subpopulation. Intn'l Patent Pub. No. WO2009/140684; Stefanich et al., Br. J. Pharmacol. 162:1855-1870 (2011).

[0014] To design therapies with a desired effect and/or efficacy, it is important to assess a patient's responsiveness to treatment with a therapeutic agent, such as a beta7 integrin antagonist. It is also important to determine optimal doses and dosing regimens of beta7 integrin antagonists that will provide or are likely to provide efficacy. Therefore, it is desirable to develop a biomarker that can be used to accurately track or monitor the responsiveness of a patient to treatment with a therapeutic agent. Such a biomarker would be particularly useful for designing effective treatment and dosing regimens for human patients in clinical trial studies and for disease treatment.

[0015] The invention described herein meets certain of the above-described needs and provides other benefits.

[0016] All references cited herein, including patent applications and publications, are incorporated by reference in their entirety for any purpose.

SUMMARY

[0017] The methods of the disclosure~~invention~~ are based, at least in part, on the discovery that receptor occupancy and cell surface expression of integrin beta7-subunit containing receptors, including αEβ7, on lymphocytes obtained from colon tissue of integrin beta7 antagonist (e.g., anti-beta7 antibody)-treated patients are capable of being assessed by flow cytometry methods. In addition, surprisingly, anti-beta7 antibody serum concentrations that were capable of saturating lymphocyte beta7 receptors in the periphery of treated patients were essentially the same as the anti-beta7 antibody serum concentrations that were capable of saturating lymphocyte beta7 receptors at the site of disease (in the colon). Accordingly, beta7 receptor occupancy in the peripheral blood is a surrogate indicator of beta7 receptor occupancy in colonic tissue. Additionally, the methods of the disclosure~~invention~~ are based, at least in part, on the discovery that levels of gene expression of integrin receptor ligands, lymphocyte genes, and cytokine genes, as well as the numbers of alphaE-positive cells in intestinal crypt epithelium change after treatment with an integrin beta7 antagonist.

[0017a] In one aspect, there is provided a method of determining or monitoring the efficacy or, or of aiding in determining or monitoring the efficacy of, an integrin beta7 antagonist for treatment of a gastrointestinal inflammatory disorder in a patient, the method comprising comparing the amount of a biomarker in a sample obtained from the patient after or during treatment with the antagonist, to an amount of the biomarker in a sample obtained

from the patient before the treatment, wherein a change in the amount of the biomarker after or during the treatment, as compared to before the treatment, is indicative of the efficacy of, or in combination with one or more additional biomarkers of efficacy is indicative of the efficacy of, the antagonist for treatment of the gastrointestinal disorder in the patient, and wherein the biomarker is integrin beta7 subunit-containing receptor occupancy by the antagonist on colonic lymphocytes, or gene expression levels of one or more integrin receptor ligands, or gene expression levels of one or more lymphocyte genes, or gene expression levels of one or more cytokines, or the number of alphaE-positive cells in intestinal crypt epithelium.

[0018] Disclosed are~~In one aspect,~~ methods of determining or monitoring the efficacy or of aiding in determining or monitoring the efficacy of an integrin beta7 antagonist for treatment of a gastrointestinal inflammatory disorder in a patient~~ are provided~~. In certain embodiments, the methods comprise comparing the amount of a biomarker in a sample obtained from the patient after or during treatment with the antagonist, to an amount of the biomarker in a

embodiments, the gene expression level of the integrin receptor ligand, MadCAM-1, is measured. In some embodiments, the level of the integrin receptor ligand, E-Cadherin, is measured. In some embodiments, the level of gene expression of one or more lymphocyte genes selected from CD19, CD8, and CD3epsilon is measured. In some embodiments, the level of gene expression of one or more cytokines selected from IL-1β, IL-6, IL-12-p40, IL-17A, IL-17-F, IL-23A, IFNγ and TNFα is measured. In certain embodiments, the level of gene expression is measured in colonic biopsy tissue. In certain embodiments, the level of gene expression is measured by qPCR. In certain embodiments, the change in the biomarker is an increase or a decrease. In certain embodiments, the biomarker is measured within 100 days after receiving a first dose of the antagonist. In certain embodiments, the biomarker is measured at day 43 and at day 71 after receiving a first dose of the antagonist or the biomarker is measured at week 6 and at week 10 after receiving a first dose of the antagonist. In one embodiment, the gastrointestinal inflammatory disorder is an inflammatory bowel disease. Exemplary inflammatory bowel diseases include ulcerative colitis and Crohn's disease. In certain embodiments, the patient is human.

[0018a] In another aspect, there is provided a method of determining or monitoring the responsiveness of, or of aiding in determining or monitoring the responsiveness of, a patient having a gastrointestinal inflammatory disorder to treatment with an integrin beta7 antagonist, the method comprising comparing the amount of a biomarker in a sample obtained from the patient after or during treatment with the antagonist, to the amount of the biomarker in a sample obtained from the patient before the treatment, wherein a change in the amount of the biomarker after or during the treatment, as compared to before the treatment is indicative of the responsiveness of, or in combination with one or more additional biomarkers of responsiveness is indicative of the responsiveness of, the patient to treatment with the antagonist, and wherein the biomarker is integrin beta7 subunit-containing receptor occupancy by the antagonist on colonic lymphocytes, or gene expression levels of one or more integrin receptor ligands, or gene expression levels of one or more lymphocyte genes, or gene expression levels of one or more cytokines, or the number of alphaE-positive cells in intestinal crypt epithelium.

[0019] Disclosed are~~In another aspect,~~ methods of determining or monitoring the responsiveness or of aiding in determining or monitoring the responsiveness of a patient having a gastrointestinal inflammatory disorder to treatment with an integrin beta7 antagonist ~~are provided~~. In certain embodiments, the methods comprise comparing the amount of a

biomarker in a sample obtained from the patient after or during treatment with the antagonist, to the amount of the biomarker in a sample obtained from the patient before the treatment, where a change in the amount of the biomarker after or during the treatment, as compared to before the treatment is indicative of the responsiveness of the patient to treatment with the antagonist, and where the biomarker is integrin beta7 subunit-containing receptor occupancy by the antagonist on colonic lymphocytes. In certain embodiments, the biomarker is selected from gene expression levels of one or more integrin receptor ligands, gene expression levels of one or more lymphocyte genes, gene expression levels of one or more cytokines, and the number of alphaE-positive cells in intestinal crypt epithelium. In certain embodiments, combinations of the above biomarkers are assessed. In certain embodiments, the afore-mentioned methods using one or more of the biomarkers selected from integrin beta7 subunit-containing receptor occupancy by the antagonist on colonic lymphocytes, gene expression levels of one or more integrin receptor ligands, gene expression levels of one or more lymphocyte genes, gene expression levels of one or more cytokines, and the number of alphaE-positive cells in

disease. Exemplary inflammatory bowel diseases include ulcerative colitis and Crohn's disease. In certain embodiments, the patient is human.

[0019a] In another aspect, there is provided method of determining or monitoring the efficacy of an integrin beta7 antagonist for treatment of a gastrointestinal inflammatory disorder in an antagonist-treated patient in a placebo-controlled clinical trial, the method comprising comparing the amount of a biomarker in a sample obtained from the patient after or during treatment with the antagonist, to an amount of the biomarker in a sample obtained from a placebo-treated patient, wherein a change in the amount of the biomarker in the antagonist-treated patient after or during treatment, as compared to the amount of the biomarker in the placebo-treated patient, is indicative of the efficacy of the antagonist for treatment of the gastrointestinal disorder in the antagonist-treated patient, and wherein the biomarker is integrin beta7 subunit-containing receptor occupancy by the antagonist on colonic lymphocytes, or gene expression levels of one or more integrin receptor ligands, or gene expression levels of one or more lymphocyte genes, or gene expression levels of one or more cytokines, or the number of alphaE-positive cells in intestinal crypt epithelium.

[0020] Disclosed are~~In yet another aspect,~~ methods of determining or monitoring the efficacy of an integrin beta7 antagonist for treatment of a gastrointestinal inflammatory disorder in an antagonist-treated patient in a placebo-controlled clinical trial~~ are provided~~. In certain embodiments, the methods comprise comparing the amount of a biomarker in a sample obtained from the patient after or during treatment with the antagonist, to an amount of the biomarker in a sample obtained from a placebo-treated patient, where a change in the amount of the biomarker in the antagonist-treated patient after or during treatment, as compared to the amount of the biomarker in the placebo-treated patient, is indicative of the efficacy of the antagonist for treatment of the gastrointestinal disorder in the antagonist-treated patient, and where the biomarker is integrin beta7 subunit-containing receptor occupancy by the antagonist on colonic lymphocytes. In certain embodiments, the biomarker is selected from gene expression levels of one or more integrin receptor ligands, gene expression levels of one or more lymphocyte genes, gene expression levels of one or more cytokines, and the number of alphaE-positive cells in intestinal crypt epithelium. In certain embodiments, the integrin beta7 subunit-containing receptor is $\alpha E\beta 7$ receptor or $\alpha 4\beta 7$ receptor. In one embodiment, the integrin beta7 subunit-containing receptor occupancy on colonic lymphocytes is determined by measuring integrin beta7 subunit-containing receptor occupancy on peripheral blood lymphocytes, where the integrin beta7 subunit-containing

receptor occupancy on peripheral blood lymphocytes was previously determined to be essentially the same as the integrin beta7 subunit-containing receptor occupancy on colonic lymphocytes. In certain embodiments, the occupancy of the integrin beta7 subunit-containing receptor is determined by a method comprising incubating the lymphocytes with labeled anti-beta7 antibody, where the labeled anti-beta7 antibody binds to the same epitope as the integrin beta7 antagonist, washing the lymphocytes, and measuring the percentage of labeled lymphocytes by flow cytometry. In certain embodiments, the label is selected from fluorescein isothiocyanate (FITC), rhodamine, phycoerythrin (PE), allophycocyanin (APC), peridinin chlorophyll protein (PerCP), PE-Cy7, APC-Cy7 and APC-H7. In one embodiment, the integrin beta7 antagonist is etrolizumab and the labeled anti-beta7 antibody is etrolizumab or FIB504. In some embodiments, the gene expression level of the integrin receptor ligand, MadCAM-1, is measured. In some embodiments, the level of the integrin receptor ligand, E-Cadherin, is measured. In some embodiments, the level of gene expression of one or more lymphocyte

genes selected from CD19, CD8, and CD3epsilon is measured. In some embodiments, the level of gene expression of one or more cytokines selected from IL-1β, IL-6, IL-12-p40, IL-17A, IL-17-F, IL-23A, IFNγ and TNFα is measured. In certain embodiments, the level of gene expression is measured in colonic biopsy tissue. In certain embodiments, the level of gene expression is measured by qPCR. In certain embodiments, the change in the biomarker is an increase or a decrease. In certain embodiments, the biomarker is measured within 100 days after receiving a first dose of the antagonist. In certain embodiments, the biomarker is measured at day 43 and at day 71 after receiving a first dose of the antagonist or the biomarker is measured at week 6 and at week 10 after receiving a first dose of the antagonist. In certain embodiments, combinations of the above biomarkers are assessed. In certain embodiments, the methods further comprise assessing one or more clinical biomarkers of efficacy selected from clinical remission at week 6, clinical remission at week 10, clinical response at week 6, clinical response at week 10, endoscopy score and rectal bleeding score of 0 at week 6, endoscopy and rectal bleeding score of 0 at week 10, and time to flare of UC after achieving response or remission. In one embodiment, the gastrointestinal inflammatory disorder is an inflammatory bowel disease. Exemplary inflammatory bowel diseases include ulcerative colitis and Crohn's disease. In certain embodiments, the patient is human.

[0020a] In another aspect, there is provided a method of determining or monitoring the responsiveness of a patient having a gastrointestinal inflammatory disorder to treatment with an integrin beta7 antagonist, wherein the patient is in a placebo-controlled clinical trial, the method comprising comparing the amount of a biomarker in a sample obtained from the patient after or during treatment with the antagonist, to an amount of the biomarker in a sample obtained from a placebo-treated patient, wherein a change in the amount of the biomarker in the antagonist-treated patient after or during treatment, as compared to the amount of the biomarker in the placebo-treated patient, is indicative of the responsiveness of the patient to treatment with the antagonist, and wherein the biomarker is integrin beta7 subunit-containing receptor occupancy by the antagonist on colonic lymphocytes, or gene expression levels of one or more integrin receptor ligands, or gene expression levels of one or more lymphocyte genes, or gene expression levels of one or more cytokines, or the number of alphaE-positive cells in intestinal crypt epithelium.

[0021] Disclosed are~~In yet still another aspect,~~ methods of determining or monitoring the responsiveness of a patient having a gastrointestinal inflammatory disorder to treatment with an integrin beta7 antagonist, where the patient is in a placebo-controlled clinical trial~~are~~

~~provided~~. In certain embodiments, the methods comprise comparing the amount of a biomarker in a sample obtained from the patient after or during treatment with the antagonist, to an amount of the biomarker in a sample obtained from a placebo-treated patient, wherein a change in the amount of the biomarker in the antagonist-treated patient after or during treatment, as compared to the amount of the biomarker in the placebo-treated patient, is indicative of the responsiveness of the patient to treatment with the antagonist, and wherein the biomarker is integrin beta7 subunit-containing receptor occupancy by the antagonist on colonic lymphocytes. In certain embodiments, the biomarker is selected from gene expression levels of one or more integrin receptor ligands, gene expression levels of one or more lymphocyte genes, gene expression levels of one or more cytokines, and the number of alphaE-positive cells in intestinal crypt epithelium. In certain embodiments, the integrin beta7 subunit-containing receptor is αEβ7 receptor or α4β7 receptor. In one embodiment, the integrin beta7 subunit-containing receptor occupancy on colonic lymphocytes is

[0021a] In another aspect, there is provided a method of determining the dosing regimen of an integrin beta7 antagonist for treatment of a gastrointestinal inflammatory disorder in a patient, the method comprising adjusting the dose regimen of the antagonist based on a comparison of the amount of a biomarker in a sample obtained from the patient after or during treatment with a dosing regimen of the antagonist, to an amount of the biomarker in a sample obtained from the patient before the treatment, wherein a change in the amount of the biomarker after or during the treatment, as compared to before the treatment, is indicative of the efficacy of or responsiveness to the dose or dosing regimen of the antagonist for treatment of the gastrointestinal disorder in the patient, and wherein the biomarker is integrin beta7 subunit-containing receptor occupancy by the antagonist on colonic lymphocytes.

[0022] Disclosed are~~In yet another aspect,~~ methods of determining the dosing of an integrin beta7 antagonist for treatment of a gastrointestinal inflammatory disorder in a patient ~~are provided~~. In certain embodiments, the methods comprise adjusting the dose of the antagonist based on a comparison of the amount of a biomarker in a sample obtained from the patient after or during treatment with a dose or dosing regimen of the antagonist, to an amount of the biomarker in a sample obtained from the patient before the treatment, where a change in the amount of the biomarker after or during the treatment, as compared to before the treatment, is indicative of the efficacy of or responsiveness to the dose or dosing regimen of the antagonist for treatment of the gastrointestinal disorder in the patient, and where the biomarker is integrin beta7 subunit-containing receptor occupancy by the antagonist on colonic lymphocytes. In certain embodiments, the integrin beta7 subunit-containing receptor is $\alpha E\beta 7$ receptor or $\alpha 4\beta 7$ receptor. In one embodiment, the integrin beta7 subunit-containing receptor occupancy on colonic lymphocytes is determined by measuring integrin beta7 subunit-containing receptor occupancy on peripheral blood lymphocytes, where the integrin beta7 subunit-containing receptor occupancy on peripheral blood lymphocytes was previously determined to be essentially the same as the integrin beta7 subunit-containing receptor occupancy on colonic lymphocytes. In certain embodiments, the occupancy of the integrin beta7 subunit-containing receptor is determined by a method comprising incubating the lymphocytes with labeled anti-beta7 antibody, where the labeled anti-beta7 antibody binds to the same epitope as the integrin beta7 antagonist, washing the lymphocytes, and measuring the percentage of labeled lymphocytes by flow cytometry. In certain embodiments, the label is selected from fluorescein isothiocyanate (FITC), rhodamine, phycoerythrin (PE), allophycocyanin (APC), peridinin chlorophyll protein (PerCP), PE-Cy7,

APC-Cy7 and APC-H7. In one embodiment, the integrin beta7 antagonist is etrolizumab and the labeled anti-beta7 antibody is etrolizumab or FIB504. In one embodiment, the change in the occupancy is an increase or decrease. In one embodiment, the occupancy is measured within 100 days after receiving a first dose of the antagonist. In one embodiment, the occupancy is measured at day 43 and at day 71. In one embodiment, the gastrointestinal inflammatory disorder is an inflammatory bowel disease. Exemplary inflammatory bowel diseases include ulcerative colitis and Crohn's disease. In certain embodiments, the patient is human. In one embodiment, the antagonist is an anti-beta7 antibody and the dosing or dosing regimen determined as indicative of the efficacy of or responsiveness to the dose or dosing regimen comprises subcutaneous administration of a first loading dose of 420 mg anti-beta7 antibody

followed two weeks later by subcutaneous administration of a first maintenance dose of 315 mg anti-beta7 antibody (or nominal dose of 300 mg) followed by subcutaneous administration of one or more subsequent maintenance doses of 315 mg anti-beta7 antibody (or nominal dose of 300 mg), where each subsequent maintenance dose is administered four weeks after the prior maintenance dose. In one embodiment, the dosing or dosing regimen determined as indicative of the efficacy of or responsiveness to the dose or dosing regimen comprises subcutaneous administration of 105 mg anti-beta7 antibody (or nominal dose of 100 mg) every four weeks or 50 mg anti-beta7 antibody (nominal dose) every two weeks. In one embodiment, the anti-beta7 antibody is etrolizumab.

[0023] Disclosed are~~In still yet another aspect,~~ methods of determining the dosing regimen of an integrin beta7 antagonist for treatment of a gastrointestinal inflammatory disorder in a patient~~ are provided~~. In certain embodiments, the methods comprise adjusting the dose regimen of the antagonist based on a comparison of the amount of a biomarker in a sample obtained from the patient after or during treatment with a dosing regimen of the antagonist, to an amount of the biomarker in a sample obtained from the patient before the treatment, where a change in the amount of the biomarker after or during the treatment, as compared to before the treatment, is indicative of the efficacy of or responsiveness to the dose or dosing regimen of the antagonist for treatment of the gastrointestinal disorder in the patient, and where the biomarker is integrin beta7 subunit-containing receptor occupancy by the antagonist on colonic lymphocytes. In certain embodiments, the integrin beta7 subunit-containing receptor is αEβ7 receptor or α4β7 receptor. In one embodiment, the integrin beta7 subunit-containing receptor occupancy on colonic lymphocytes is determined by measuring integrin beta7 subunit-containing receptor occupancy on peripheral blood lymphocytes, where the integrin beta7 subunit-containing receptor occupancy on peripheral blood lymphocytes was previously determined to be essentially the same as the integrin beta7 subunit-containing receptor occupancy on colonic lymphocytes. In certain embodiments, the occupancy of the integrin beta7 subunit-containing receptor is determined by a method comprising incubating the lymphocytes with labeled anti-beta7 antibody, where the labeled anti-beta7 antibody binds to the same epitope as the integrin beta7 antagonist, washing the lymphocytes, and measuring the percentage of labeled lymphocytes by flow cytometry. In certain embodiments, the label is selected from fluorescein isothiocyanate (FITC), rhodamine, phycoerythrin (PE), allophycocyanin (APC), peridinin chlorophyll protein (PerCP), PE-Cy7, APC-Cy7 and APC-H7. In one embodiment, the integrin beta7 antagonist is etrolizumab and

the labeled anti-beta7 antibody is etrolizumab or FIB504. In one embodiment, the change in the occupancy is an increase or decrease. In one embodiment, the occupancy is measured within 100 days after receiving a first dose of the antagonist. In one embodiment, the occupancy is measured at day 43 and at day 71. In one embodiment, the gastrointestinal inflammatory disorder is an inflammatory bowel disease. Exemplary inflammatory bowel diseases include ulcerative colitis and Crohn's disease. In certain embodiments, the patient is human. In one embodiment, the antagonist is an anti-beta7 antibody and the dosing or dosing regimen determined as indicative of the efficacy of or responsiveness to the dose or dosing regimen comprises subcutaneous administration of a first loading dose of 420 mg anti-beta7 antibody followed two weeks later by subcutaneous administration of a first maintenance dose of 315 mg anti-beta7 antibody (or nominal dose of 300 mg) followed by subcutaneous administration of one or more subsequent maintenance doses of 315 mg anti-beta7 antibody (or nominal dose of 300 mg), where each subsequent maintenance dose is administered four weeks after the prior maintenance dose. In one embodiment, the dosing or dosing regimen determined as indicative of the efficacy of or responsiveness to the dose or dosing regimen comprises subcutaneous administration of 105 mg anti-beta7 antibody (or nominal dose of 100 mg) every four weeks or 50 mg anti-beta7 antibody (nominal dose) every two weeks. In one embodiment, the anti-beta7 antibody is etrolizumab.

[0024] Disclosed ~~I~~in certain ~~aspects~~ embodiments of the above-described methods, the integrin beta7 antagonist is a monoclonal anti-beta7 antibody. In certain such embodiments, the anti-beta7 antibody is selected from a chimeric antibody, a human antibody, and a humanized antibody. In certain embodiments, the anti-beta7 antibody is an antibody fragment. In certain embodiments, the anti-beta7 antibody comprises six hypervariable regions (HVRs), wherein:

(i) HVR-L1 comprises amino acid sequence A1-A11, wherein A1-A11 is RASESVDTYLH (SEQ ID NO:1); RASESVDSLLH (SEQ ID NO:7), RASESVDTLLH (SEQ ID NO:8), or RASESVDDLLH (SEQ ID NO:9) or a variant of SEQ ID NOs:1, 7, 8 or 9 (SEQ ID NO:26) wherein amino acid A2 is selected from the group consisting of A, G, S, T, and V and/or amino acid A3 is selected from the group consisting of S, G, I, K, N, P, Q, R, and T, and/or A4 is selected from the group consisting of E, V, Q, A, D, G, H, I, K, L, N, and R, and/or amino acid A5 is selected from the group consisting of S, Y, A, D, G, H, I, K, N, P, R, T, and V, and/or amino acid A6 is selected from the group consisting of V, R, I, A, G, K, L, M, and Q, and/or amino acid A7 is selected from the group consisting of D, V, S, A, E, G, H, I, K, L, N, P, S, and T, and/or amino acid A8 is selected from the group consisting of D, G, N, E, T, P and S, and/or amino acid A9 is selected from the group consisting of L, Y, I and