1 from more than one JCV strain, for example, from one or more of strains 1A, 1B, 2A, 2B, 3, 4, and 7. After preparation of VP1, *e.g.,* recombinantly synthesized VP1, the VP1 for use in the assays described herein is then further purified through standard biochemical methods including density-gradient/ultracentrifugation methods, or a series of chemical precipitation steps, concentration/diafiltration and ion-exchange chromatography. The purification methods typically include a step to remove smaller proteins including monomer VP1 polypeptides, or pentamer VP1. The removal of these smaller particles can be done in, for example, in one step or in two steps *(e.g.,* a first filtration step to remove VP1 monomers, and then a second filtration step to remove pentamer VP1 particles). Such biochemical purification methods are known to those in the art. Examples 1 and 7 provide two different methods of JCV VP1-VLP purification.

[0141] An HPVLP preparation (HPVLPs) according to one aspect of the present invention does not contain significant amounts of VP1 monomer (*e.g.*, has been purified to remove monomers). An HPVLP preparation according to another aspect of the present invention does not contain significant amounts of VP1 molecules in configurations the size of a VP1 pentamer, or smaller (including monomer). The HPVLP can be prepared from recombinant VP1 or naturally-occurring VP1 (*e.g.,* isolated from virus or virus capsid). In some embodiments, additional JCV components, such as one or both of the minor coat proteins from JC virus, *e.g.,* VP2 or VP3, are included in the HPVLP particle or are associated with the substrate.

[0142] In some cases, recombinantly expressed VP1 may not assemble into pentamers or HPVLPs that resemble naturally-occurring viral capsids, for example, recombinantly expressed VP1 may assemble into tubes or other non-spherical geometries. Accordingly, the invention relates to methods of producing HPVLPs that are substantially spherical in geometry. The invention includes HPVLP preparations where at least about 10%, about 15%, about 20%, about 25%, about 50%, about 60%, about 65%, about 70%, about 80%, about 90%, about 95%, or about 99% of the HPVLPs in the preparation resemble the naturally-occurring JCV capsid *(e.g.,* are in an icosahedral or substantially spherical configuration). In some embodiments, an HPVLP preparation contains at least 10%, at least 15%, at least 20%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% of the HPVLPs in the preparation resemble the naturally-occurring JCV capsid. Such methods can include expressing viral proteins under conditions that result in such a preparation and/or isolating and purifying expressed viral proteins as described herein to produce such a preparation.

[0143] *Methods of Making HPVLPs.* HPVLPs can be made, for example, by transforming a baculovirus with a vector expressing a VP1 gene, such as a VP1 gene from a JC virus. The baculovirus is used to infect a cell culture, such as an insect cell culture *(e.g.,* SF9 cells) or a mammalian cell culture, and the cells express the VP1 protein. HPVLPs are isolated by lysing the cells, and purifying the particles through a series of centrifugation and ultrafiltration steps. In general, the purification is performed using methods such as sucrose cushion sedimentation, isopycnic ultracentrifugation and extensive ultrafiltration or other methods known to those in the art. In certain embodiments, the purification will include twice centrifuging the particles through a sucrose cushion. In an alternative purification method, cells are lysed, and particles are isolated by a series of precipitation and concentration/diafiltration steps with a final ion-exchange step. In yet another alternative method, the HPVLPs are purified by chromatographic methods, and without centrifugation steps.

[0144] Purity can be assessed using any suitable techniques known in the art, for example, analytical ultracentrifugation, electron microscopy, PAGE analysis, mass spectrometry, protein concentration, or activity in an ELISA with control sera. Insufficiently purified VLPs result in a high background yielding falsely high anti-JCV antibody levels or calculated exposure rates.

[0145] In some embodiments, the HPVLPs contain VP1 as the sole JC virus protein.

[0146] In some embodiments, the HPVLPs are heterogeneous particles, and therefore include VP1 protein, and at least one of the minor coat proteins of JC virus, *e.g.,* VP2 or VP3. In another embodiment, the HPVLP includes VP1,

VP2 and VP3 proteins. An HPVLP that includes VP1 and VP2 can be produced using methods known in the art, for example, by transforming a baculovirus with a nucleic acid including a VP1 and a VP2 gene, such as under the control of the same or different promoters. A cell culture is infected with the baculovirus, and the cells express VP1 and VP2, and HPVLPs form which include both types of proteins. In one embodiment, the VP1 and VP2 genes are on different DNA molecules, the DNA molecules are transformed into different baculoviruses and the baculoviruses are used to transfect cells in the same culture. The cells express the VP1 and VP2 proteins, and HPVLPs form which include both types of protein. In some cases, a heterogeneous HPVLP will include, *e.g,* one or two VP2 polypeptides for every five VP1 polypeptides. In general, an HPVLP will contain more VP1 polypeptides than VP2 polypeptides, as is the case in naturally-occurring JC virus.

[0147] An HPVLP that includes both VP1 and VP3 or both VP1 and VP2 molecules can be produced, for example, by transforming a baculovirus with a nucleic acid including a VP1 and a VP3 gene or a VP1 and VP2 gene, respectively, under the control of the same or different promoters. A cell culture is infected with the baculovirus, and the cells express VP1 and VP3 or VP1 and VP2, and HPVLPs form which include both types of proteins. In some embodiments, the VP1 and VP3 or VP1 and VP2 genes are on different DNA molecules, the DNA molecules are transformed into different baculoviruses, and the baculoviruses are used to transfect cells in the same culture. The cells express the VP1 and VP3 proteins or VP1 and VP2 genes, respectively, and HPVLPs form which include both types of protein. HPVLP particles can be isolated from such preparations using methods known in the art such as those used to isolate JCV capsids.

[0148] Typically, a VP1 pentamer that is in a heterogeneous HPVLP will include, *e.g,* five VP1 polypeptides and one VP3 polypeptide and/or one VP2 polypeptide, depending on whether a VP3 gene or VP2 gene was used to make the constructs. There will typically be more VP1 polypeptides than VP3 or VP2 polypeptides in an HPVLP. In some embodiments, the VP2 or VP3 is from a polyoma virus that is not a JC virus, *e.g.,* a BK virus polypeptide.

[0149] An HPVLP that includes all three of VP1 and VP2 and VP3 molecules can be produced by transforming a baculovirus with a nucleic acid *(e.g.,* a circular DNA, *e.g.,* < 5.5 kb) including a VP1, VP2 and VP3 gene, such as under the control of the same or different promoters. A cell culture, such as a mammalian cell culture, is infected with the baculovirus, and the cells express VP1, VP2 and VP3 proteins. HPVLPs consequently form which include all three types of proteins. In one embodiment, the VP1, and either or both of the VP2 and VP3 genes are on different DNA molecules, the DNA molecules are transformed into the same or different baculovirus, and the baculovirus are used to infect cells in the same or separate cultures. The cells express the VP1, VP2 and VP3 proteins, and HPVLPs form which include both types of protein. A heterogeneous HPVLP can include, *e.g,* five VP1 polypeptides and one each of VP2 and VP3 polypeptides, although the ratios may vary within a preparation. There will typically be more VP1 polypeptides than VP2 and VP3 polypeptides in an HPVLP.

[0150] In some embodiments, the HPVLP will be greater in size than a VP1 pentamer. By greater in size, it is meant that the mass of protein contained in an HPVLP particle is greater than a pentamer containing solely VP1.

[0151] In other embodiments, the method of preparing a solution of HPVLP can include removing from the solution particles (*e.g.,* VP1 monomers or small VP1 containing particles) that are the size of a VP1 pentamer or smaller. Methods such as centrifugation and size-exclusion chromatography can be used to perform this purification step. In some embodiments, other methods known in the art, *e.g.,* ion exchange chromatography, can be used in the preparation of HPVLPs that are larger than a VP1 pentamer. In general, an HPVLP preparation suitable for use in an assay will contain at least 20% HPVLPs, at least 25% HPVLPs, at least 40% HPVLPs, at least 60% HPVLPs, at least 65% HPVLPs, at least 70% HPVLPs, at least 80% HPVLPs, at least 85% HPVLPs, at least 90% HPVLPs, at least 95% HPVLPs, or at least 99% HPVPLs compared to non-HLVLP particles (*e.g.,* by percent of pentamers compared to VP1 monomers and aggregates containing fewer than five VP1 molecules).

[0152] *Methods of Evaluating Samples and/or Subjects.* As used herein, methods of evaluating or analyzing a subject or biological sample from a subject include one or more of performing the analysis of the sample, requesting analysis of the sample, requesting results from analysis of the sample, or receiving the results from analysis of the sample. (Generally herein, determination (or determining), analysis or evaluation (or evaluating) can include one or both of performing the underlying method or receiving data from another who has performed the underlying method.)

[0153] The analysis or evaluation requires a transformation of material, *e.g.,* biological material or assay components. For example, a biological sample can be evaluated for the presence of anti-JCV antibodies, anti-JCV antibody titer and percent inhibition of JCV antibodies. The evaluation can be performed before or after or at the same time the patient is receiving treatment, such as for MS. The evaluation is based, at least in part, on analysis of a sample from the subject. The presence of anti-JCV antibodies can be determined by contact with a specific binding agent, *e.g.,* a JCV protein, such as VP1. The binding agent can be a JCV protein, *e.g.,* VP1 in the form of a particle, *e.g.,* a HPVLP.

[0154] In one embodiment, an assay to detect the presence of anti-JCV antibodies is a two-step assay, such as described herein. The assay utilizes HPVLPs under conditions suitable for binding an anti-JCV antibody. The assay is capable of detecting any isotype of anti-JCV antibody (including IgG, IgM, IgA, and IgE). The assay is also highly sensitive and can detect anti-JCV antibodies at a concentration of, for example, 2.0 μg/mL or less, *e.g.* 1.5 μg/mL or less, 1.25 μg/mL or less, 1.0 μg/mL or less, 0.5 μg/mL or less, 50 ng/mL or less, 10 ng/mL or less, 5 ng/mL or less, 1.7 ng/mL or

less, or 1 ng/mL or less.

[0155] The sample can be analyzed for the level of JCV nucleic acid present in the sample. For example, nucleic acids can be isolated from the sample and used for PCR amplification or a Next-Generation (Nex-Gen) Sequencing technique.

[0156] For example, a crude lysate of the biological sample is subject to an amplification method, such as PCR, and the amplified product is analyzed by one or more of electrophoresis, restriction fragment mapping, hybridization or sequencing to identify whether JCV DNA or RNA is present in the sample and how much is in the sample.

[0157] The biological sample can be removed from the patient and analyzed.

[0158] In some embodiments, the patient sample, can be stored prior to testing for JCV antibodies The patient sample, e.g., the patient sample containing JCV antibodies can be stored for 1-21 days, *e.g.,* 1-14 days or 1-7 days or longer *(e.g.,* one day, two days, three days, five days, seven days, ten days, 14 days, 21 days or longer); for one to six weeks, *e.g.,* one to three weeks or one to two weeks or longer *(e.g.,* up to one week, up to two weeks, up to three weeks, up to six weeks, or longer); or for one to six months, *e.g.,* one to three months or one to two months or longer *(e.g.,* up to one month, up to two months, up to three months, up to six months or longer). The sample can be stored, for example, frozen *(e.g.,* at -80°C to -20°C), at 2-8°C, at ambient temperature (18°C-25°C) or warmer, e.g., at 37°C.

[0159] As used herein, the term "acquire" or "acquiring" refers to obtaining possession of a physical entity, or a value, *e.g.,* a numerical value, by "directly acquiring" or "indirectly acquiring" the physical entity or value, *e.g.,* the status of a patient, such as prior exposure to anti-VLA-4 therapy or other immunosuppressants, or JCV status. "Directly acquiring" means performing a process (*e.g.,* performing a synthetic or analytical method) to obtain the physical entity or value. "Indirectly acquiring" refers to receiving the physical entity or value from another party or source *(e.g.,* a third party laboratory that directly acquired the physical entity or value). Directly acquiring a physical entity includes performing a process that includes a physical change in a physical substance, *e.g.,* a starting material. Exemplary changes include making a physical entity from two or more starting materials, shearing or fragmenting a substance, separating or purifying a substance, combining two or more separate entities into a mixture, performing a chemical reaction that includes breaking or forming a covalent or non-covalent bond. Directly acquiring a value includes performing a process that includes a physical change in a sample or another substance, *e.g.,* performing an analytical process which includes a physical change in a substance, *e.g.,* a sample, analyte, or reagent (sometimes referred to herein as "physical analysis"), performing an analytical method, *e.g.,* a method which includes one or more of the following: separating or purifying a substance, *e.g.,* an analyte, or a fragment or other derivative thereof, from another substance; combining an analyte, or fragment or other derivative thereof, with another substance, *e.g.,* a buffer, solvent, or reactant; or changing the structure of an analyte, or a fragment or other derivative thereof, *e.g.,* by breaking or forming a covalent or non covalent bond, between a first and a second atom of the analyte; or by changing the structure of a reagent, or a fragment or other derivative thereof, *e.g.,* by breaking or forming a covalent or non covalent bond, between a first and a second atom of the reagent.

[0160] At least one or both of determining a patient's status (*e.g.,* JCV status), or an activity level, and determining if the status has a preselected relationship with a reference criterion, includes one or more of analyzing a sample, requesting analysis of the sample, requesting results from analysis of the sample, or receiving the results from analysis of the sample. (Generally, analysis can include one or both of performing the underlying method (*e.g.,* an immunoassay) or receiving data from another who has performed the underlying method.)

[0161] *Anti-VLA-4 therapy.* An anti-VLA-4 therapy is a molecule, *e.g.,* a small molecule compound or protein biologic *(e.g.,* an antibody or fragment thereof, such as an antigen-binding fragment thereof) that blocks VLA-4 activity. The molecule that is the anti-VLA-4 therapy is a VLA-4 antagonist. A VLA-4 antagonist includes any compound that inhibits a VLA-4 integrin from binding a ligand and/or receptor. An anti-VLA-4 therapy can be an antibody (*e.g.,* natalizumab (TYSABRI®)) or fragment thereof, or a soluble form of a ligand. Soluble forms of the ligand proteins for α4 integrins include soluble VCAM-I or fibronectin peptides, VCAM-I fusion proteins, or bifunctional VCAM-I/Ig fusion proteins. For example, a soluble form of a VLA-4 ligand or a fragment thereof may be administered to bind to VLA-4, and in some instances, compete for a VLA-4 binding site on cells, thereby leading to effects similar to the administration of antagonists such as anti-VLA-4 antibodies. For example, soluble VLA-4 integrin mutants that bind VLA-4 ligand but do not elicit integrin-dependent signaling are suitable for use in the described methods. Such mutants can act as competitive inhibitors of wild type integrin protein and are considered "antagonists." Other suitable antagonists are "small molecules."

[0162] "Small molecules" are agents that mimic the action of peptides to disrupt VLA-4/ligand interactions by, for instance, binding VLA-4 and blocking interaction with a VLA-4 ligand *(e.g.,* VCAM-I or fibronectin), or by binding a VLA-4 ligand and preventing the ligand from interacting with VLA-4. One exemplary small molecule is an oligosaccharide that mimics the binding domain of a VLA-4 ligand (*e.g.,* fibronectin or VCAM-I) and binds the ligand-binding domain of VLA-4. (See, Devlin et al., Science 249: 400-406 (1990); Scott and Smith, Science 249:386-390 (1990); and U.S. Pat. No. 4,833,092 (Geysen.)

[0163] A "small molecule" may be chemical compound, *e.g.,* an organic compound, or a small peptide, or a larger peptide-containing organic compound or non-peptidic organic compound. A "small molecule" is not intended to encompass an antibody or antibody fragment. Although the molecular weight of small molecules is generally less than 2000

Daltons, this figure is not intended as an absolute upper limit on molecular weight.

[0164] *Combination Therapy or Alternatives to Anti-VLA-4 Therapy.* The anti-VLA-4 therapy, *e.g.,* natalizumab, can be administered with a second agent, or an alternative therapy can be administered instead of the anti-VLA-4 therapy, such as when a patient is determined to be at higher risk for PML.

[0165] Non-limiting examples of second agents for treating multiple sclerosis in combination with the anti-VLA-4 therapy, or alternative agents for use instead of the anti-VLA-4 therapy, include: fumaric acid salts, such as dimethyl fumarate; Sphingosine 1-phosphate (S1P)-antagonists, such as the SIB-blocking antibody Sphingomab; interferons, such as human interferon beta-la *(e.g.,* AVONEX® or Rebif®)) and interferon P-1b (BETASERON® human interferon β substituted at position 17; Berlex/Chiron); glatiramer acetate (also termed Copolymer 1, Cop-1; COPAXONE® Teva Pharmaceutical Industries, Inc.); an antibody or a fragment thereof (such as an antigen-binding fragment thereof), such as an anti-CD20 antibody, *e.g.,* Rituxan® (rituximab), or an antibody or fragment thereof that competes with or binds an overlapping epitope with rituximab; mixtoxantrone (NOVANTRONE®, Lederle); a chemotherapeutic agent, such as clabribine (LEUSTATIN®), azathioprine (IMURAN®), cyclophosphamide (CYTOXAN®), cyclosporine-A, methotrexate, 4-aminopyridine, and tizanidine; a corticosteroid, such as methylprednisolone (MEDRONE®, Pfizer), or prednisone; CTLA4 Ig; alemtuzumab (MabCAMPATH®) or daclizumab (an antibody that binds CD25); statins; and TNF antagonists.

[0166] Glatiramer acetate is a protein formed from a random chain of amino acids (glutamic acid, lysine, alanine and tyrosine (hence GLATiramer)). Glatiramer acetate can be synthesized in solution from these amino acids at a ratio of approximately 5 parts alanine to 3 parts lysine, 1.5 parts glutamic acid and 1 part tyrosine using N-carboxyamino acid anhydrides.

[0167] Additional second agents, or agents for use in place of the anti-VLA-4 therapy, include antibodies or antagonists of other human cytokines or growth factors, for example, TNF, LT, IL-1, IL-2, IL-6, IL-7, IL-8, IL-12 IL-15, IL-16, IL-18, EMAP-11, GM-CSF, FGF, and PDGF. Still other exemplary second agents include antibodies to cell surface molecules such as CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD80, CD86, CD90 or their ligands. For example, daclizubmab is an anti-CD25 antibody that may ameliorate multiple sclerosis.

[0168] Still other exemplary antibodies include antibodies that provide an activity of an agent described herein, such as an antibody that engages an interferon receptor, *e.g.,* an interferon beta receptor. Typically, in implementations in which the second agent includes an antibody, it binds to a target protein other than VLA-4 or other than an α4 integrin, or at least an epitope on VLA-4 other than one recognized by natalizumab.

[0169] Still other additional exemplary second agents include: FK506, rapamycin, mycophenolate mofetil, leflunomide, non-steroidal anti-inflammatory drugs (NSAIDs), for example, phosphodiesterase inhibitors, adenosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, agents that interfere with signaling by proinflammatory cytokines as described herein, IL- 1β converting enzyme inhibitors (*e.g.,* Vx740), anti-P7s, PSGL, TACE inhibitors, T-cell signaling inhibitors such as kinase inhibitors, metalloproteinase inhibitors, sulfasalazine, azathloprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors and derivatives thereof, as described herein, anti-inflammatory cytokines (*e.g.* IL-4, IL-10, IL-13 and TGF).

[0170] A second agent may be used to treat one or more symptoms or side effects of MS. Such agents include, *e.g.,* amantadine, baclofen, papaverine, meclizine, hydroxyzine, sulfamethoxazole, ciprofloxacin, docusate, pemoline, dantrolene, desmopressin, dexamethasone, tolterodine, phenytoin, oxybutynin, bisacodyl, venlafaxine, amitriptyline, methenamine, clonazepam, isoniazid, vardenafil, nitrofurantoin, psyllium hydrophilic mucilloid, alprostadil, gabapentin, nortriptyline, paroxetine, propantheline bromide, modafinil, fluoxetine, phenazopyridine, methylprednisolone, carbamazepine, imipramine, diazepam, sildenafil, bupropion, and sertraline. Many second agents that are small molecules have a molecular weight between 150 and 5000 Daltons.

[0171] Examples of TNF antagonists include chimeric, humanized, human or *in vitro* generated antibodies (or antigen-binding fragments thereof) to TNF *(e.g.,* human TNF α), such as D2E7, (human TNFα antibody, U.S. Patent No. 6,258,562; BASF), CDP-571/CDP-870/BAY-10-3356 (humanized anti-TNFα antibody; Celltech/Pharmacia), cA2 (chimeric anti-TNFα antibody; REMICADE™, Centocor); anti-TNF antibody fragments (*e.g.*, CPD870); soluble fragments of the TNF receptors, *e.g.,* p55 or p75 human TNF receptors or derivatives thereof, *e.g.,* 75 kd TNFR-IgG (75 kD TNF receptor-IgG fusion protein, ENBREL™; Immunex; see, *e.g.,* Arthritis & Rheumatism 37:S295, 1994; J. Invest. Med. 44:235A, 1996), p55 kdTNFR-IgG (55 kD TNF receptor-IgG fusion protein (LENERCEPT™)); enzyme antagonists, *e.g.,* TNFα converting enzyme (TACE) inhibitors (*e.g.*, an alpha-sulfonyl hydroxamic acid derivative, WO 01/55112, and N-hydroxyformamide TACE inhibitor GW 3333, -005, or -022); and TNF-bp/s-TNFR (soluble TNF binding protein; see, *e.g.,* Arthritis & Rheumatism 39:S284, 1996; Amer. J. Physiol. - Heart and Circulatory Physiology 268:37-42, 1995).

[0172] In one implementation, the anti-VLA-4 therapy and the second agent are provided as a co-formulation, and the co-formulation is administered to the subject. It is further possible, *e.g.,* at least 24 hours before or after administering the co-formulation, to administer separately one dose of the anti-VLA-4 therapy formulation and then one dose of a formulation containing the second agent. In another implementation, the anti-VLA-4 therapy and the second agent are provided as separate formulations, and the step of administering includes sequentially administering the anti-VLA-4 therapy and the second agent. The sequential administrations can be provided on the same day (*e.g.*, within one hour

of one another or at least 3, 6, or 12 hours apart) or on different days.

[0173] The anti-VLA-4 therapy and the second agent each can be administered as a plurality of doses separately in time. The anti-VLA-4 therapy and the second agent are typically each administered according to a regimen. The regimen for one or both may have a regular periodicity. The regimen for the anti-VLA-4 therapy can have a different periodicity from the regimen for the second agent, *e.g.,* one can be administered more frequently than the other. In one implementation, one of the anti-VLA-4 therapy and the second agent is administered once weekly and the other once monthly. In another implementation, one of the anti-VLA-4 therapy and the second agent is administered continuously, *e.g.,* over a period of more than 30 minutes but less than 1, 2, 4, or 12 hours, and the other is administered as a bolus. The anti-VLA-4 therapy and the second agent can be administered by any appropriate method, *e.g.,* subcutaneously, intramuscularly, or intravenously.

[0174] Each of the anti-VLA-4 therapy and the second agent can be administered at the same dose as each is prescribed for monotherapy. The anti-VLA-4 therapy can be administered at a dosage that is equal to or less than an amount required for efficacy if administered alone. Likewise, the second agent can be administered at a dosage that is equal to or less than an amount required for efficacy if administered alone.

[0175] *Kits.* Reagents for performing an anti-JCV antibody assay can be provided in the form of a kit. Except for the patient sample, some or all materials required for the assay can be provided in the kit. A kit can include for example, a substrate, such as a plate with wells coated with JCV antigen substrate, *e.g.,* HPVLP. The plate can be for example a 6-well plate, a 12-well plate, a 24-well plate, a 48-well plate, a 96-well plate or a 384 well plate. The plates provided in a kit can be pre-coated with JCV VLP antigen, such as at 0.4 μg/mL. The kit can include materials and reagents for use with high-throughput systems such as SPR (Solid Phase Receptacle) tips for use with bioMerieux systems.

[0176] The kit can also include JCV antigen, *e.g.,* HPVLP lyophilized or in solution, such as for use with the confirmation step of the assay. The kit can include a JCV cut-off calibrator, an anti-JCV antibody positive control and a JCV negative control, which are samples of sera, such as human sera. Solutions containing JCV antigen and sera can include a preservative, such as sodium azide, *e.g.,* 0.05%, 0.1%, 1.5%, and 2% sodium azide. A kit can include one or more reagents for detecting a complex containing anti-JCV antibodies bound to antigen, such as HPVLP. Reagents for detecting the complex include, for example, a JCV conjugate, a casein sample, a detectable reagent, such as TMB (tetramethylbenzidine), a wash buffer, and a stop reagent.

[0177] The JCV substrate can be, for example, an anti-human antibody, such as an enzyme-conjugated anti-human antibody. The JCV conjugate can be an affinity-purified and peroxidase-conjugated donkey anti-human antibody. The casein solution can contain casein, a surfactant and a non-azide preservative in buffer (*e.g.*, phosphate buffered saline (PBS)). The TMB substrate solution can include TMB and hydrogen peroxide in buffer. The kit can include a wash buffer, and the wash buffer can contain, for example, surfactant in PBS with non-azide preservatives. The stop reagent can be, for example, an acid, such as sulfuric acid (*e.g.,* 1 M sulfuric acid).

[0178] The solutions provided in the kit can be provided at concentrated levels such that dilution is required before use. The HPVLP for use in solution binding to anti-JCV antibody in a biological sample, such as in the confirmation step of the two-step assay, can be provided as a concentration of 2 mg/mL, 1.5 mg/mL, 1 mg/mL, 0.5 mg/mL, for use at, for example, 10 μg/mL, 5 μg/mL, 1 μg/mL, 0.8 μg/mL, 0.4 μg/mL, 0.2 μg/mL The wash buffer, for example, can be provided at 10x concentration. The JCV substrate (such as an affinity-purified and peroxidase-conjugated donkey anti-human antibody) can be provided at, for example, 1 mg/mL, 0.8 mg/mL or 0.6 mg/mL, for dilution by, *e.g.,* 1:40,000, 1:30,000, 1:20,000 or 1:20,000 prior to use in an anti-JCV antibody detection assay.

[0179] Materials for sealing the reaction mixes, such as sealing tape, can also be included in the kit.

[0180] *Reporting of results.* The results of the risk-assessment analysis can be reported, such as to a treatment center, or a healthcare provider, or an insurance provider. The results of the risk-assessment can be stored in a database. Informational material can be provided for performing and interpreting the risk assessment. The informational material can provide guidance as to where to report the results of the assessment, such as to a treatment center or healthcare provider or database provider. The informational material can be provided in a kit or a packet, and can include forms for reporting the results of the assessment, including each prong of the assessment (information regarding prior treatment with anti-VLA-4 therapies, prior treatment with immunosuppressants, and JCV status), and address and contact information regarding where to send such forms or other related information; or a URL (Uniform Resource Locator) address for reporting the results in an online database or an online application (*e.g.*, an "app"). The informational material can include guidance regarding whether a patient should receive treatment with an anti-VLA-4 therapy, depending on the patient's risk of PML according to the results of the risk assessment.

[0181] The kit or packet may also include instructions and items for the collection or transport of a patient sample to a healthcare provider, or for receiving a sample from a healthcare provider, or for performing the evaluative methods described herein. For example, besides instructional information, a kit or packet featured in the invention can include one or more of a swab or scraper, or a vessel (*e.g.*, a cup, a test tube, an ampoule, or a bag) for collecting, and storing and transporting a biological sample. The kit or packet may also contain supplies for performing an immunoassay or a sequencing assay for detection of JCV antibodies or nucleic acids, respectively.

[0182] A kit can include one or more containers for the reagents required for an assay, *e.g.,* a JCV-detection assay. The reagents can be provided in a concentration suitable for use in the assay or with instructions for dilution for use in the assay. The kit can contain separate containers, dividers or compartments for the assay components, and the informational material. For example, the assay components can be contained in a bottle or vial, and the informational material can be contained in a plastic sleeve or packet. The separate elements of the kit can be are contained within a single, undivided container. For example, an assay reagent is contained in a bottle or vial that has attached thereto the informational material in the form of a label. The kit can include a plurality (*e.g.,* a pack) of individual containers, each containing one or more unit forms (*e.g.*, for use with one assay) of an assay component. For example, the kit includes a plurality of ampoules, foil packets, or blister packs, each containing a single unit of assay reagent for use in a screening or confirmatory assay. The containers of the kits can be air tight and/or waterproof. The container can be labeled for use.

[0183] The informational material of a kit or packet is not limited in its form. In many cases, the informational material, *e.g.,* instructions, is provided in printed matter, *e.g.,* a printed text, drawing, and/or photograph, *e.g.,* a label or printed sheet. However, the informational material can also be provided in other formats, such as computer readable material, video recording, or audio recording. The informational material of the kit can be contact information, *e.g.,* a physical address, email address, website, or telephone number, where a user of the kit or packet can obtain substantive information about how to find the information required for the risk assessment analysis, *e.g.,* where and how to identify prior treatments administered to a subject, and how to perform an assay to determine the JCV status of a patient. The informational material can also be provided in any combination of formats.

[0184] A biological sample can be provided to an assay provider, *e.g.,* a service provider (such as a third party facility) or a healthcare provider, who evaluates the sample in an assay and provides a read out. For example, an assay provider receives a biological sample from a subject, such as a plasma, blood or serum sample, and evaluates the sample using an assay described herein, and determines that the sample contains JCV antibodies or nucleic acid. The assay provider, *e.g.,* a service provider or healthcare provider, can further determine, *e.g.,* by contacting a healthcare provider or a database service provider, the amount of prior anti-VLA-4 therapy that a patient has received or whether a patient has previously received treatment with an immunomodulator. The assay provider can further determine that the subject is not a candidate to receive treatment with an anti-VLA-4 therapy, such as natalizumab, or that the subject is a candidate to receive treatment with an immunomodulator, or that the subject may be a candidate who should have enhanced monitoring as compared to a subject who is determined to have a negative JCV status (e.g., who tests negative for JCV nucleic acid or anti-JCV antibodies). For example, a candidate who has received prior treatment with an anti-VLA-4 therapy for 24 months or less, and who has not received prior therapy with an immunosuppressant, but who is determined to be JCV positive, can be selected as a candidate to receive further anti-VLA-4 therapy, but with a recommendation to monitor the patient more frequently for the development of adverse symptoms, such as symptoms that may indicate the development of PML. The assay provider can perform an assessment for PML risk as described herein and determines that subject is a candidate to receive treatment with an anti-VLA-4 therapy, such as natalizumab. The assay provider can inform a healthcare provider that the subject is a candidate for treatment with the anti-VLA-4 therapy, and the candidate is administered the anti-VLA-4 therapy. For example, the assay provider may determine that a patient is at a lower risk for PML and subsequently inform the healthcare provider of the determination of the lower risk and that the subject is a candidate for treatment with the anti-VLA-4 therapy.

[0185] In another example, the assay provider determines that a patient is at a higher risk for PML and subsequently informs a healthcare provider of the determination of the higher risk, and recommends that the patient is a candidate for treatment with the anti-VLA-4 therapy, but that the patient should undergo increased testing for PML and, optionally, JCV status. The assay provider can inform the healthcare provider that the patient is at higher risk of PML and therefore the patient should receive an alternative to anti-VLA-4 therapy, or the patient is a candidate to receive anti-VLA-4 therapy with increased testing for PML and, optionally, JCV status.

[0186] The assay provider can provide the results of the risk assessment, and optionally, conclusions regarding one or more of diagnosis, prognosis, or appropriate therapy options to, for example, a healthcare provider, or patient, or an insurance company, in any suitable format, such as by mail or electronically, or through an online database. The information collected and provided by the assay provider can be stored in a database. A healthcare provider or insurance provider or another entity can recommend, *e.g.,* to the patient or a second healthcare provider, that a patient undergo a risk assessment for PML as described herein.

[0187] PML risk stratification tools are useful as one component in making individual benefit-risk treatment decisions for patients taking or considering taking a VLA4 inhibitor or other therapeutics known to increase risk of developing PML. Quantification of a patient's PML risk can be used, for example, in benefit-risk analysis.

[0188] Headings, *e.g.,* (a), (b), (i) *etc,* are presented merely for ease of reading the specification and claims. The use of headings in the specification or claims does not require the steps or elements be performed in alphabetical or numerical order or the order in which they are presented.

[0189] The invention is further illustrated by the following examples, which should not be construed as further limiting.

EXAMPLES

Example 1. PML risk in MS patients was quantified for the first time using the two established risk factors and anti-JCV antibody status as determined by a unique, two-step VP1 VLP-based ELISA.

Methods

Patients, samples and data collection

[0190] Due to the infrequent occurrence of PML, data on natalizumab-treated PML patients were collected from several sources including post-marketing data from the Biogen Idec global natalizumab safety database and clinical trials as of March 4, 2011. Prior immunosuppressant use data were not available for all patients exposed to natalizumab; therefore, the proportion of patients with and without prior immunosuppressant use in the TYSABRI® Global Observational Program in Safety (TYGRIS; NCT00477113, NCT00483847) was used as an estimate for the overall natalizumab-treated population. TYGRIS is an observational cohort study designed to obtain long term safety data in natalizumab-treated MS patients in a clinical practice setting. Assessment of anti-JCV antibody prevalence in the general MS population was based on a single baseline plasma or serum sample collected from patients from four sources, including ongoing or completed natalizumab clinical studies (AFFIRM (Polman et al., N. Engl. J. Med. 354:899-910, 2006; STRATIFY-1 (NCT01070823), TYGRIS-US, and an independent MS registry in Sweden (available on the internet at msreg.net/cms/sv/home, accessed February 3, 2011)). A clinical plan was developed for wide-scale collection of serum and plasma samples obtained prior to PML diagnosis, including both clinical trial and post-marketing cases.

Identification of natalizumab treatment duration as a risk factor for PML

[0191] Estimates of PML incidence since natalizumab market reintroduction were calculated based on natalizumab post marketing exposure through February 28, 2011, and the number of confirmed PML cases as of May 2011. PML incidence for each time period (cumulative duration or 12-month treatment interval) was calculated using the number of patients that developed PML during that time period divided by the number of patients ever exposed to natalizumab for that amount of time.

Identification of prior immunosuppressant use as a risk factor for PML

[0192] Immunosuppressant treatment histories of natalizumab-treated MS patients that developed PML in the post-marketing setting and clinical trials were obtained from the Biogen Idec global natalizumab safety database as of November 2, 2010, and compared to the data obtained from TYGRIS. This cut-off date for prior immunosuppressant use history was chosen (versus March 4, 2011 for all other data) as it is expected that the inclusion of this as a risk factor in labeling (December 2010 in the EU) (Package Insert. TYSABRI® (natalizumab). Biogen Idec. Weston, MA July 2010; Summary of Product Characteristics. TYSABRI® (natalizumab). Biogen Idec. Weston, MA, December 13, 2010) would be a confounding factor.

Identification of anti-JCV antibody status as a risk factor for PML

[0193] The overall prevalence of anti-JCV antibodies in the general MS population was determined using a unique two-step VP1 VLP-based ELISA as previously described (Gorelik et al., Ann. Neurology, 2010). The prevalence of anti-JCV antibodies in MS patients with PML where pre-PML serum or plasma samples were available prior to diagnosis was also determined using this assay and compared to the overall prevalence in the general MS population.

Estimation of PML incidence by anti-JCV antibody serostatus

[0194] The incidence of PML in anti-JCV antibody positive and negative patients was estimated using the overall global incidence of PML, and the incidence after 25-48 infusions (the time point after which the increase in PML incidence was most pronounced in this analysis), the anti-JCV antibody prevalence in the general MS population, and the number of MS patients with PML who had pre-PML samples available that tested anti-JCV antibody positive prior to diagnosis. A one-sided Fisher's exact test was used to compare the estimated incidence of PML in anti-JCV antibody positive and anti-JCV antibody negative patients. To provide a conservative estimate of PML incidence in anti-JCV antibody negative patients, a sensitivity analysis was performed to assess the impact of a hypothetical anti-JCV antibody negative PML case on this estimate. Additionally, a sensitivity analysis was performed to assess the statistical certainty of this estimate by varying the number of anti-JCV antibody positive PML cases.

Quantification of PML risk: prior immunosuppressant use, natalizumab treatment duration, and anti-JCV antibody positive status

[0195] Risk factor algorithms were developed to estimate PML incidence in patients with and without certain risk factors for natalizumab associated PML and anti-JCV antibody serostatus. These algorithms estimated PML risk by prior immunosuppressant use (yes or no), natalizumab treatment duration (1-24 months and 25-48 months), and anti-JCV antibody status. These risk algorithms were based upon PML incidence data by natalizumab treatment duration (1-24 or 25-48 months) and estimates of prior immunosuppressant use in natalizumab-treated patients from TYGRIS and in those with PML. In addition, the estimated overall prevalence of anti-JCV antibodies in the general MS population was used to impute the incidence of PML associated with serostatus for the three-factor risk algorithm, assuming that all confirmed cases of natalizumab-associated PML were anti-JCV antibody positive prior to diagnosis. A sensitivity analysis was performed to quantify the effect of varying the estimates used to develop this three factor algorithm based upon the highest and lowest values observed.

Results

Identification of natalizumab treatment duration as a risk factor for PML

[0196] On a worldwide basis, 102 confirmed cases of PML were identified as of March 4, 2011. Overall, PML risk increased with increasing treatment duration (FIG. 1A), with the greatest increase in risk occurring after two years of therapy, peaking at 1.68 cases per 1000 patients in year three (FIG. 1B). Data beyond four year were limited.

Identification of prior immunosuppressant use as a risk factor for PML

[0197] Prior immunosuppressant use was more common in natalizumab treated patients who developed PML compared to patients enrolled in TYGRIS, which represented the overall patient population receiving natalizumab. Forty-five percent of natalizumab-treated PML patients had received one or more immunosuppressant therapies prior to initiating treatment with natalizumab, compared with 20.3% in natalizumab-treated patients (13.9% in the US and 23.6% in the EU) from TYGRIS. The most common prior immunosuppressants used in both the natalizumab-treated PML population and in TYGRIS included mitoxantrone, methotrexate, cyclophosphamide, azathioprine, and mycophenolate with no specific pattern being observed in the type of immunosuppressant, duration of use, or wash-out period between discontinuation of the immunosuppressant and initiation of natalizumab (Table 1).

Table 1. Prior immunosuppressant use history in natalizumab-associated PML patients and patients enrolled in TYGRIS.

Characteristics	All post-marketing confirmed PML cases with prior immunosuppressant use (N = 32)	TYGRIS patients with prior immunosuppressant use (N=792)
Prior immunosuppressants		
Mitoxantrone	18 (56%)	344 (43%)
Methotrexate	5 (16%)	45 (6%)
Azathioprine	5 (16%)	133 (17%)
Cyclophosphamide	6 (19%)	71 (9%)
Mycophenolate	4 (13%)	48 (6%)
Other	3 (9%)	201 (25%)
Duration of Prior immunosuppressant use	0.03-204 months	<1-24 months
Range	30·6 months	10·1 months
Mean		
Wash-out period		
Range	2-93 months	<1-24 months
Mean	24·7 months	8·5 months

Quantification of PML risk: natalizumab treatment duration and prior immunosuppressant use

[0198] When patients were stratified by natalizumab treatment duration (1-24 or 25-48 months) and prior immunosuppressant use (yes or no), four distinct subgroups of patients were identified with respect to incidence of PML (FIG. 2). Three of these subgroups had an estimated PML incidence of less than or approximately equal to 1 per 1000. PML risk was lowest in patients who were treated with natalizumab for 1-24 months, and who had not received prior immunosuppressant therapy, 0.19 per 1000 (95% CI: 0·10-0·33). The fourth subgroup, including patients who had both of these risk factors for PML, had the highest risk, with an estimated PML incidence of 4·3 per 1000 (95% CI: 2·9-6·2).

Identification of anti-JCV antibody status as a risk factor for PML

[0199] 5,896 patients from AFFIRM, TYGRIS-US, STRATIFY-1, and the Swedish MS Registry had a baseline sample available for anti-JCV antibody testing. Demographics, including natalizumab treatment duration and prior immunosuppressant use history, where available, were similar between these data sources (Table 2). The overall anti-JCV antibody prevalence in the general MS population assessed in this study was 55% (95% CI: 54-56%).

Table 2. Anti-JCV antibody prevalence and demographic data of general MS population.

	AFFIRM (N=823)	**TYGRIS-US (N=1480)**	**STRATIFY-1 (N=1096)**	**Swedish MS Patients (N=2497)**
Anti-JCV Antibody Prevalence	54·6% (51·1-58·0)	47·6% (45·0-50·1)	56·0% (53·0-59·0)	59·0% (57·0-60·9)
Age (years)				
Range	18-50	18-75	12-75	12-75
Mean	35.9	44.3	44.4	37.5
Median	36	44	45	37
Gender (%)				
Male	30·6%	24·1%	24·3%	28·1%
Female	69-4%	75·9%	75·7%	71·9%
Geography	North America and EU/ Rest of World	US and Canada	US	Sweden
Prior immunosuppressant				
Use (%)				
Yes	3·6%	8·8%	3·8%	NA
No	96-4%	91·2%	96·2%	NA

[0200] In the TYGRIS-US dataset, 1451 of the 1480 patients had age, gender and prior immunosuppressant information available. In the STRATIFY-1 dataset, 988 of 1096 patients had prior immunosuppressant information available. In the Swedish MS dataset, 2464 of 2497 patients had age information available and 2494 of 2497 patients had gender information available. Prior immunosuppressant use was not available (NA) in the Swedish MS patients.

[0201] One or more pre-PML samples were obtained from 25 natalizumab-treated MS patients 6.5-187 months prior to PML diagnosis. As shown in Supplemental Table 1, these 25 patients had clinical characteristics that were similar to the 102 confirmed PML cases worldwide, indicating no obvious selection bias. All patients for whom multiple pre-PML samples were available tested anti-JCV antibody positive at all time points, including those samples collected prior to initiation of natalizumab treatment. The 100% (25 out of 25) anti-JCV antibody positive prevalence in MS natalizumab-treated PML patients prior to PML diagnosis was significantly different from the expected 55% prevalence observed in the general MS population ($p<0·0001$), demonstrating the ability of anti-JCV antibody status to serve as an additional PML risk stratification tool.

Supplemental Table 1. Clinical characteristics of 25 MS PML patients with Pre-PML samples compared to all 102 post-marketing PML cases.

Characteristics		MS patients with pre-PML samples - all tested positive for anti-JCV antibody (N = 25)	All post-marketing confirmed PML cases (N = 102)
Geographic distribution			
	US	4(16%)	42(41%)
	Europe/ROW	21 (84%)	60 (59%)
Age at diagnosis			
	Range	27 - 55	23 - 67
	Mean	40·7	44·6
	Median	41	44
Gender			
	Male	8 (32%)	32 (31%)
	Female	17 (68%)	70 (69%)
Duration of MS at diagnosis (years)			
	Range	1·5-21	1·5-23
	Mean	12·2	11·9
	Median	12·3	11·1
TYSABRI® exposure (month)			
	Range	17-51	12-52
	Mean	33·0	30·8
	Median	32	30
Prior immunosuppressants			
	Yes	9(38%)	39 (42%)
	No	15 (63%)	54 (58%)

[0202] MS onset date was unknown for 5 patients in the anti-JCV antibody positive group and 38 patients in all PML group. Prior immunosuppressant use status was unknown for 1 patient in anti-JCV antibody positive group and 9 patients in the overall PML group.

Estimation of PML incidence by anti-JCV antibody serostatus

[0203] The incidence of PML in patients who were anti-JCV antibody positive was estimated to be almost 2-fold that of the overall natalizumab-treated population (Table 4). To estimate the overall incidence of PML by anti-JCV antibody status, the following method was used: Based on the 25 natalizumab-treated MS patients with pre-PML samples available, it was estimated that the 25 MS patients with PML came from approximately 20276 patients receiving natalizumab treatment, based upon the overall rate of PML, 1.23 per 1000 patients (FIG. 1). Assuming that 55% of these 20276 patients were anti-JCV antibody positive (*i.e.,* 11152 patients) and 45% were anti-JCV antibody negative (*i.e.,* 9124 patients), the incidence of PML in anti-JCV antibody positive patients was estimated to be 2.24 cases per 1000 patients treated (=1000×25/11152), 95% CI: 1.45-3.31, similar to the rate estimated in the literature (Tyler, Ann. Neurol. 68:271-274, 2010). Conversely, the estimated incidence of PML in anti-JCV antibody negative patients was 0 cases per 1000 patients (95% CI: 0-0·40), significantly different from the estimated incidence in anti-JCV antibody positive patients, p<0·0001.

Table 4. Estimated incidence of PML by anti-JCV antibody status based on 25 cases of PML that were anti-JCV antibody positive prior to the onset of PML.

	Number of PML Cases	Total Patients Treated	Incidence per 1000 patients (95% CI)
Overall PML incidence in natalizumab-treated MS patients			
Anti-JCV Antibody Positive	25	11152	2·24 (1·45, 3·31)

(continued)

	Number of PML Cases	Total Patients Treated	Incidence per 1000 patients (95% CI)
Overall PML incidence in natalizumab-treated MS patients			
Anti-JCV Antibody Negative	0	9124	0(0, 0·40)
Total	25	20276	1·23 (0·80, 1·82)
P-value		<0·0001	
RR (95% CI)		∞ (6·44, ∞)	
PML incidence after 25-48 months of natalizumab therapy			
Anti-JCV Antibody Positive	18	4533	3.97 (2·36, 6·27)
Anti-JCV Antibody Negative	0	3709	0 (0, 0·99)
Total	18	8242	2·18 (1·30, 3·45)
P-value		<0·0001	
RR (95% CI)		∞ (5·63, ∞)	
Sensitivity analysis: assumption of 1 anti-JCV antibody negative hypothetical PML patient			
Anti-JCV Antibody Positive	25	11598	2·16 (1·40, 3·18)
Anti-JCV Antibody Negative	1	9489	0·11 (0·00, 0·59)
Total	26	21087	1·23 (0·81, 1·81)
P-value		<0·0001	
RR (95% CI)		20·5 (3·35, 842)	

[0204] The effect of natalizumab treatment duration on this estimate was assessed in an analysis using the PML incidence after 25-48 months of natalizumab (the duration of therapy after which the increase in incidence was most pronounced). The risk of PML after 25-48 months of therapy in anti-JCV antibody positive patients was 3.97 per 1000 (95% CI: 2.36-6·27) and in anti-JCV antibody negative patients was 0 per 1,000 (95% CI: 0-0.99), Table 3.

[0205] At the time of this writing, the incidence of PML in anti-JCV antibody negative patients could not be fully ascertained because no PML case has tested anti-JCV antibody negative prior to diagnosis. Therefore, to estimate the incidence of PML in anti-JCV antibody negative patients, a sensitivity analysis was performed assuming a hypothetical case of PML had occurred in an anti-JCV antibody negative patient, thus allowing for determination of a conservative estimate for which the rate is likely lower. This analysis demonstrated an estimated incidence of PML in anti-JCV antibody negative patients of at least 20-fold lower than in anti-JCV antibody positive patients, p<0·0001 (Table 3).

[0206] Sensitivity analysis of the effect of increasing the number of anti-JCV antibody positive PML patients with Pre-PML samples available that tested anti-JCV antibody positive demonstrated that statistical certainty regarding the increased risk of PML in anti-JCV antibody positive patients was not improved beyond 25 available Pre-PML samples (Table 5).

Table 5. Effect of increasing anti-JCV antibody positive PML case numbers on statistical certainty of PML incidence estimations in anti-JCV antibody positive patients.

Number of anti-JCV antibody positive PML cases that tested prior to PML diagnosis	PML Incidence per 1000 Patients				1-sided p-value:
	Anti-JCV antibody negative		Anti-JCV antibody positive		
	Incidence	95% CI	Incidence	95% CI	
10		0, 1·01		1·08, 4·12	p=0025
25	0	0, 0·40	2·24	1·45, 3·31	P<0·0001
30		0, 0·34		1·51, 3·20	
40		0, 0·25		1·60, 3·05	
50		0, 0·20		1·66, 2·95	
60		0, 0·17		1·71, 2·88	

Quantification of PML risk: natalizumab treatment duration, prior immunosuppressant use, and anti-JCV antibody status

[0207] A combined, quantitative PML risk algorithm was developed for natalizumab-treated MS patients based on natalizumab treatment duration, prior immunosuppressant use, and anti-JCV antibody status (FIG. 3). Because JCV exposure is a requirement for PML, patients who are anti-JCV antibody negative represented the lowest risk sub-group in the PML risk stratification algorithm, with an estimated risk of ≤ 0.11 per 1000 (95% CI: 0-0·59), based on the conservative estimate determined in the sensitivity analysis. Conversely, the highest risk group consists of those patients that are anti-JCV antibody positive, with prior immunosuppressant use, and who have been treated with natalizumab for 25-28 months. This algorithm assumed that all 102 confirmed cases of PML were anti-JCV antibody positive prior to PML diagnosis. For the higher risk sub-group (patients who had all three risk factors) the estimated PML risk was approximately 7.8 per 1000 (95% CI: 5·2-11·3). For patients who were anti-JCV antibody positive with no prior immunosuppressant use, PML risk was consistent with risk in the overall natalizumab-treated population at similar time points (FIGs. 1A and 1B). Sensitivity analysis of the effect of varying the estimates used to develop this algorithm resulted in minimum and maximum values that were generally consistent with the original estimates seen in the base case scenario (see FIGs. 4A and 4B). The risk in anti-JCV antibody negative patients was determined by assuming a hypothetical case of PML had occurred in an anti-JCV antibody negative patient.

[0208] This analysis varied anti-JCV antibody prevalence in the general MS population from 48% (as seen in TYGRIS-US) to 59% (as seen in the independent Swedish Registry), prior immunosuppressant use in the natalizumab-treated MS population from 14-24% (based upon US and EU estimates in TYGRIS, respectively), and natalizumab 25-48 month exposure estimates from 35-45% (based upon the current estimate of 40% and an approximate 10% increase over the past year). Plots represent point estimates and 95% confidence intervals for each scenario (base case, minimum, and maximum). In general, the base case scenario was relatively consistent with the minimum and maximum estimates.

[0209] Since JCV infection is required for PML development, the lowest risk of PML was determined to be in patients who were anti-JCV antibody negative, ≤ 0·11 cases per 1000 natalizumab-treated patients (95% CI 0-0.59), irrespective of other risk factors, at least 20-fold lower than in anti-JCV antibody positive patients, p<0.0001. Although there have been no cases of PML in anti-JCV antibody negative patients to date, the true risk of developing PML cannot be zero because the anti-JCV antibody assay has an estimated analytical false-negative rate between 2.5-3.2%.[1, 19] Conversely, PML risk was highest in patients who possessed all three risk factors (natalizumab treatment for 25-48 months, prior immunosuppressant use, and anti-JCV antibody positive status) with an estimated incidence of 7.8 cases per 1000 patients (95% CI: 5·2-11·3).

Example 2. The anti-JCV antibody ELISA was validated at clinical laboratories to demonstrate the robustness of the method.

[0210] A novel, 2-step enzyme-linked immunosorbent assay (ELISA) that detects anti-JCV antibodies in human serum or plasma was recently described (see PCT/US2011/020832). The key attributes of the assay include both direct binding and in-solution competition components; use of well characterized preparations of JC virus-like particles (VLP); inclusion of appropriate quality control (QC) samples; statistical determination of assay cut points using a large number of longitudinally collected clinical samples; normalization of the signal of the assay; and detection of several isotypes of anti-JCV antibodies (including IgG, IgM, IgA, and IgE).

[0211] The anti-JCV antibody ELISA was validated at three clinical laboratories in order to demonstrate the robustness of the method. Analytical validation was performed by evaluation of intra- and inter-assay precision, analytical specificity and sensitivity, matrix interference, robustness, and reagent stability.

[0212] Stability of anti-JCV antibodies in serum and plasma samples was demonstrated by using assay QC samples prepared from pooled human sera as well as serum and plasma samples from individual donors. Anti-JCV antibodies were shown to be stable in serum or plasma through 6 freeze/thaw cycles, and for 14 days when stored both at ambient (18-25°C) temperature and at 2-8°C. Additionally, stability of anti-JCV antibodies in whole blood stored at 2-8°C, at ambient temperatures (18-25°C), or at 37°C for 7 days, 7 days, and 3 days, respectively, prior to processing was also shown using both serum and plasma collection tubes. Stability of the JC VLP was shown through 4 freeze/thaw cycles and for 18 months at 2-8°C.

[0213] The analytical validation demonstrated that the assay is sensitive, specific, and precise. The assay sensitivity was estimated at 1.7 ng/mL using a humanized anti-JCV monoclonal antibody control, and was estimated at 1.25 μg/mL using a purified polyclonal antibody from anti-JCV antibody positive sera. The sensitivity to detect JCV infection was estimated to be 97.5%. The specificity of the assay to discriminate JCV specific antibodies from antibodies directed to BK virus, a related polyomavirus, was also demonstrated. The average inter- and intra-assay precision was approximately 6.4% and 12.2% for the screening step and 2.6% and 5.3% for the confirmation step. Results obtained for plasma and serum were highly congruent, and assay robustness was demonstrated by the highly concordant results generated by

3 laboratories testing a panel of 100 blinded samples.

Example 3. A refined two-step JCV assay (the Gen2 Assay) provides more accurate results than the original assay (Gen1Assay).

[0214] The two-step anti-JCV antibody assay was modified following optimization rounds. The new assay differs from the first in at least the follow ways:

- HPVLP is used at a substrate concentration of 0.4 μg/mL on plates in the first step, and in solution in the confirmatory assay, as opposed to 1 μg/mL used in the Gen1 assay;
- Patient serum is diluted 1:101 prior to applying to HPVLP on plates in the first step of the assay, or to HPVLP in solution in the confirmatory assay, as opposed to 1:200 in the Gen1 assay;
- The secondary reagent (anti-human IgG) conjugated to HRP is typically diluted 1:20,000 (but may have to be readjusted for new lots to match signal to previous lot), and the incubation time with the conjugate is only 30 min. In the Gen1 assay, the same reagent was diluted 1:80,000 and incubation time was 60 min;
- the binding reaction is assayed by incubating the HRP substrate TMB for 20 minutes ± 2 minutes, whereas in Gen1, the TMB incubation was for 20 minutes ± 5 minutes;
- In the confirmation assay, 10 μl of sample is added to 1 mL of confirmation buffer (1:101 dilution), and the reaction proceeds for 10 to 20 minutes. In the Gen1 assay, a 2x concentration of sample (1:100 dilution) and HPVLP (2 μg/mL) was mixed in equal proportion and then incubated for 60 minutes.
- The cut-off calibrator (CO) is adjusted to have a reactivity index of about nOD 1.0, and a positive control (PC) is adjusted to have a reactivity index of about nOD 1.3). The CO and PC are made by mixing an anti-JCV antibody positive serum and an anti-JCV antibody negative serum. For the negative control (NC), which is typically bottle negative sera, the reactivity index target is about 0.1;. Qualitatively, the controls come from different pools of human serum, but from an assay target concentration, they are similar to the Gen 1 control levels.

[0215] The JCV Gen2 clinical agreement study results are summarized in Table 6 below. All Gen1 testing was performed at a Focus Diagnostics reference laboratory (Cypress, CA), and the testing sites for the Gen2 assay included Denver (n: overall= 275; on TYSABRI®= 149; Naïve= 126); New York (n: overall= 275; on TYSABRI®= 136; Naïve= 139); and Focus Diagnostics (n= overall 262; on TYSABRI®= 95; Naïve= 167). Percent agreement is expressed as (Gen2/Gen1), lower and upper bound of the 96% confidence interval (95% CI: LB to UB).

Table 6 . JCV Gen2 Clinical Agreement Study Results

	Overall		**Patients on TYSABRI®**		**Naïve Patients**	
Testing Site	Negative % Agreement (NPA)	Positive % Agreement (PPA)	Negative % Agreement (NPA)	Positive % Agreement (PPA)	Negative % Agreement (NPA)	Positive % Agreement (PPA)
Denver	86.5% (115/133) 95% CI:79.6 to 91.3%	97.2% (138/142) 95% CI:93 to 98.9%	85.7% (60/70) 95% CI:75.7 to 92.1%	94.9%(75/79) 95% CI:87.7 to 98%	87.3% (55/63) 95% CI:76.9 to 93.4%	100% (63/63) 95% CI:94.3 to 100%
New York	88.8% (119/134) 95% CI:82.4 to 93.1%	100% (141/141) 95% CI:97.3 to 100%	89.5% (51/57) 95% CI:78.9 to 95.1%	100%(79/79) 95% CI:95.4 to 100%	88.3% (68/77) 95% CI:79.3 to 93.7%	100% (62/62) 95% CI:94.2 to 100%

(continued)

	Overall		Patients on TYSABRI®		Naïve Patients	
Testing Site	Negative % Agreement (NPA)	Positive % Agreement (PPA)	Negative % Agreement (NPA)	Positive % Agreement (PPA)	Negative % Agreement (NPA)	Positive % Agreement (PPA)
Focus Diagnostics	90.2% (101/112) 95% CI:83.3 to 94.4%	100% (150/150) 95% CI:97.5 to 100%	87.2% (34/39) 95% CI:73.3 to 94.4%	100%(56/56) 95% CI:93.6 to 100%	91.8% (67/73) 95% CI:83.2 to 96.2%	100% (94/94) 95% CI:96.1 to 100%

Example 4. Anti-JCV antibody status can be used to categorize a patient's risk for PML.

[0216] We hypothesized that anti-JCV antibody positive patients could be further stratified for the risk of developing PML based on anti-JCV antibody titers (nOD or index) and anti-JCV antibody avidity/affinity (% inhibition). This hypothesis was derived from the observation that patients having an anti-JCV antibody titer and % inhibition below a predetermined level ("a clinical cut-point") are at lower risk for developing PML compared to the overall anti-JCV antibody positive population. To determine the relative risk of PML suggested by the status of antibody titer and percent inhibition, anti-JCV antibody titers and percent inhibition could be made prior to initiation of TYSABRI® (natalizumab) or when patients are already on TYSABRI®.

[0217] To determine the relative risk of PML suggested by the status of antibody titer and percent inhibition in combination with other risk factors, pre-existing data from two different anti-JCV antibody assays ("Generation I" and "Generation II") was collected and analyzed. The pre-existing data included anti-JCV antibody titer information expressed as "nOD" or "index."

[0218] In the Generation I assay, 22% (77/356) of anti-JCV antibody positive patients had nODs > 1.0 (C-1801), and 34% (13/38) of anti-JCV antibody positive PML patients had nODs >1.0 (C-1801). Thus ~1.5 fold more PML patients have an nOD > 1.0 as compared to non-PML patients. This translates to a 2-3-fold risk ratio associated with an nOD>1.0.

[0219] 6% of TYSABRI® non-PML patients were also observed to have >2 fold change in anti-JCV antibody titer (nOD) for longitudinal samples collected over >2 years (C-1801). However, the majority of PML patients with longitudinal samples collected at informative timepoints (>1 year before PML diagnosis, and within 6 month of PML diagnosis and at PML diagnosis) demonstrated a >2 fold increase in anti-JCV antibody titer (nOD). This suggests that patients who do not exhibit a significant change in JCV titer over time are at a lower risk of developing PML.

[0220] Exemplary nOD and antibody titers are provided in FIGs. 5A-10B. Patient data is summarized in Table 7.

[0221] A graph of the statistical analysis is shown in FIG. 11.

[0222] Statistical analysis indicated that for percent inhibition, ~17% of antibody-positive samples are less than 0.502, and ~0% of PML samples are less than 0.502. ~30% of antibody-positive samples are less than 70% inhibition, and ~0% of PML samples (with index <3.0) are less than 70% inhibition.

Table 7. anti-JCV antibody titers and nOD measurements in patients.

Patient	Diagnosis	nODs or Index	Titers
1	09Oct09	Ratio: 1.077/0.258= ~4 fold between first and second test	Ratio: 5400/600= 9 fold between first and second test
2	16Feb05	Consistently high (>1.0)	Consistently high (16200)
3	08Oct09	Ratio: 0.385/0.129= ~3 fold between first and second test (increased)	Ratio: 600/200= 3 between first and second test (increased)
4	16Feb10	Ratio: 0.628/0.309= ~2 fold between first and second test (increased)	Ratio: 1800/600= 3 between first and second test (increased)
5	14Jun09	No increase	No increase
6	Unknown	No increase	No increase

(continued)

Patient	Diagnosis	nODs or Index	Titers
7	11Jul08	Ratio: 0.639/0.226= ~2.8 between first and second test (increased)	Ratio: 1800/200= 9 between first and second test (increased)

Reference Example 5. An analytically validated anti-JCV-antibody assay has been introduced into clinical practice to stratify MS patients for higher or lower risk of PML.

[0223] The aim of the below study was to assess anti-JCV-antibody titer changes prior to and after initiation of treatment with natalizumab.

[0224] The anti-JCV antibody assay (Gorelik et al., Ann. Neurol. 2010) was applied to samples of Swedish MS patients treated with natalizumab, including five PML positive patients. Normalized OD (nOD) values of the anti-JCV antibody assay were studied before and during treatment with natalizumab. Positive samples were diluted in 1:3 dilution steps to determine titer levels. A proportion of the same patients was also tested for antibodies towards a nuclear human cytomegalovirus (CMV) antigen (Schmitz et al., J. Clin. Microbiol. 1977), and antibodies towards the recombinant varicella-zoster (VZV) glycoprotein E antigen (Thomsson, J. Virol. Methods 2011).

Table 8. Patients tested for anti-JCV antibodies.

Characteristics	Female	Male	All
Patients (n, %)	603 (70%)	258 (30%)	861
Age (median, range)	37 (13-60)	36 (12-63)	36 (12-63)
Time between paired sampling (median, range)	12 (1-38)	12 (1-36)	12 (1-38)
Patients with time between paired sampling 1 to 8 month (n, % all patients)			244 (28%)
Patients with time between paired sampling 8 to 18 month (n, % all patients)			296 (34%)
Patients with time between paired sampling >18 month (n, % all patients)			321 (37%)

[0225] After initiation of natalizumab treatment, the anti-JCV antibody levels remained relatively stable with a mild decline of nOD levels observed in the anti-JCV positive patients. The apparent decline in anti-JCV antibody levels (nOD) was observed when patients were on natalizumab treatment (n=471), but not during the preceding interferon beta therapy (n=210). This indicates a potential effect of natalizumab therapy on the anti-JCV antibody levels, without significantly affecting the serological status and the seropositivity rate (pre: 56%; post 55%).

[0226] After initiation of natalizumab treatment, the anti-VZV (OD) (n=715), but not the anti-CMV (n=502) antibody levels declined slightly.

[0227] For the 5 patients who developed PML, the observed change in levels of anti-JCV antibodies (nOD) in serum is summarized in the below Table 9. Levels of anti-JCV antibodies (nOD) in serum increased at the time of PML diagnosis compared with the baseline values.

Table 9. Change in nOD during natalizumab treatment

PML Patient	Months treated with natalizumab prior to PML diagnosis	∆nOD between time of initiation of natalizumab treatment and time of PML diagnosis
1	-25	0.348
2	-29	0.284
3	-34	0.190
4	-49	0.932
5	-25	0.175

[0228] From this study we concluded that the therapy with natalizumab may lead to a mild decrease in anti-JCV

antibody levels (nOD) without affecting the JCV seropositivity rate. Notably, only 5% of the anti-JCV positive reference population demonstrated a change in nOD values (ΔnOD) above 0.151 (95%-percentile), while this was observed in all the 5 Swedish cases of PML at time of diagnosis compared with the baseline values. Thus, the investigation of an increase in anti-JCV antibody levels during natalizumab therapy, prior to PML diagnosis, in the context of PML risk stratification is warranted.

Example 6. Use of a clinical cut-off distinct from an analytical cut-off to delineate high and low risk groups among anti-JCV antibody positive patients.

[0229] Results from the Stratify I study were used to determine a clinical cut-off distinct from an analytical cut-off to delineate high and low risk groups among anti-JCV antibody positive patients. Thus, a patient's risk of PML would be initially based on baseline anti-JCV antibody titer levels. The Generation II anti-JCV antibody assay was used in this study.

[0230] TYSABRI® non-PML patients (Stratify I, n= 1044) and PML patients (> 6 months prior to PML diagnosis (n= 38) were evaluated (FIG. 12). In the Generation II assay, 17% of anti-JCV antibody positive patients had titers (index) below the lowest titer (index) observed for samples from PML patients collected >6 months prior to PML diagnosis, suggesting that those patients may have lower risk for developing PML (like anti-JCV antibody negative patients). Additionally 50% of anti-JCV antibody positive patients had titers (index) below index 1.5, compared to only 13% PML patients from whom samples were collected >6 months prior to PML diagnosis had index <1.5. Also, only 4.4% of the PML patients not known to previously receive immunosuppressant agents had samples with index <1.5, suggesting that those patients may have lower risk for developing PML compared to patient5s with high anti-JCV antibody titer (nOD or index).

[0231] Patients having an nOD < 0.5 (109/1044 (10.4% of total samples) or 109/549 (20% of anti-JCV antibody positive patients)) were determined to be in the lowest PML risk group (potentially as low as anti-JCV antibody negative patients), as no PML patients had an index<0.5. Patients having index >0.5 but <1.5 were determined to be in the lower risk zone, as 50% of non-PML anti-JCV antibody positive patients and only 13% of PML patients, respectively, had samples in this zone. Additionally, only 4% of PML patients who were not known to receive prior immunosuppressive therapies, had samples with index <1.5 (FIG. 13). Patients having an index >1.5 (271/549 (50%) of anti-JCV antibody positive population) were determined to be at higher risk for PML. Forty seven percent of patients were anti-JCV antibody negative.

[0232] Post-PML diagnosis, patients are subjected to immune-adsorption (IA) or plasma exchange (PLEX) to remove circulating natalizumab and to restore immune function. The anti-JCV antibody levels are rapidly restored to pre-procedure levels in these patients.

Example 7. Proposed statistical methodology for assigning stratified risks to Multiple Sclerosis (MS) patients undergoing TYSABRI® treatment who have already tested positive for anti-JCV antibodies in the refined two-step anti-JCV assay described herein.

[0233] For the STRATIFY-II (American Academy of Neurology (AAN) Meeting, April 21-28, 2012, abstract S041.002) study, two alternative strategies (denoted as Strategies 1 and 2) for assigning PML risk to anti-JCV sero-positives will be evaluated. Strategy-1, the more conservative of the two methods, will be a refinement of one of the nonparametric bivariate tolerance regions provided in the attached report. Strategy-2, whose statistical methodology is sketched below, should assign a higher proportion of future anti-JCV sero-positives to low risk of developing PML compared with Strategy-1.

[0234] Strategy-2 devises a lower simultaneous tolerance region around a fitted equation that measures %-inhibition vs. index for a PML patient sample. Whereas Strategy-1 constructs a low risk region based on the %-inhibition/index measurements from the two-step anti-JCV assay in STRATA (Ann. Neurol., 68:295-303, 2010) and STRATIFY-I (Ann. Neurol., 70:742-750, 2011) patients (almost all of which are assumed to have very low risk of developing PML), Strategy-2 constructs a high risk region based on measurements collected from MS patients prior to their dates of PML diagnosis. Though our limited collection of PML samples may not be representative of the entire universe of Tysabri treated MS patients infected with PML, Strategy-2 assumes that the %-inhibition vs. index relationship in these samples is representative of the PML universe prior to diagnosis. This assumption was statistically supported by anti-JCV PML data showing a %-inhibition vs. index relationship parallel to that of the STRATIFY-1+STRATA. It is this parallelism that exploited by Strategy-2 to model the PML %-inhibition vs. index relationship.

[0235] The relationship between %-inhibition and index will be first statistically modeled for the combined set of STRATIFY-1/STRATA/PML samples. The fitted equation to %-inhibition vs. index will distinguish between PML and STRATIFY-1/STRATA samples. A lower simultaneous 95% or 99% tolerance region will then be constructed around the fitted equation constrained for PML samples. Future anti-JCV sero-positives with %-inhibition/index measurements falling inside this tolerance region will be assigned higher risk of developing PML; this tolerance region should guarantee that at least 95% (or 99%) of samples from PML patients prior to their diagnosis will be assigned to higher risk. Note that

future samples with index measurements > 2.5 will automatically be assigned to higher risk of developing PML.

[0236] *Statistical Details*. The following mixed model (or some refinement of it) will be first fit using the SAS MIXED procedure to a combined set of Gen-2 anti-JCV STRATIFY-1 + STRATA + PML sample %-inhibition/index measurements.

$$Y_{ij} = \beta_0 + \delta \times W + \beta_1 \times X^+ + \beta_{11} \times X^+ \times X^+ + \pi_i + \varepsilon_{ij} \quad (1)$$

where

Y (or log-ratio)= $-\log_e\{1- \%inhibition/100\}$;
X^+ = index if index< x_0
= x_0 for $x_0 \leq$ index ≤ 2.5;
W=0 if the sample was collected from a STRATIFY-1 or STRATA patient
=1 if the sample was collected from a PML patient prior to diagnosis;
π_i =random effect for patient i;
ε_{ij} =random assay measurement+longitudinal error for jth time point of ith patient;

[0237] ε_{ij} 's are assumed normally and independently distributed with mean zero and variance σ^2; π_i's are assumed normally and independently distributed with mean zero and variance σ_p^2 ; ε_{ij}'s and π_i's are assumed independent. Thus for mixed model in equation (1), X^+ and W are fixed effects, while π_i and ε_{ij} are random effects. A preliminary estimate of x_0 was 1.77, but that will be refined. A lower 95 or 99% simultaneous tolerance region around the fitted equation will be constructed (refs. 1-5). Lower boundaries of the tolerance region as a function of index level will then be back transformed into %-inhibition. Future samples whose %-inhibition/index measurements either fall into the tolerance region or have index measurements > 2.5 will be judged at higher risk of developing PML.

[0238] The below Table 10 provides estimated percentages of anti-JCV antibody positives that will be classified as lower risk based on different nODs.

Table 10. Estimated percentages of anti-JCV antibody positives that will be classified as lower risk based on different nODs.

Index Measurement Rule for Assignment to Lower PML Risk	Empirical Percentage of non-PML STRATIFY-1 Sero-positives Assigned to Lower Risk of Developing PML	Estimated Percentage of Future non-PML Sero-positives Assigned to Lower PML Risk with 95% Confidence	Estimated Proportion of Pre-PML Sero-positives misclassified at Lower PML Risk (based on a fitted Weibull to 39 independent patient samples collected prior to diagnosis)**	Estimated Percentage of Future pre-PML Sero-positives Misclassified at Lower PML Risk (with 95% Confidence certainty)	Empirical Percentage of In-house collection of pre-PML Sero-positives misclassified at lower PML risk *
≤0.40	11.1% (66/595)	≥9.2%	0.4%	≤1.2%	0% (0/153)
≤0.50	16.0% (95/595)	≥13.7%	0.8%	≤2.0%	0.65% (1/153)
≤0.65	22.7% (135/595)	≥20.0%	1.7%	≤3.6%	4.58% (7/153)
≤0.70	25.2% (205/595)	≥22.5%	2.0%	≤4.2%	4.58% (7/153)
≤0.75	25.7% (153/595)	≥22.9%	2.4%	≤5.0%	8.50% (13/153)
≤1.00	34.5% (205/595)	≥31.4%	5.4%	≤9.6%	11.76% (18/153)

(continued)

Index Measurement Rule for Assignment to Lower PML Risk	Empirical Percentage of non-PML STRATIFY-1 Sero-positives Assigned to Lower Risk of Developing PML	Estimated Percentage of Future non-PML Sero-positives Assigned to Lower PML Risk with 95% Confidence	Estimated Proportion of Pre-PML Sero-positives misclassified at Lower PML Risk (based on a fitted Weibull to 39 independent patient samples collected prior to diagnosis)**	Estimated Percentage of Future pre-PML Sero-positives Misclassified at Lower PML Risk (with 95% Confidence certainty)	Empirical Percentage of In-house collection of pre-PML Sero-positives misclassified at lower PML risk *
≤1.25	41.0% (244/595)	≥37.8%	9.7%	≤15.7%	15.69% (24/153)
≤1.50	46.9% (279/595)	≥43.6%	15.6%	≤22.2%%	22.22% (34/153)
*Biased estimates of pre-PML population due to multiple and unequal numbers of measurements within patient donors. ** Estimated fit based on average of 1000 simulations where 1 time point per pre-PML patient was randomly selected.					

Claims

1. A method of evaluating a patient's risk of developing Progressive Multifocal Leukoencephalopathy (PML), the method comprising:

   i) determining, in a serum or plasma sample of the patient, an anti-JC Virus (JCV) antibody titer, wherein the anti-JCV antibody titer is determined by an ELISA assay comprising the following steps:

   (a) forming a reaction mixture comprising an aliquot of sample and a substrate on which is disposed Highly Purified Viral-Like Particles (HPVLPs), and
   (b) detecting the level of anti-JCV antibody bound to said substrate on which is disposed HPVLPs;

   wherein the anti-JCV antibody titer is expressed as an index value, wherein the index value is determined by normalizing an optical density (OD) value of the sample to a cut-off calibrator adjusted to have an nOD of 1, and a positive control is adjusted to have an nOD of 1.3; wherein the cut-off calibrator and positive control comprise a mixture of serum positive for anti-JCV antibodies and serum negative for anti-JCV antibodies, and wherein a negative control comprises anti-JCV antibody negative serum and has an nOD of 0.1;
   and
   ii) determining the patient to be at high risk of developing PML if the anti-JCV antibody index value is determined to be > 1.5.

2. The method of claim 1, wherein

   the anti-JCV antibody titer is expressed as an index value for a first reaction mixture comprising a first aliquot of the serum or plasma sample of the patient and a substrate on which is disposed HPVLP; and
   in a second step, a % inhibition indicative of a degree to which incubation with soluble-phase HPVLP reduces a level of unbound anti-JCV antibody that binds to HPVLP disposed on a substrate as compared to the first reaction mixture, is determined in a second reaction mixture comprising a second aliquot of the serum or plasma sample of the patient and a substrate on which is disposed HPVLP; and
   determining the patient to be at high risk of developing PML if the anti-JCV antibody index value is determined to be > 1.5 and % inhibition is determined to be > 70%.

3. The method according to claim 1 or claim 2, wherein the anti-JCV antibody titer or % inhibition is determined prior to an administration of natalizumab.

4. The method according to any one of claims 1 to 3, wherein the anti-JCV antibody titer or % inhibition is determined after the patient has initiated a treatment with natalizumab.

5. The method according to any one of claims 1 to 4, further comprising:

(a) determining if the patient has received treatment with natalizumab for longer than 24 months; or
(b) determining if the patient has received a non-anti-VLA-4 immunosuppressant therapy, wherein the non-anti-VLA-4 immunosuppressant therapy is selected from mitoxantrone, methotrexate, azathioprine, cyclophosphamide, mycophenolate, anti-CD20 therapy, anti-CD11a therapy, and mycophenolate mofetil.

6. The method according to any one of claims 1 to 5, wherein the anti-JCV antibody titer or % inhibition is retested at 6 month or 12 month intervals.

7. The method according to claim 6, wherein an increase in anti-JCV antibody titer or % inhibition indicates an increase in the patient's risk of developing PML.

8. The method according to any one of claims 1 to 7, wherein the patient has multiple sclerosis.

9. The method according to any one of claims 1 to 8, wherein the patient determined to be at high risk of developing PML is determined to be at higher risk of developing PML if the patient has received natalizumab for longer than 24 months and has not previously received a non-anti-VLA-4 immunosuppressant therapy, wherein the non-anti-VLA-4 immunosuppressant therapy is selected from mitoxantrone, methotrexate, azathioprine, cyclophosphamide, mycophenolate, anti-CD20 therapy, anti-CD11a therapy, and mycophenolate mofetil.

Patentansprüche

1. Verfahren zum Beurteilen des Risikos eines Patienten, progressive multifokale Leukenzephalopathie (PML) zu entwickeln, wobei das Verfahren umfasst:

i) Bestimmen, in einer Serum- oder Plasmaprobe des Patienten, eines Anti-JC-Virus(JCV)-Antikörperspiegels, wobei der Anti-JCV-Antikörperspiegel durch ein ELISA-Assay bestimmt wird, das folgende Schritte umfasst:

(a) Bilden eines Reaktionsgemischs, das eine Teilprobe der Probe und einen Träger umfasst, auf dem hochreine virenartige Partikel (HPVLP) angeordnet sind,
(b) Nachweisen des Aktivitätsmaßes von Anti-JCV-Antikörper, der an den Träger gebunden ist, auf dem HPVLP angeordnet sind;
wobei der Anti-JCV-Antikörperspiegel als ein Indexwert ausgedrückt ist, wobei der Indexwert durch Normieren eines Werts der optischen Dichte (OD) der Probe auf einen Cut-off-Kalibrator, der auf eine nOD von 1 eingestellt ist, und eine positive Kontrolle auf eine nOD von 1,3 eingestellt ist; wobei der Cut-off-Kalibrator und die positive Kontrolle ein Gemisch an Serum, das für Anti-JCV-Antikörper positiv ist, und an Serum, das für Anti-JCV-Antikörper negativ ist, umfassen, und wobei eine negative Kontrolle ein anti-JCV-Antikörper-negatives Serum umfasst und eine nOD von 0,1 aufweist;
und

ii) Bestimmen, dass der Patient ein hohes Risiko zur Entwicklung von PML aufweist, wenn bestimmt wird, dass der Anti-JCV-Antikörper-Indexwert > 1,5 ist.

2. Verfahren nach Anspruch 1, wobei:

der Anti-JCV-Antikörperspiegel als ein Indexwert für ein erstes Reaktionsgemisch ausgedrückt wird, das eine erste Teilprobe der Serum- oder Plasmaprobe des Patienten und einen Träger umfasst, auf dem HPVLP angeordnet sind; und
in einem zweiten Schritt eine prozentuale Inhibition, die einen Grad angibt, zu dem verglichen mit dem ersten Reaktionsgemisch Inkubation mit HPVLP der löslichen Phase eine Menge an nicht gebundenem Anti-JCV-Antikörper reduziert, der an HPVLP anbindet, das auf einem Träger angeordnet ist, in einem zweiten Reaktionsgemisch bestimmt wird, das eine zweite Teilprobe der Serum- oder Plasmaprobe des Patienten und einen Träger umfasst, auf dem HPVLP angeordnet sind; und
Bestimmen, dass der Patient ein hohes Risiko zur Entwicklung von PML aufweist, wenn bestimmt wird, dass der Anti-JCV-Antikörper-Indexwert > 1,5 ist, und bestimmt wird, dass die prozentuale Inhibition > 70 % ist.

3. Verfahren nach Anspruch 1 oder Anspruch 2, wobei der Anti-JCV-Antikörperspiegel oder die prozentuale Inhibition vor einer Verabreichung von Natalizumab bestimmt wird.

4. Verfahren nach einem der Ansprüche 1 bis 3, wobei der Anti-JCV-Antikörperspiegel oder die prozentuale Inhibition bestimmt wird, nachdem der Patient eine Behandlung mit Natalizumab begonnen hat.

5. Verfahren nach einem der Ansprüche 1 bis 4, ferner umfassend:

(a) Bestimmen, ob der Patient für mehr als 24 Monate eine Behandlung mit Natalizumab erhalten hat; oder
(b) Bestimmen, ob der Patient eine Nicht-Anti-VLA-4-Immunsuppressivumtherapie erhalten hat, wobei die Nicht-Anti-VLA-4-Immunsuppressivumtherapie ausgewählt wird aus Mitoxantron, Methotrexat, Azathioprin, Cyclophosphamid, Mycophenolat, Anti-CD20-Therapie, Anti-CD11a-Therapie und Mycophenolat-Mofetil.

6. Verfahren nach einem der Ansprüche 1 bis 5, wobei der Anti-JCV-Antikörperspiegel oder die prozentuale Inhibition in Abständen von 6 Monaten oder 12 Monaten erneut getestet wird.

7. Verfahren nach Anspruch 6, wobei ein Anstieg des Anti-JCV-Antikörperspiegels oder der prozentualen Inhibition einen Anstieg des Risikos des Patienten zur Entwicklung von PML angibt.

8. Verfahren nach einem der Ansprüche 1 bis 7, wobei der Patient multiple Sklerose hat.

9. Verfahren nach einem der Ansprüche 1 bis 8, wobei für den Patienten, bei dem bestimmt wird, dass er ein hohes Risko zur Entwicklung von PML hat, bestimmt, wird, dass er ein höheres Risko zur Entwicklung von PML hat, wenn der Patient für mehr als 24 Monate Natalizumab erhalten hat und zuvor keine Nicht-Anti-VLA-4-Immunsuppressivumtherapie erhalten hat, wobei die Nicht-Anti-VLA-4-Immunsuppressivumtherapie ausgewählt wird aus Mitoxantron, Methotrexat, Azathioprin, Cyclophosphamid, Mycophenolat, Anti-CD20-Therapie, Anti-CD11a-Therapie und Mycophenolat-Mofetil.

Revendications

1. Procédé d'évaluation du risque d'un patient de développer une leucoencéphalopathie multifocale progressive (LEMP), le procédé comprenant :

i) la détermination, dans un échantillon de sérum ou de plasma du patient, d'un titre d'anticorps anti-virus JC (JCV), dans lequel le titre d'anticorps anti-JCV est déterminé par un test ELISA comprenant les étapes suivantes :

(a) formation d'un mélange réactionnel comprenant une aliquote d'échantillon et un substrat sur lequel sont disposées des particules virales hautement purifiées (HPVLP), et
(b) détection du taux d'anticorps anti-JCV lié audit substrat sur lequel sont disposées des HPVLP ;
dans lequel le titre d'anticorps anti-JCV est exprimé sous la forme d'une valeur d'indice, dans lequel la valeur d'indice est déterminée en normalisant une valeur de densité optique (OD) de l'échantillon à un calibrateur seuil réglé pour avoir une nOD de 1, et un témoin positif est réglé pour avoir une nOD de 1,3 ; dans lequel le calibrateur seuil et le témoin positif comprennent un mélange de sérum positif pour les anticorps anti-JCV et de sérum négatif pour les anticorps anti-JCV, et dans lequel un témoin négatif comprend un sérum négatif pour les anticorps anti-JCV et a une nOD de 0,1 ;
et

ii) la détermination que le patient présente un risque élevé de développer une LEMP si la valeur d'indice d'anticorps anti-JCV est déterminée comme étant supérieure à 1,5.

2. Procédé selon la revendication 1, dans lequel :

le titre d'anticorps anti-JCV est exprimé sous la forme d'une valeur d'indice pour un premier mélange réactionnel comprenant une première aliquote de l'échantillon de sérum ou de plasma du patient et un substrat sur lequel est disposé une HPVLP ; et
dans une seconde étape, un pourcentage d'inhibition indicatif du degré auquel l'incubation avec une HPVLP en phase soluble réduit un taux d'anticorps anti-JCV non lié qui se lie à une HPVLP disposée sur un substrat

par rapport au premier mélange réactionnel, est déterminé dans un second mélange réactionnel comprenant une seconde aliquote de l'échantillon de sérum ou de plasma du patient et un substrat sur lequel est disposée une HPVLP ; et
la détermination que le patient présente un risque élevé de développer une LEMP si la valeur d'indice d'anticorps anti-JCV est déterminée comme étant supérieure à 1,5 et un pourcentage d'inhibition est déterminé comme étant supérieur à 70 %.

3. Procédé selon la revendication 1 ou la revendication 2, dans lequel le titre d'anticorps anti-JCV ou le pourcentage d'inhibition est déterminé avant une administration de natalizumab.

4. Procédé selon l'une quelconque des revendications 1 à 3, dans lequel le titre d'anticorps anti-JCV ou le pourcentage d'inhibition est déterminé après que le patient a initié un traitement avec le natalizumab.

5. Procédé selon l'une quelconque des revendications 1 à 4, comprenant en outre :

(a) la détermination que le patient a reçu un traitement avec du natalizumab pendant plus de 24 mois ; ou
(b) la détermination que le patient a reçu une thérapie immunosuppressive non anti-VLA-4, dans lequel la thérapie immunosuppressive non anti-VLA-4 est choisie parmi la mitoxantrone, le méthotrexate, l'azathioprine, le cyclophosphamide, le mycophénolate, une thérapie anti-CD20, une thérapie CD11a et le mycophénolate mofétil.

6. Procédé selon l'une quelconque des revendications 1 à 5, dans lequel le titre d'anticorps anti-JCV ou le pourcentage d'inhibition est retesté à des intervalles de 6 mois ou 12 mois.

7. Procédé selon la revendication 6, dans lequel une augmentation du titre d'anticorps anti-JCV ou du pourcentage d'inhibition indique une augmentation du risque du patient de développer une LEMP.

8. Procédé selon l'une quelconque des revendications 1 à 7, dans lequel le patient est atteint de sclérose en plaques.

9. Procédé selon l'une quelconque des revendications 1 à 8, dans lequel le patient déterminé comme présentant un risque élevé de développer une LEMP est déterminé comme présentant un risque plus élevé de développer une LEMP si le patient a reçu du natalizumab pendant plus de 24 mois et n'a pas a déjà reçu une thérapie immunosuppressive non anti-VLA-4, dans lequel la thérapie immunosuppressive non anti-VLA-4 est choisie parmi la mitoxantrone, le méthotrexate, l'azathioprine, le cyclophosphamide, le mycophénolate, une thérapie anti-CD20, une thérapie anti-CD11a et le mycophénolate mofétil .

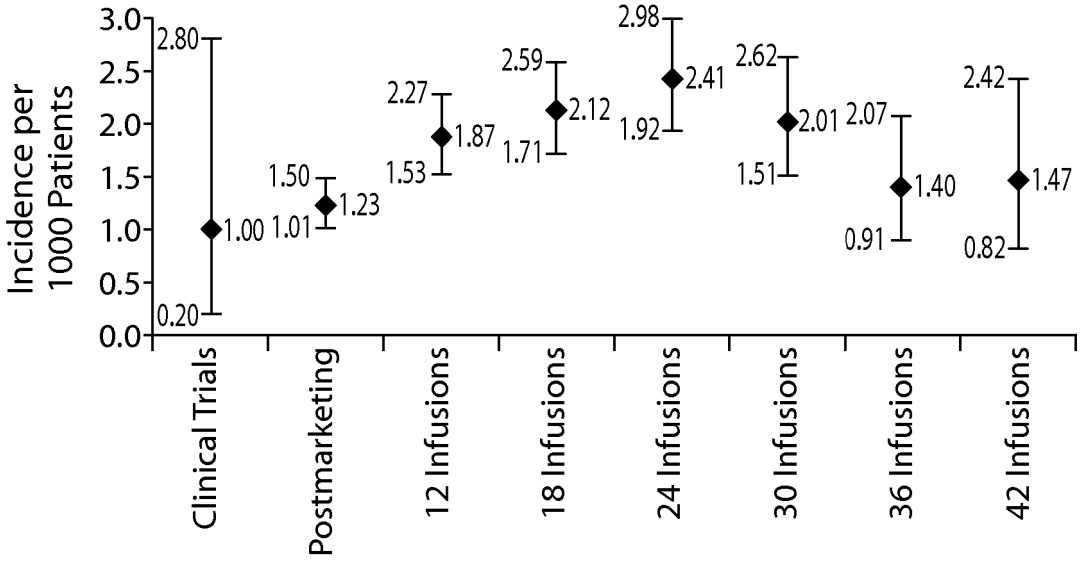

Incidence per 1000 Patients
3.0
2.5
2.0
1.5
1.0
0.5
0.0
2.80
1.00
0.20
1.50
1.01
1.23
2.27
1.53
1.87
2.59
1.71
2.12
2.98
1.92
2.41
2.62
1.51
2.01
2.07
0.91
1.40
2.42
0.82
1.47
Clinical Trials
Postmarketing
12 Infusions
18 Infusions
24 Infusions
30 Infusions
36 Infusions
42 Infusions

Fig. 1A

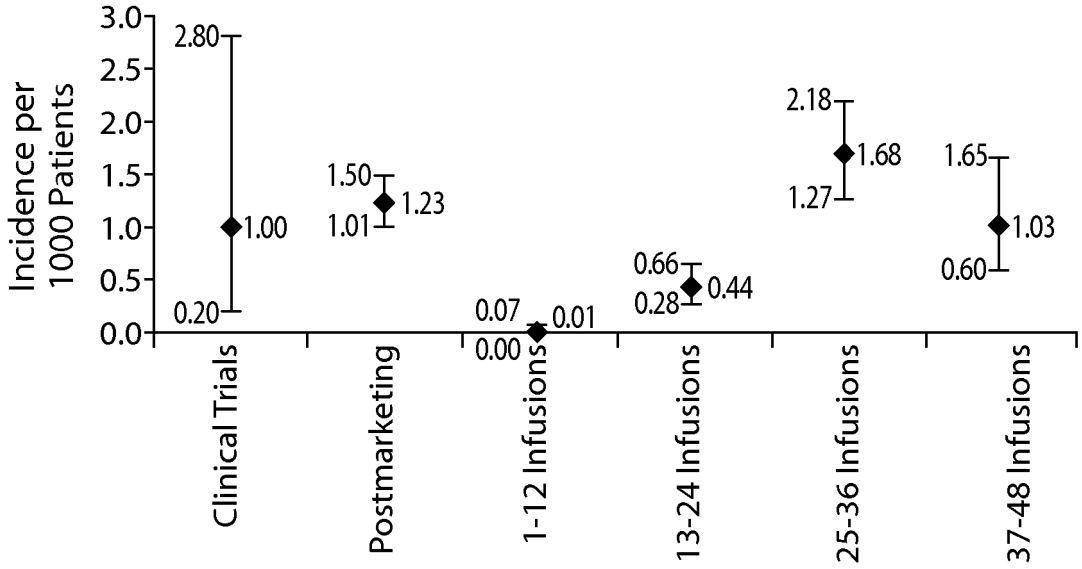

Incidence per 1000 Patients
3.0
2.5
2.0
1.5
1.0
0.5
0.0
2.80
1.00
0.20
1.50
1.01
1.23
0.07
0.00
0.01
0.66
0.28
0.44
2.18
1.27
1.68
1.65
0.60
1.03
Clinical Trials
Postmarketing
1-12 Infusions
13-24 Infusions
25-36 Infusions
37-48 Infusions

Fig. 1B

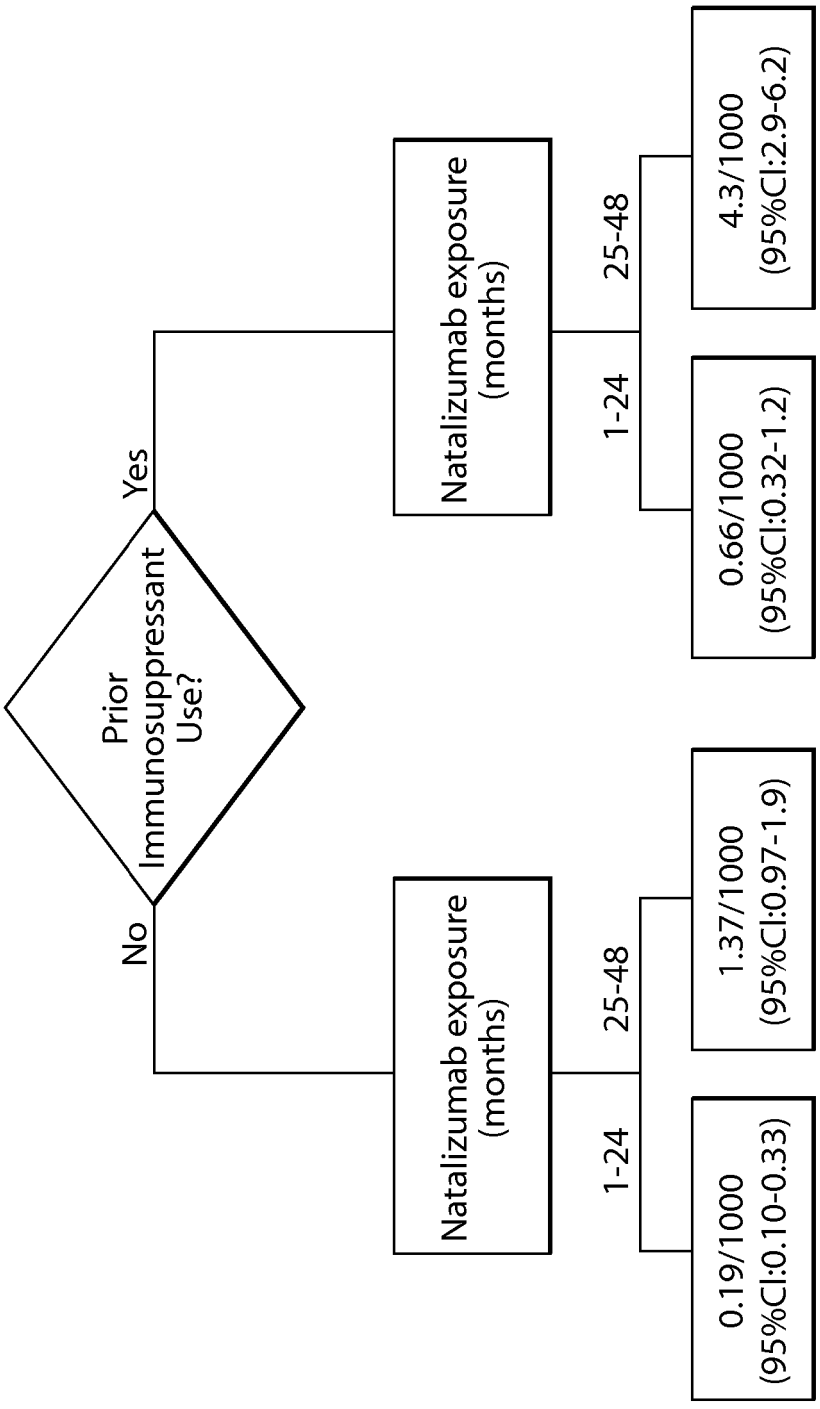
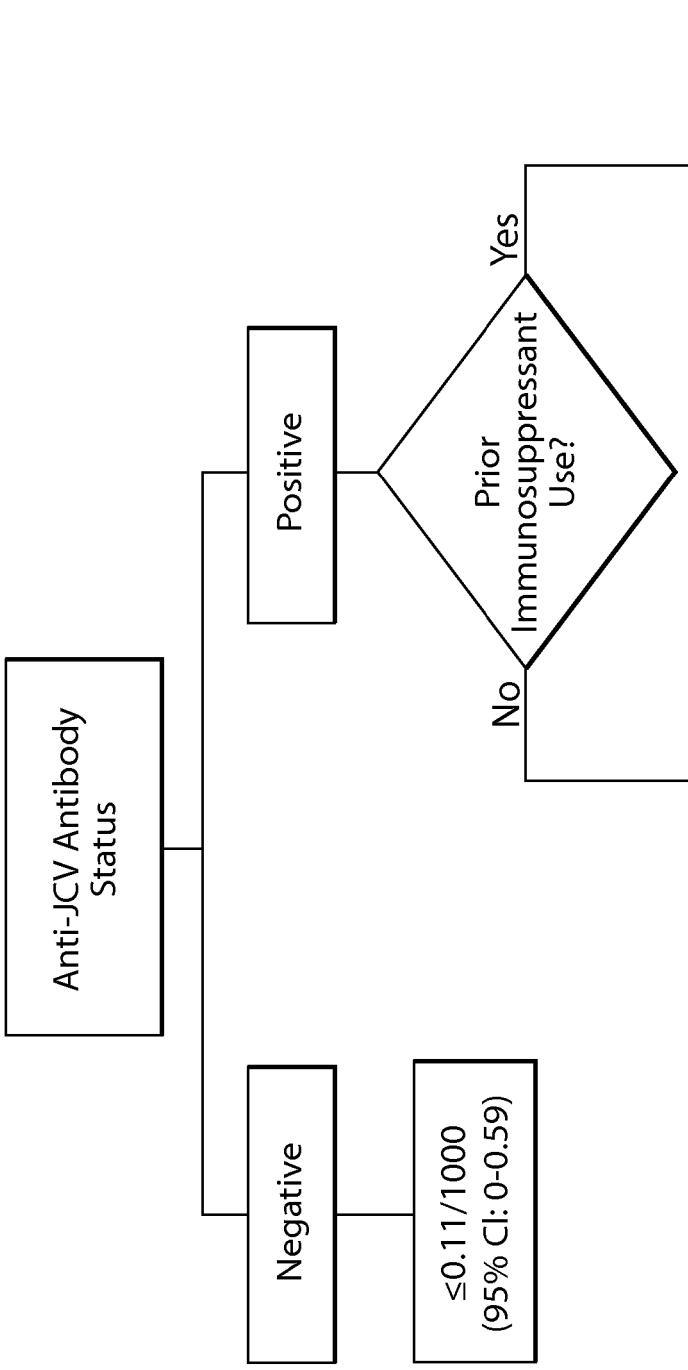

Prior Immunosuppressant Use?
Yes
No
Natalizumab exposure (months)
Natalizumab exposure (months)
1-24
25-48
1-24
25-48
0.66/1000 (95%CI:0.32-1.2)
4.3/1000 (95%CI:2.9-6.2)
0.19/1000 (95%CI:0.10-0.33)
1.37/1000 (95%CI:0.97-1.9)

Fig. 2



Natalizumab Exposure	Anti-JCV Antibody Positive	Anti-JCV Antibody Positive with Prior Immunosuppressant Use
1-24 months	0.35/1000 (95% CI: 0.19-0.60)	1.2/1000 (95% CI: 0.58-2.2)
25-48 months	2.5/1000 (95% CI: 1.8-3.4)	7.8/1000 (95% CI: 5.2-11.3)

Fig. 3

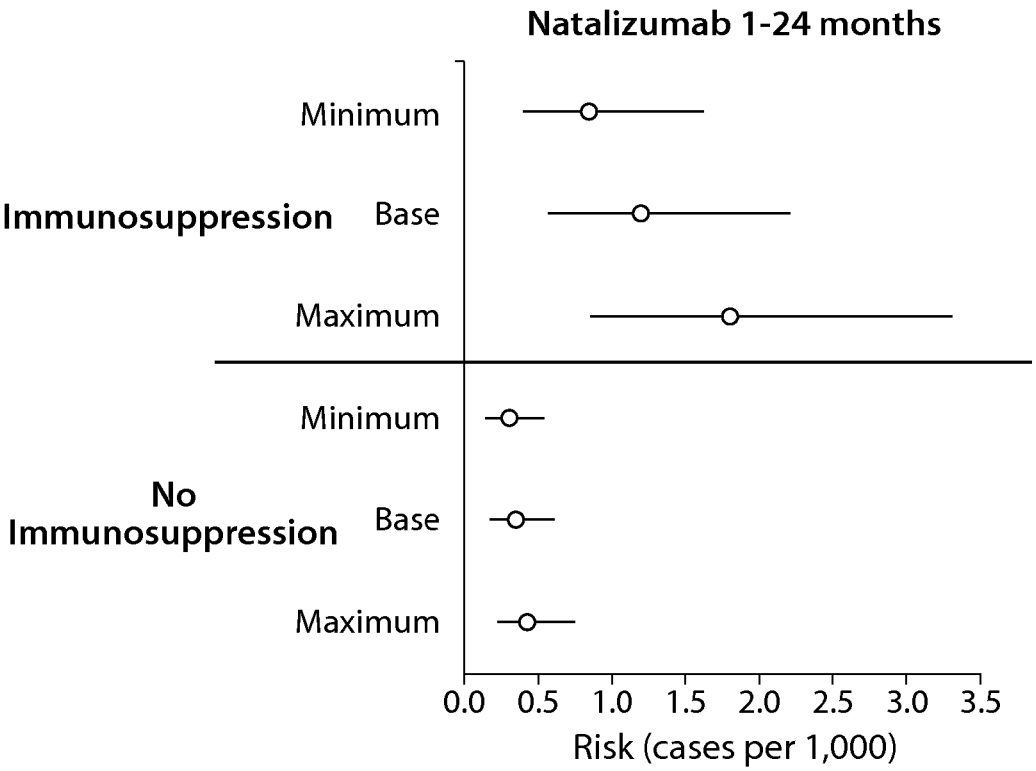

Natalizumab 1-24 months
Immunosuppression
Minimum
Base
Maximum
No Immunosuppression
Minimum
Base
Maximum
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5
Risk (cases per 1,000)

Fig. 4A

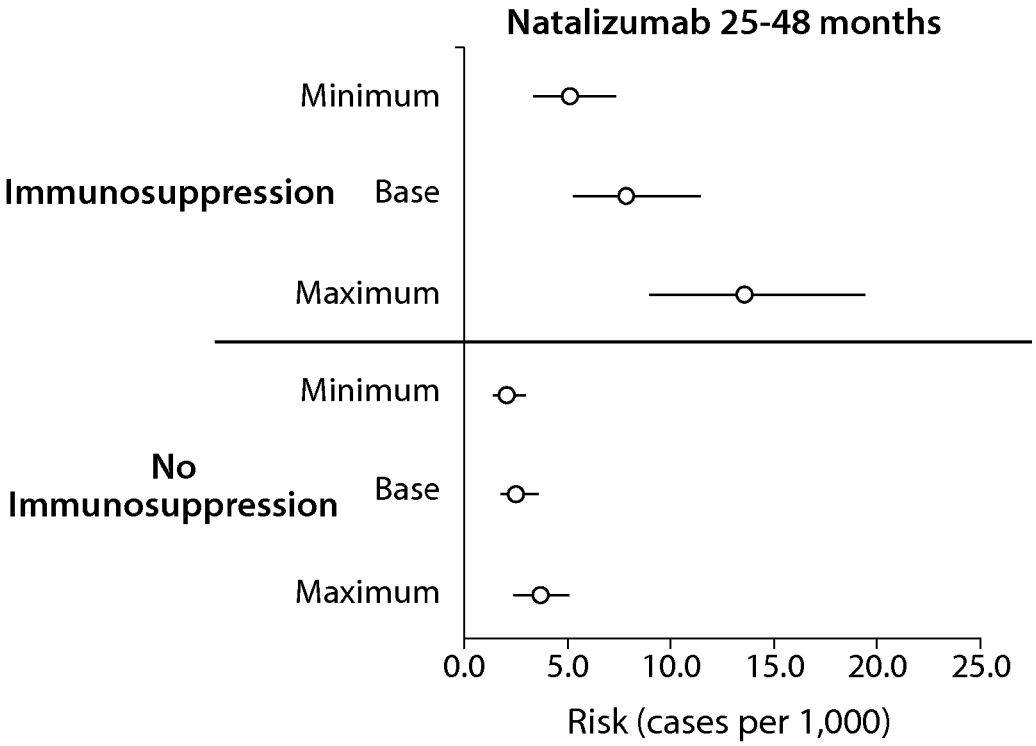

Natalizumab 25-48 months
Immunosuppression
Minimum
Base
Maximum
No Immunosuppression
Minimum
Base
Maximum
0.0 5.0 10.0 15.0 20.0 25.0
Risk (cases per 1,000)

Fig. 4B

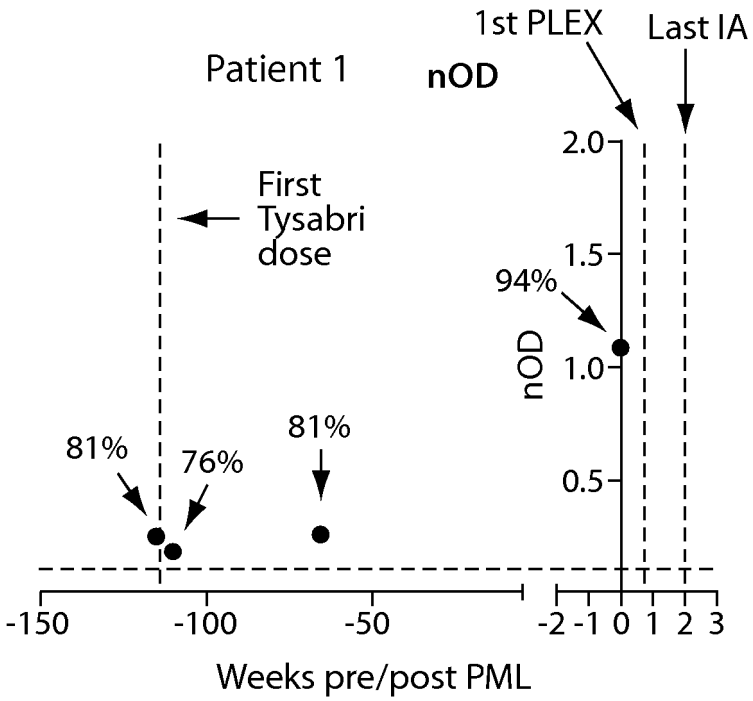

Patient 1
nOD
1st PLEX
Last IA
First Tysabri dose
94%
81%
76%
81%
2.0
1.5
1.0
0.5
nOD
-150
-100
-50
-2 -1 0 1 2 3
Weeks pre/post PML

Fig. 5A

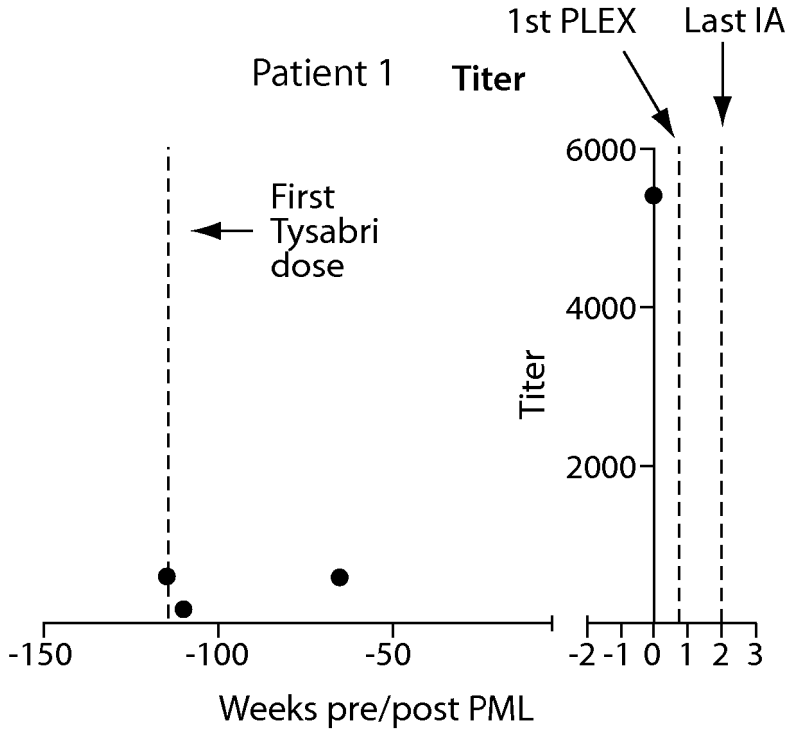

Patient 1
Titer
1st PLEX
Last IA
First Tysabri dose
6000
4000
2000
Titer
-150
-100
-50
-2 -1 0 1 2 3
Weeks pre/post PML

Fig. 5B

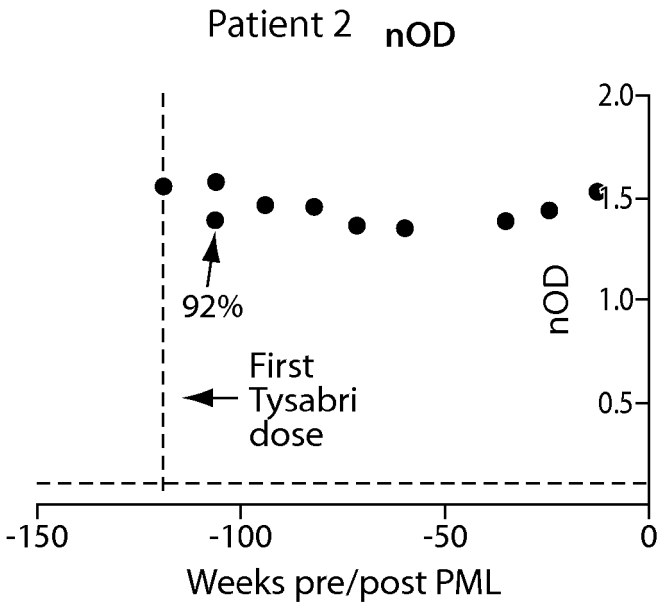

Patient 2
nOD
2.0
1.5
1.0
0.5
nOD
92%
First
Tysabri
dose
-150
-100
-50
0
Weeks pre/post PML

Fig. 6A

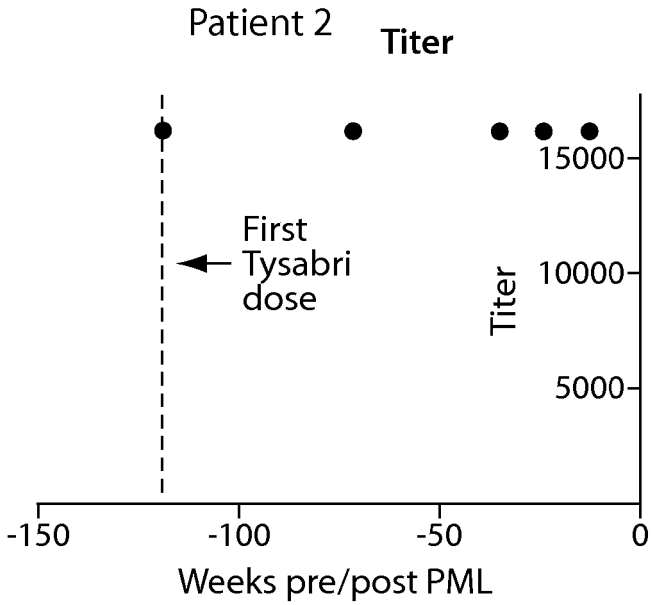

Patient 2
Titer
15000
10000
5000
Titer
First
Tysabri
dose
-150
-100
-50
0
Weeks pre/post PML

Fig. 6B

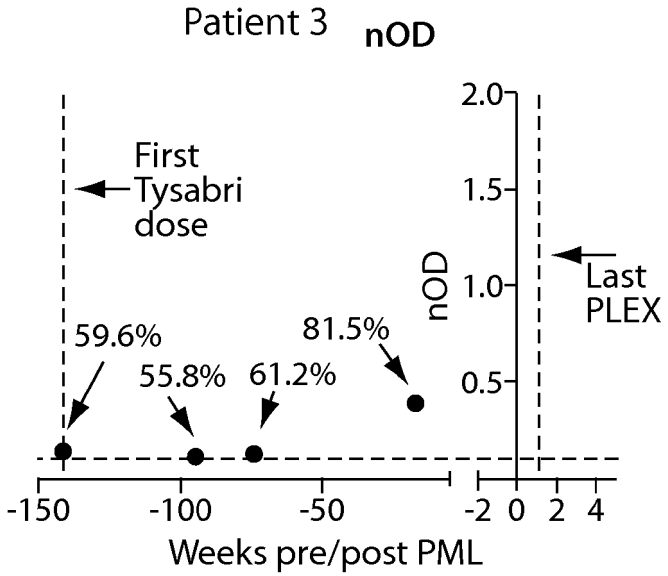

Patient 3
nOD
2.0
1.5
1.0
0.5
nOD
First
Tysabri
dose
Last
PLEX
59.6%
55.8%
61.2%
81.5%
-150
-100
-50
-2
0
2
4
Weeks pre/post PML

Fig. 7A

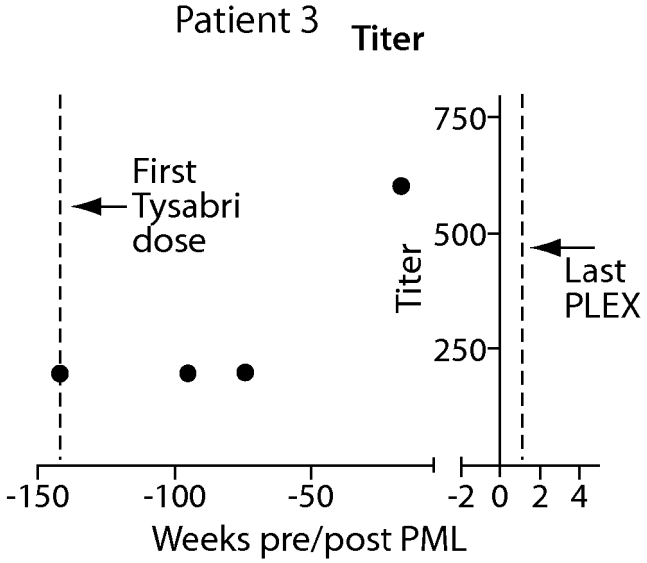

Patient 3
Titer
750
500
250
Titer
First
Tysabri
dose
Last
PLEX
-150
-100
-50
-2
0
2
4
Weeks pre/post PML

Fig. 7B

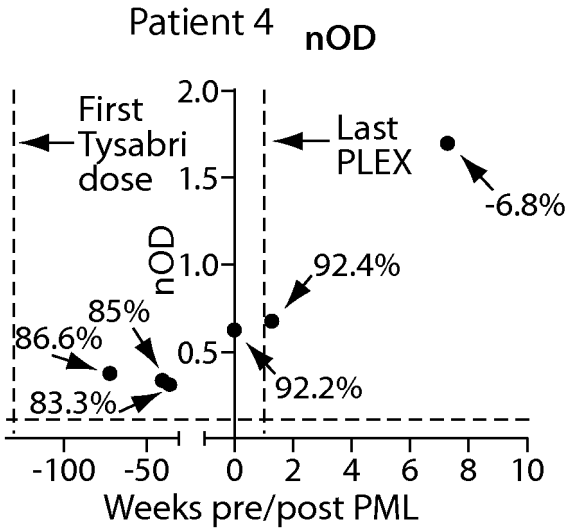

Patient 4
nOD
2.0
1.5
1.0
0.5
nOD
First
Tysabri
dose
Last
PLEX
-6.8%
92.4%
85%
86.6%
83.3%
92.2%
-100
-50
0
2
4
6
8
10
Weeks pre/post PML

Fig. 8A

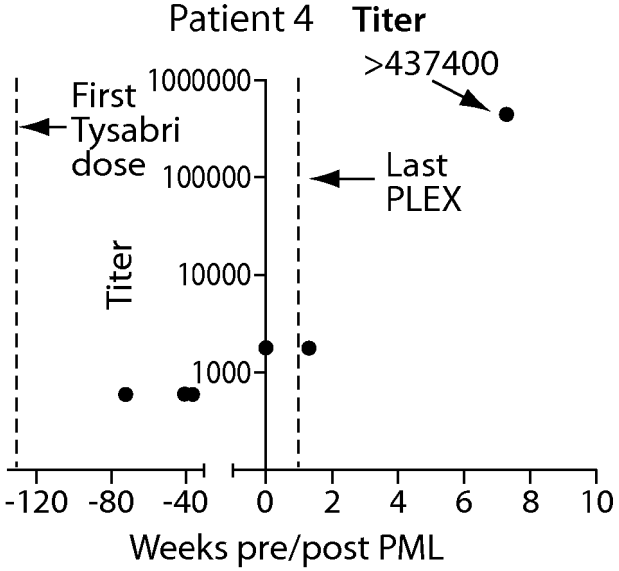

Patient 4
Titer
>437400
1000000
100000
10000
1000
Titer
First
Tysabri
dose
Last
PLEX
-120
-80
-40
0
2
4
6
8
10
Weeks pre/post PML

Fig. 8B

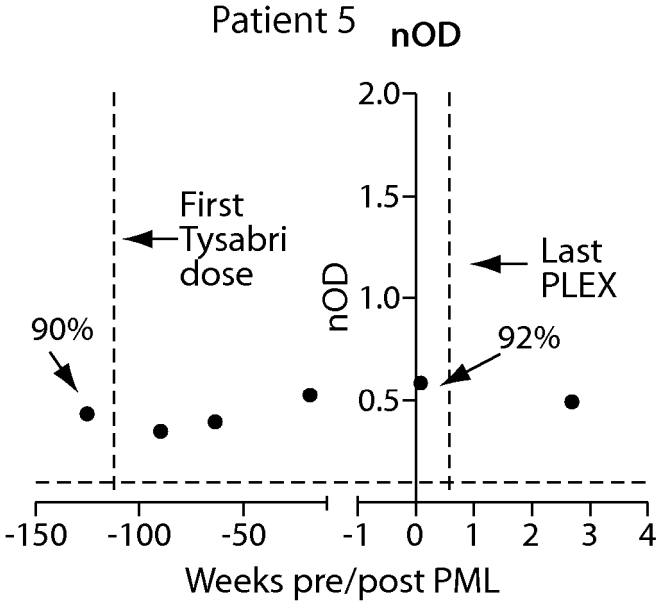

Patient 5
nOD
2.0
1.5
1.0
0.5
nOD
First
Tysabri
dose
Last
PLEX
90%
92%
-150
-100
-50
-1
0
1
2
3
4
Weeks pre/post PML

Fig. 9A

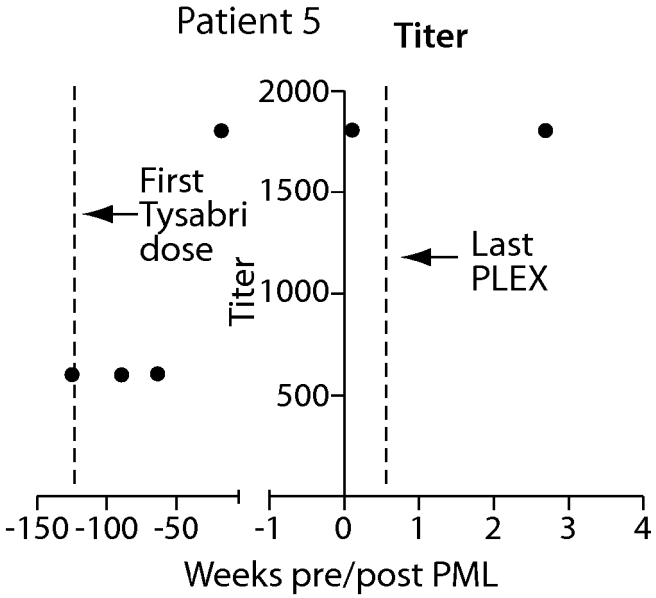

Patient 5
Titer
2000
1500
1000
500
Titer
First
Tysabri
dose
Last
PLEX
-150
-100
-50
-1
0
1
2
3
4
Weeks pre/post PML

Fig. 9B

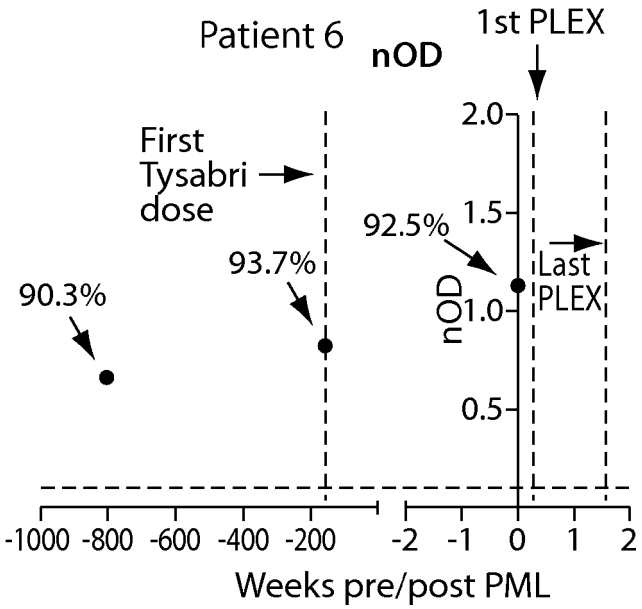

Patient 6
nOD
1st PLEX
First
Tysabri
dose
93.7%
90.3%
92.5%
Last
PLEX
nOD
2.0
1.5
1.0
0.5
-1000 -800 -600 -400 -200
-2 -1 0 1 2
Weeks pre/post PML

Fig. 9C

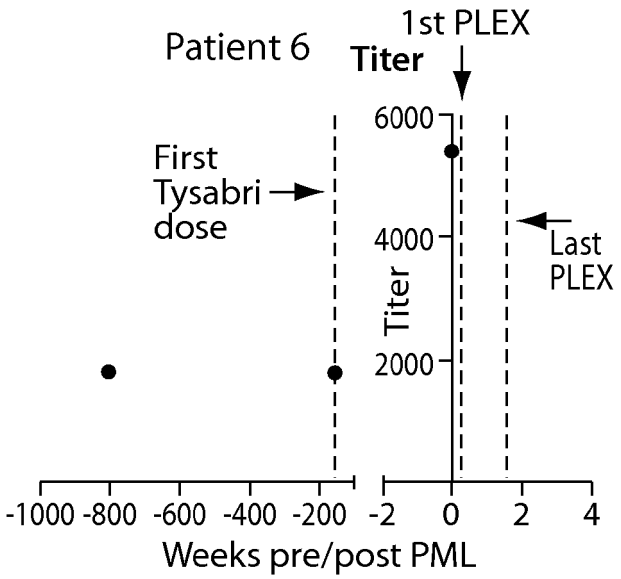

Patient 6
Titer
1st PLEX
First
Tysabri
dose
Last
PLEX
Titer
6000
4000
2000
-1000 -800 -600 -400 -200
-2 0 2 4
Weeks pre/post PML

Fig. 9D

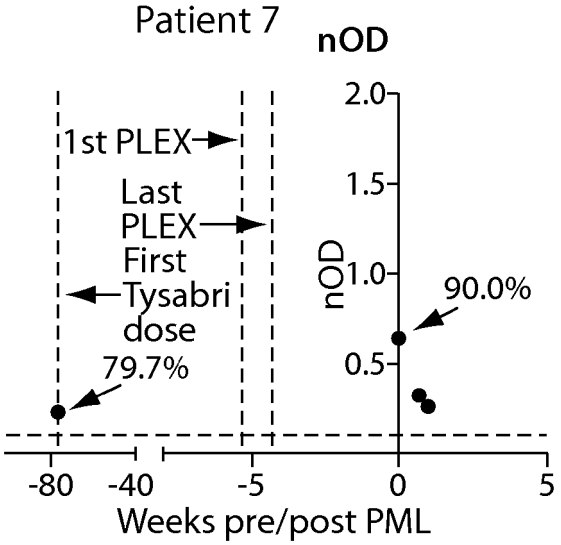


Fig. 10A

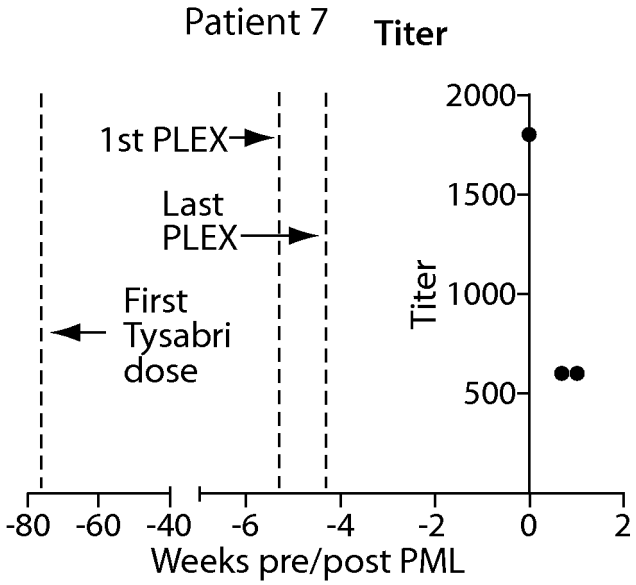


Fig. 10B

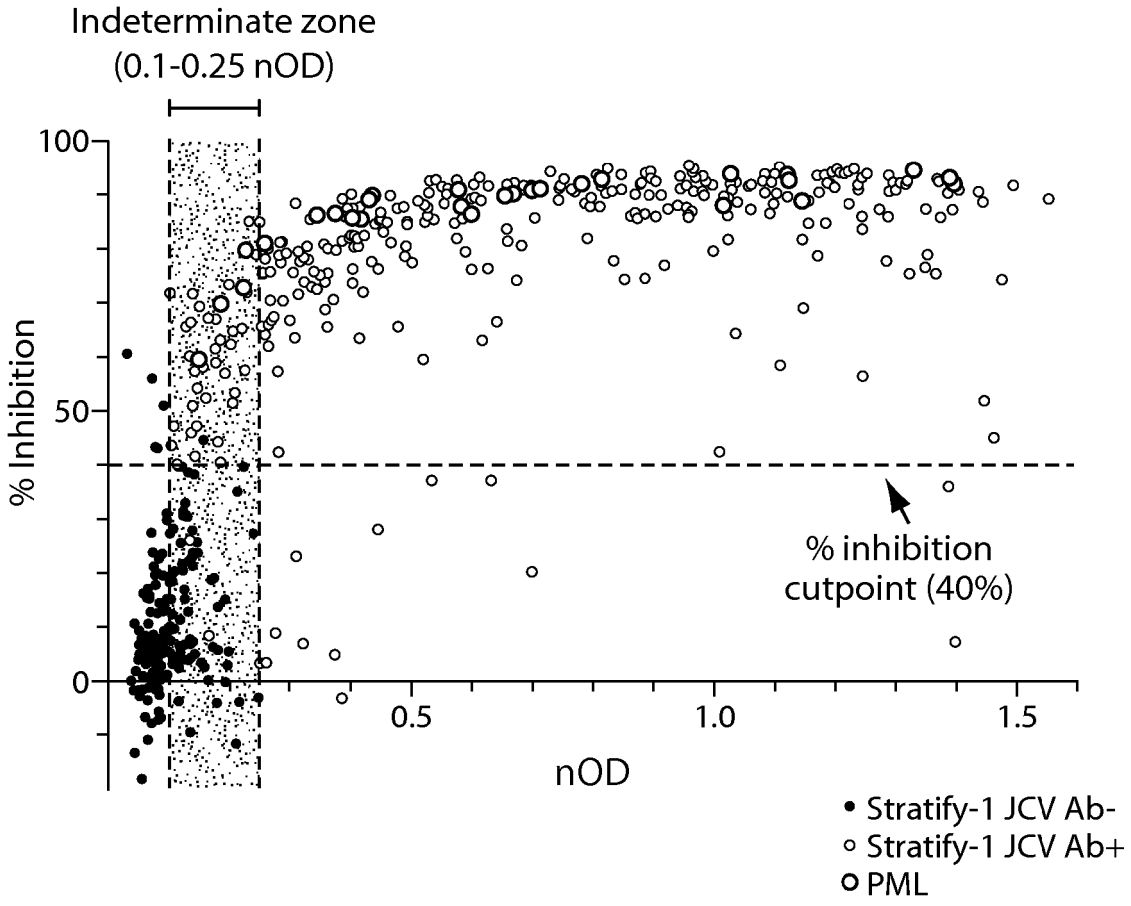

Indeterminate zone
(0.1-0.25 nOD)
100
50
0
% Inhibition
0.5
1.0
1.5
nOD
% inhibition
cutpoint (40%)
Stratify-1 JCV Ab-
Stratify-1 JCV Ab+
PML

Fig. 11

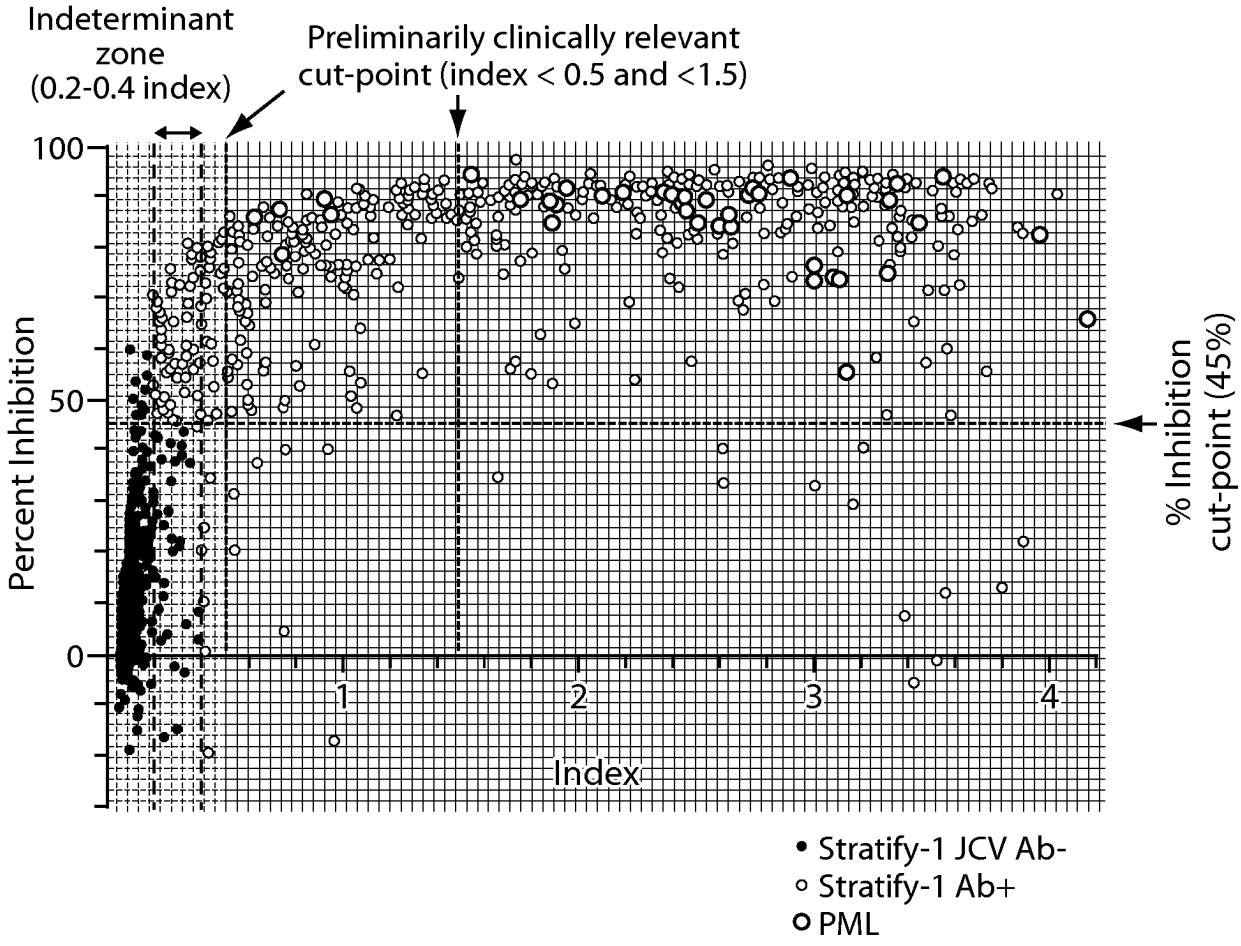
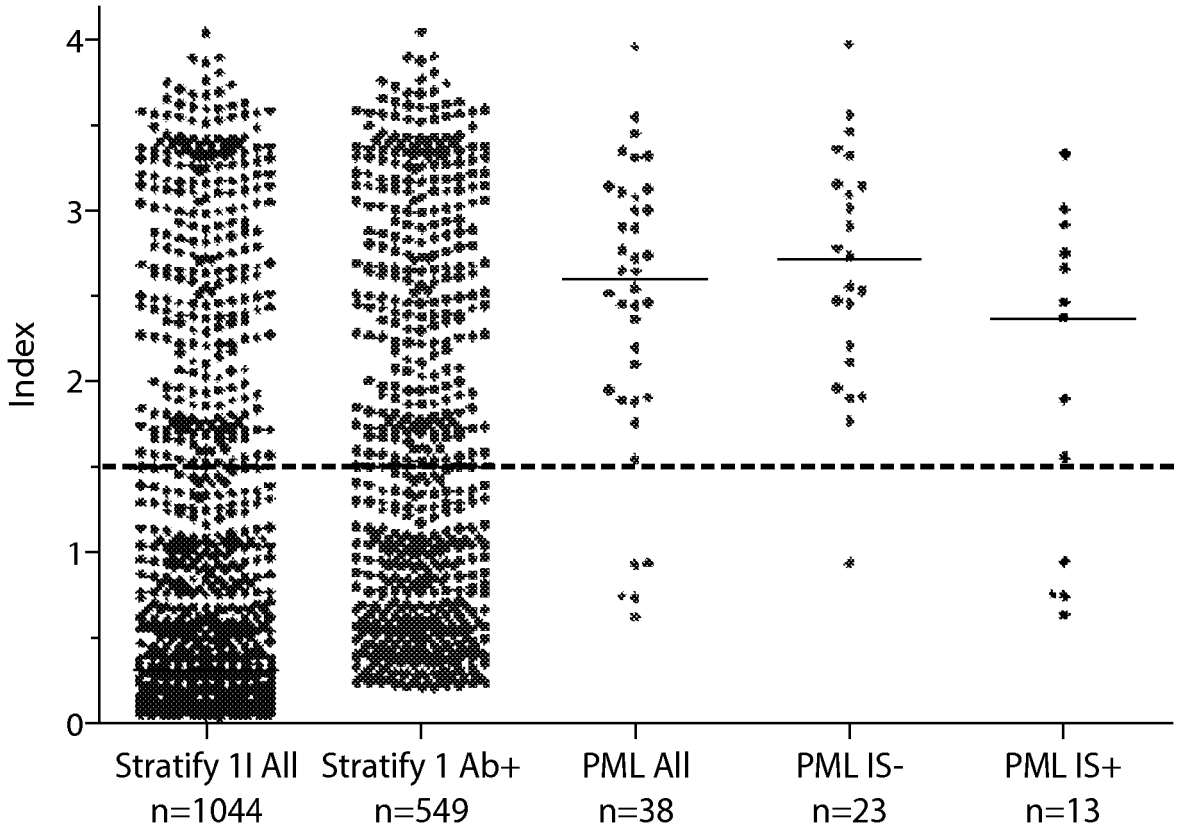

Indeterminant zone (0.2-0.4 index)
Preliminarily clinically relevant cut-point (index < 0.5 and <1.5)
100
50
0
Percent Inhibition
1
2
3
4
Index
% Inhibition cut-point (45%)
• Stratify-1 JCV Ab-
∘ Stratify-1 Ab+
O PML

Fig. 12



50% (278/549) of Ab+ patients have index <1.5
4.4% (1/23) of PML IS- patients have index <1.5

Fig. 13

REFERENCES CITED IN THE DESCRIPTION

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