METHODS FOR PREDICTING AND MONITORING MUCOSAL HEALING

~~CROSS-REFERENCES TO RELATED APPLICATIONS~~

~~**[0001]** This application claims priority to U.S. Provisional Application No. 61/710,491, filed October 5, 2012, and U.S. Provisional Application No. 61/824,959, filed May 17, 2013, the disclosures of which are hereby incorporated by reference in their entirety for all purposes.~~

BACKGROUND OF THE INVENTION

[0002] Inflammatory bowel disease (IBD) which includes Crohn's disease (CD) and ulcerative colitis (UC) is a chronic idiopathic inflammatory disorder affecting the gatrointestine tract. Disease progression of CD and UC includes repeated episodes of inflammation and ulceration of the intestine, leading to complications requiring hospitalization, surgery and escalation of therapy (Peyrin-Biroulet *et al*., *Am. J. Gastroenterol,*. 105: 289-297 (2010); Langholz E., *Dan. Med. Bull.*, 46: 400-415 (1999)). Current treatments such as anti-tumor necrosis factor-alpha (TNF-α) biologics (*e.g.*, infliximab (IFX), etanercept, adalimumab (ADL) and certolizumab pegol), thiopurine drugs (*e.g.*, azathioprine (AZA), 6-mercaptopurin (6-MP)), anti-inflammatory drugs (*e.g.*, mesalazine), and steroids (*e.g.*, corticosteroids) have been shown to reduce disease activity. In some clinical trials of CD, mucosal healing (MH) which is described as the absence of intestinal ulcers, was induced in patients receiving combination therapy of corticosteroids and IFX or ADL. Furthermore, MH was maintained in patients receiving IFX.

[0003] Other studies have shown that mucosal healing can be a hallmark of suppression of bowel inflammation and can predict long-term disease remission (Froslie *et al*., G*astroenterology*, 133: 412-422 (2007); Baert *et al.*, *Gastroenterology*, (2010)). Long-term mucosal healing has been associated with a decreased risk of colectomy and colorectal cancer in UC patients, a decreased need for corticosteroid treatment in CD patients, and possibly a decreased need for hospitalization (Dave *et al*., *Gastroenterology & Hepatology*, 8(1): 29-38 (2012)).

[0004] The process of mucosal healing can be divided into three phase, beginning with bleeding (*e.g.*, degradation of the endothelial layers of the blood vessels) and inflammation, then progression to cell and tissue proliferation, and finally tissue remodeling. At the inflammation stage, cytokines, chemokines and other inflammatory signaling molecules are secreted by immune cells in the gut mucosa. During proliferation, tissue repair and remodeling growth factors activate intestinal epithelial cells to proliferate, migrate to the sites of injury and repair the damaged tissue. At the remodeling phase, structural and functional improvements occur to the intestinal mucosal barrier. The present invention is based on the identification of novel markers of mucosal healing that are predictive of the phases of the process.

[0004a] US 2004/197304 relates to methods of determining efficacy of a treatment of inflammatory diseases of the bowel in mammals. Dave et al: Gastroenterology & hepatology, 2012:pp. 29-38 relates to mucosal healing in inflammatory bowel diease. Karel et al: Current medical research and opinion, Vol.21, nr. 11, 2005: pp 1741-1754 relates to endoscopic and histological evidence of persistent mucosal healing and correlation with clinical improvement following sustained infliximab treatment for crohns's disease. Daperno et al. Journal of Crohn's and Colitis, vol. 5, no. 5, pp.484-498 relates to measures and markers of predicition to achieve, detect and monitor intestinal healing in inflammatory bowel disease. Scaldaferri, 2010: World journal of gastroenterology, vol.16, no.21 relates to mucosal biomarkers in inflmmatory bowel disease.

[0005] There is an unmet need in the art for non-invasive methods for predicting the likelihood of mucosal healing and/or monitoring the progression of mucosal healing in patients with IBD or other inflammatory diseases. This information enables the personalized therapeutic management of the disease, such as allowing for appropriate selection and/or administration of therapy. The present invention satisfies this need and provides related advantages as well.

BRIEF SUMMARY OF THE INVENTION

[0006] Provided herein is a method for predicting the likelihood of mucosal healing in a subject. The method comprises the steps of: (a) measuring a first set of markers to form an inflammatory phase marker score; (b) measuring a second set of markers to form a proliferation phase marker score; (c) comparing the inflammatory phase marker score to the proliferation

phase marker score; and (d) predicting the likelihood of mucosal healing based upon the comparison in step (c).

[0007] In some embodiments, the subject has an inflammatory bowel disease (IBD). In some instances, the inflammatory bowel disease is Crohn's disease or ulcerative colitis.

[0008] In ~~some embodiments,~~one aspect, the present invention relates to a method for predicting the likelihood or monitoring the progression of mucosal healing in a subject having or suspected of having an inflammatory bowel disease (IBD), the method comprising:

(a) measuring a first set of markers to form an inflammatory phase marker score wherein the first set of markers comprises one or more of TWEAK, CRP, ICAM, SAA, VCAM, IL-2, IL-8, IL-12p70, IL-1β, GMCSF, IFNγ, IL-6, TNFα, ASCA-A, ASCA-G, CBir1, Fla2, FlaX, OmpC, and an anti-drug antibody (ADA), and ~~combinations thereof. In some instances,~~wherein each of the first set of markers ~~comprises one~~ is assigned a value based upon the concentration or ~~more of GMCSF, IL-2, and VCAM~~level of the marker relative to a standard or a set of standards or based upon a quantile level of the marker;.

~~[0009]~~ ~~In some embodiments,~~(b) measuring a second set of markers to form a proliferation phase marker score wherein the second set of markers comprises one or more of AREG, EREG, HBEGF, HGF, HRGB, BTC, EGF, TGFA, FGF1, FGF2, FGF4, FGF7, FGF9, FGF19,

SCF, PDGFA, PDGFB, PDGFC, VEGFA, VEGFB, VEGFC, VEGFD, TGFB1, IL-10, and an anti-TNFα antibody~~. In some instances, the second set of markers comprises HGF.~~, and

wherein each of the second set of markers is assigned a value based upon the concentration or level of the marker relative to a standard or a set of standards or based upon a quantile level of the marker;

(c) comparing the inflammatory phase marker score to the proliferation phase marker score; and

(d) predicting the likelihood of mucosal healing or monitoring the progression based upon the comparison in step (c).

[0008a] In one embodiment, the the first set of markers comprises one or more of GMCSF, IL-2, and VCAM and/or wherein the second set of markers comprises HGF.

~~**[0010]**~~**[0008b]** In ~~some~~ one embodiment~~s~~, the value assigned to each marker is ~~assigned a value of~~ selected from 0 to 6 based upon the concentration or level of the marker~~.~~

~~**[0011]** In some embodiments,~~ , and optionally wherein the concentration or level of the marker is relative to the level of the same marker in a patient population without mucosal healing.

~~**[0012]** In some embodiments~~ **[0008c]** In one embodiment, the method as described in paragraph [0008a] wherein

(i) the value for each marker in the first set of markers is summed to form the inflammatory phase marker score and/or~~. In some embodiments,~~

(ii) the value for each marker in the second set of markers is summed to form the proliferation phase marker score.

6. The method of any one of claims 1 to 5, wherein

~~**[0013]** In some embodiments,~~ (i) the comparison in step (c) comprises applying an algorithm incorporating the inflammatory phase marker score and the proliferation phase marker score~~.~~ and/or

~~**[0014]** In some embodiments,~~ (ii) the algorithm comprises subtracting the inflammatory phase marker score from the proliferation phase marker score to form a biomarker score of the subject.

~~[0015]~~ **[0008d]**~~In some embodiments,~~ In one embodiment, the method as described in paragraph [0008c] wherein the method comprises a method for predicting the likelihood of mucosal healing in a subject, wherein the subject has an increased likelihood of having complete improvement of mucosal healing without relapse when the biomarker score of the subject is higher than the biomarker score of a patient population without mucosal healing.

~~[0016]~~ **[0008e]** In ~~some embodiments~~one embodiment, the method as described in paragraph [0008c] wherein the method comprises a method for monitoring the progression of mucosal healing in a subject, wherein the subject is progressing through the phases of mucosal healing when the biomarker score of the subject increases at each time point over the plurality of time points and wherein the subject is progressing from a phase of mucosal healing selected from an inflammatory phase and a proliferation phase onto the next phase of mucosal healing.

[0008e] In one embodiment, the method as described in paragraph [0008c] wherein ~~In some embodiments,~~ the algorithm monitors the progression or predicts the likelihood of mucosal healing independent of clinical confounders~~. In some instances~~ and, wherein the clinical confounders comprise one or more selected from the group consisting of age of diagnosis, age of last sample, disease location, anal involvement, smoking, and surgery.

~~[0017]~~ **[0008f]** In ~~some embodiments~~one embodiment, the method as described in paragraph [0008c-e] wherein the algorithm predicts the likelihood or monitors the progression of mucosal healing ~~by~~ excluding serology markers~~. In other words, the algorithm can predict the likelihood of mucosal healing without the use of serology markers. In particular embodiments,~~ and wherein the excluded serology markers comprise one or more selected from the group consisting of ASCA-A, ASCA-G, CBir1, Fla2, FlaX, and OmpC.

~~[0018]~~ **[0008g]**In ~~some embodiments~~one embodiment, the method as described in paragraph [0008a-f] wherein the subject is receiving an anti-TNFα antibody~~. In some embodiments~~ wherein, the anti-TNFα antibody comprises one or more of REMICADE™ (infliximab), ENBREL™ (etanercept), HUMIRA™ (adalimumab), and CIMZIA® (certolizumab pegol).

[0019] **[0008h]** In some embodiments, In one embodiment, the method as described in paragraph [0008a-g] wherein the marker is measured in a sample selected from the group consisting of serum, plasma, whole blood, stool, peripheral blood mononuclear cells (PBMC), polymorphonuclear (PMN) cells, and a tissue biopsy, and combinations thereof.

[0020] **[00008i]** Also provided herein is In one embodiment, the method as described in paragraph [0008a-h] wherein the method comprises a method for monitoring the progression of mucosal healing in a subject further comprising optimizing therapeutic efficacy of an anti-TNFα antibody therapy based upon the progression of mucosal healing in the subject. The method comprises the steps of: (a) measuring a first set of markers at a plurality of time points to form a plurality of inflammatory phase marker scores; (b) measuring a second set of markers at a plurality of time points to form a plurality of proliferation phase marker scores; (c) comparing the inflammatory phase marker score to the proliferation phase marker score at each time point and across the plurality of time points; and (d) monitoring the progression of mucosal healing based upon the comparison in step (c).

[0021] In some embodiments, the subject has an inflammatory bowel disease (IBD). In some instances, the inflammatory bowel disease is Crohn's disease or ulcerative colitis.

[0022] In some embodiments, the first set of markers comprises one or more of TWEAK, CRP, ICAM, SAA, VCAM, IL-2, IL-8, IL-12p70, IL-1β, GMCSF, IFNγ, IL-6, TNFα, ASCA-A, ASCA-G, CBir1, Fla2, FlaX, OmpC, and an anti-drug antibody (ADA). In some instances, the first set of markers comprises one or more of GMCSF, IL-2, and VCAM.

[0023] In some embodiments, the second set of markers comprises one or more of AREG, EREG, HBEGF, HGF, HRGB, BTC, EGF, TGFA, FGF1, FGF2, FGF4, FGF7, FGF9, FGF19, SCF, PDGFA, PDGFB, PDGFC, VEGFA, VEGFB, VEGFC, VEGFD, TGFB1, IL-10, and an anti-TNFα antibody. In some instances, the second set of markers comprises HGF.

[0024] In some embodiments, each marker is assigned a value of from 0 to 6 based upon the concentration or level of the marker.

[0025] In some embodiments, the concentration or level of the marker is relative to the level of the same marker in a patient population without mucosal healing.

~~**[0026]** In some embodiments, the value for each marker in the first set of markers is summed to form the inflammatory phase marker score. In some embodiments, the value for each marker in the second set of markers is summed to form the proliferation phase marker score.~~

[0027] In some embodiments, the comparison in step (c) comprises applying an algorithm incorporating the inflammatory phase marker score and the proliferation phase marker score.

[0028] In some embodiments, the algorithm comprises subtracting the inflammatory phase marker score from the proliferation phase marker score to form a biomarker score of the subject at each time point.

[0029] In some embodiments, the subject is progressing through the phases of mucosal healing when the biomarker score of the subject increases at each time point over the plurality of time points. In some instances, the subject is progressing from a phase of mucosal healing selected from an inflammatory phase and a proliferation phase onto the next phase of mucosal healing.

[0030] In some embodiments, the algorithm monitors the progression of mucosal healing independent of clinical confounders. In some instances, the clinical confounders comprise one or more selected from the group consisting of age of diagnosis, age of last sample, disease location, anal involvement, smoking, surgery, and combinations thereof.

[0031] In some embodiments, the algorithm monitors the progression of mucosal healing by excluding serology markers. In other words, the algorithm can predict the progression of mucosal healing without the use of serology markers. In certain embodiments, the excluded serology markers comprise one or more selected from the group consisting of ASCA-A, ASCA-G, CBir1, Fla2, FlaX, and OmpC.

[0032] In some embodiments, the subject is receiving an anti-TNFα antibody. In some embodiments, the anti-TNFα antibody comprises one or more of REMICADE™ (infliximab), ENBREL™ (etanercept), HUMIRA™ (adalimumab), and CIMZIA® (certolizumab pegol).

[0033] In some embodiments, the marker at each time point is measured in a sample selected from the group consisting of serum, plasma, whole blood, stool, peripheral blood mononuclear cells (PBMC), polymorphonuclear (PMN) cells, and a tissue biopsy.

[0034] In some embodiments, the method further comprises optimizing therapeutic efficacy of an anti-TNFα antibody therapy based upon the progression of mucosal healing in the subject.

[0035] In yet other embodiments, the method further comprises selecting an appropriate therapeutic regimen based upon the progression of mucosal healing in the subject.

[0008j] In one embodiment, the method as described in paragraph [0008a-g] wherein the method comprises a method for monitoring the progression of mucosal healing in a subject further comprising selecting an appropriate therapeutic regimen based upon the progression of mucosal healing in the subject.

[0036] Other objects, features, and advantages of the present invention will be apparent to one of skill in the art from the following detailed description and figures.

BRIEF DESCRIPTION OF THE DRAWINGS

[0037] FIG. 1 illustrates the three phases of mucosal healing – inflammatory phase, proliferation phase and remodeling phase.

[0038] FIG. 2 shows representative data of clinical outcome for an individual who exhibited mucosal improvement (FIG. 2A) and an individual who experienced relapse after exhibiting complete improvement (FIG 2B).

[0039] FIG. 3 depicts the clinical status of individuals who have healed (FIG. 3A) and individuals who have never healed (FIG. 3B).

[0040] FIG. 4 shows the marker distributions between two extremes in the mucosal healing continuum – individuals who have never healed and individuals who have completely healed.

[0041] FIG. 5 depicts a table of inflammatory markers that are associated with mucosal healing. For GM-CSF, IL2, VCAM and HGF, lower marker values are predictive of mucosal healing.

[0042] FIG. 6A-B show a schematic of the individual patient analysis strategy that compares the expression of various markers in “true” healed indivudals (FIG. 6A) to not healed individuals (FIG. 6B).

[0043] FIG. 7A-F show inflammatory marker data used to identify “true” healed individuals. Individuals exhibiting low inflammation were selected for the individual patient analysis study.

[0044] FIG. 8A-D show clinical data (*e.g.*, ATI and/or IFX status) and the levels (*e.g.*, concentration) of repair factor markers in samples from Patient #1, a “true” healed individual.

[0045] FIG. 9 A-D show data of inflammatory, anti-inflammatory and serology markers for Patient #1.

[0046] FIG. 10 A-D show clinical data and the levels of repair factor markers in samples from Patient #2, a “true” healed individual.

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Alpco Diagnostics (Salem, NH), Pierce Biotechnology, Inc. (Rockford, IL), and/or Abazyme (Needham, MA).

[0194] In other instances, the presence or level of various markers such as an inflammatory phase marker, a growth factor marker, a repair factor marker, an anti-inflammatory marker, a proliferation phase marker, or a serology marker is detected using a multiplexed immunoarray, such as a Collaborative Enzyme Enhanced Reactive ImmunoAssay (CEER), also known as the Collaborative Proximity Immunoassay (COPIA). CEER is described in the following patent documents ~~which are herein incorporated by reference in their entirety for all purposes~~: PCT Publication Nos. WO 2008/036802, WO 2009/012140, WO 2009/108637, WO 2010/132723, WO 2011/008990, WO 2011/050069; WO 2012/088337; WO 2012/119113; and WO 2013/033623.

[0195] Suitable anti-repair factor antibodies useful in determining the level of a selected growth factor in a sample include, without limitation, an antibody that recognizes (binds to, forms a complex with, is specific for) a selected growth factor protein, a growth factor polypeptide having substantially the same amino acid sequence as the selected growth factor protein, or a fragment thereof such as an immunoreactive fragment thereof. A growth factor polypeptide generally describes polypeptides having an amino acid sequence with greater than about 50% identity, preferably greater than about 60% identity, more preferably greater than about 70% identity, still more preferably greater than about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity with a growth factor protein, with the amino acid identity determined using a sequence alignment program such as CLUSTALW. Suitable antibodies for determining the level of a growth factor are available from, *e.g.*, Promega (Madison, WI), R&D Systems, Inc. (Minneapolis, MN), Invitrogen (Camarillo, CA), CHEMICON International, Inc. (Temecula, CA), Abcam (Cambridge, MA), Santa Cruz Biotechnology (Santa Cruz, CA), and Dako (Carpinteria, CA).

C. Inflammatory Phase Markers

[0196] In certain aspects, any of a variety of inflammatory phase markers, including but not limited to biochemical markers, serological markers, protein markers, and/or other clinical characteristics, are useful in the methods of the present invention. In particular embodiments, the methods herein facilitate predicting the likelihood of mucosal healing and monitoring the

[0260] The human stem cell factor (SCF) polypeptide sequence is set forth in, *e.g.*, NCBI Accession No. AAI26167.1. One skilled in the art will appreciate that PDGF-A is also known as kit ligand, c-kit ligand, mast cell growth factor and MGF. One skilled in the art will appreciate that SCF includes all isoform variants.

[0261] The interleukin 10 (IL-10) polypeptide sequence is set forth in, *e.g.*, NCBI Accession No. AAI04253.1. One skilled in the art will appreciate that IL-10 includes all isoform variants.

E. Anti-TNF Drug Levels & Anti-Drug Antibody (ADA) Levels

[0262] In some embodiments, the method comprises determining the presence and/or level of a drug analyte in a patient sample (*e.g.*, a serum sample from a patient on anti-TNF drug therapy) to form an inflammatory phase marker score and/or proliferation phase marker score. In certain instances, the measurements can be made at multiple time points, *e.g.*, before, during, and/or after the course of therapy. In some embodiments, the drug analyte is an anti-TNF drug (*e.g.*, level of free anti-TNFα therapeutic antibody such as infliximab) and/or an anti-drug antibody (ADA) (*e.g.*, level of autoantibody to the anti-TNF drug such as HACA, HAHA, HAMA, and combinations thereof).

[0263] In particular embodiments, the presence and/or level of an anti-TNF drug and/or ADA is determined with a homogeneous mobility shift assay using size exclusion chromatography. These methods and related technology are described in PCT Publication Nos. WO 2011/056590, WO 2012/054532, WO 2012/154253 and WO 2013/006810, and in US Provisional Application No. 61/683,681, filed August 15, 2012~~, the disclosures of which are incorporated by reference in their entirety for all purposes~~. These methods are particularly advantageous for measuring the presence or level of TNFα inhibitors as well as autoantibodies (*e.g.*, HACA, HAHA, *etc.*) that are generated against them.

[0264] The method for determining the presence or level of an anti-TNFα drug in a sample can comprise: (a) contacting a labeled TNFα with a sample having an anti-TNFα drug to form a labeled complex with the anti-TNFα drug; (b) subjecting the labeled complex to size exclusion chromatography to separate the labeled complex from free labeled TNFα and to detect an amount of the labeled complex and an amount of the free labeled TNFα; and (c) comparing the amount of the labeled complex and the amount of the free labeled TNFα detected in step (b) to a

[0331] Next, the analysis of the biomarker score was performed by excluding the serology markers. Once again, the biomarker score was significantly associated with an increased odd of having complete improvement (FIG. 31). This was also the case when the data was adjusted to control for potential clinical confounders (FIG. 32). FIG. 33 shows the distribution of the biomarker score without serology markers and partitioned by mucosal healing status.

[0332] This example describes a biomarker score approach that can be used when multivariate modeling cannot be used with the dataset. The method involves the following steps: 1) selecting an experimental biomarker set (*e.g.*, proliferation phase markers and inflammatory phase markers except ATI and IFX) to be tested; 2) calculating the p-value, OR and ROC AUC of the biomarker score as a single marker is excluded from the analysis; 3) eliminating any marker from the experimental biomarker set if that marker results in the lowest p-value when excluded in the analysis; 4) plotting the biomarker score, p-value, OR and ROC AUC; and 5) repeating steps 1-4. The trends in the statistical analysis can be used to determine which markers of the set are more predictive of the remodeling phase of mucosal healing (*e.g.*, complete improvement without relapse) (FIG. 34 A-C). This example provides a method for selecting informative markers that are predictive of the different phases of mucosal healing.

[0333] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, one of skill in the art will appreciate that certain changes and modifications may be practiced within the scope of the appended claims. ~~In addition, each reference provided herein is incorporated by reference in its entirety to the same extent as if each reference was individually incorporated by reference.~~

WHAT IS CLAIMED IS:

1. A method for predicting the likelihood of mucosal healing in a subject, the method comprising:

(a) measuring a first set of markers to form an inflammatory phase marker score;

(b) measuring a second set of markers to form a proliferation phase marker score;

(c) comparing the inflammatory phase marker score to the proliferation phase marker score; and

(d) predicting the likelihood of mucosal healing based upon the comparison in step (c).

2. The method of claim 1, wherein the subject has an inflammatory bowel disease (IBD).

3. The method of claim 2, wherein the inflammatory bowel disease is Crohn's disease or ulcerative colitis.

4. The method of any one of claims 1 to 3, wherein the first set of markers comprises one or more of TWEAK, CRP, ICAM, SAA, VCAM, IL-2, IL-8, IL-12p70, IL-1β, GMCSF, IFNγ, IL-6, TNFα, ASCA-A, ASCA-G, CBir1, Fla2, FlaX, OmpC, and an anti-drug antibody (ADA).

5. The method of claim 4, wherein the first set of markers comprises one or more of GMCSF, IL-2, and VCAM.

6. The method of any one of claims 1 to 5, wherein the second set of markers comprises one or more of AREG, EREG, HBEGF, HGF, HRGB, BTC, EGF, TGFA, FGF1, FGF2, FGF4, FGF7, FGF9, FGF19, SCF, PDGFA, PDGFB, PDGFC, VEGFA, VEGFB, VEGFC, VEGFD, TGFB1, IL-10, and an anti-TNFα antibody.

7. The method of claim 6, wherein the second set of markers comprises HGF.

8. The method of any one of claims 1 to 7, wherein each marker is assigned a value of from 0 to 6 based upon the concentration or level of the marker.

9. The method of claim 8, wherein the concentration or level of the marker is relative to the level of the same marker in a patient population without mucosal healing.

10. The method of any one of claims 1 to 9, wherein the value for each marker in the first set of markers is summed to form the inflammatory phase marker score.

11. The method of any one of claims 1 to 10, wherein the value for each marker in the second set of markers is summed to form the proliferation phase marker score.

12. The method of any one of claims 1 to 11, wherein the comparison in step (c) comprises applying an algorithm incorporating the inflammatory phase marker score and the proliferation phase marker score.

13. The method of claim 12, wherein the algorithm comprises subtracting the inflammatory phase marker score from the proliferation phase marker score to form a biomarker score of the subject.

14. The method of claim 12, wherein the subject has an increased likelihood of having complete improvement of mucosal healing without relapse when the biomarker score of the subject is higher than the biomarker score of a patient population without mucosal healing.

15. The method of any one of claims 12 to 14, wherein the algorithm predicts the likelihood of mucosal healing independent of clinical confounders.

16. The method of claim 15, wherein the clinical confounders comprise one or more selected from the group consisting of age of diagnosis, age of last sample, disease location, anal involvement, smoking, and surgery.

17. The method of any one of claims 12 to 16, wherein the algorithm predicts the likelihood of mucosal healing excluding serology markers.

18. The method of claim 17, wherein the excluded serology markers comprise one or more selected from the group consisting of ASCA-A, ASCA-G, CBir1, Fla2, FlaX, and OmpC.

19. The method of any one of claims 1 to 18, wherein the subject is receiving an anti-TNFα antibody.

20. The method of claim 19, wherein the anti-TNFα antibody comprises one or more of REMICADE™ (infliximab), ENBREL™ (etanercept), HUMIRA™ (adalimumab), and CIMZIA® (certolizumab pegol).

21. The method of any one of claims 1 to 20, wherein the marker is measured in a sample selected from the group consisting of serum, plasma, whole blood, stool, peripheral blood mononuclear cells (PBMC), polymorphonuclear (PMN) cells, and a tissue biopsy.

22. A method for monitoring the progression of mucosal healing in a subject, the method comprising:

(a) measuring a first set of markers at a plurality of time points to form a plurality of inflammatory phase marker scores;

(b) measuring a second set of markers at a plurality of time points to form a plurality of proliferation phase marker scores;

(c) comparing the inflammatory phase marker score to the proliferation phase marker score at each time point and across the plurality of time points; and

(d) monitoring the progression of mucosal healing based upon the comparison in step (c).

23. The method of claim 22, wherein the subject has an inflammatory bowel disease (IBD).

24. The method of claim 23, wherein the inflammatory bowel disease is Crohn's disease or ulcerative colitis.

25. The method of any one of claims 22 to 24, wherein the first set of markers comprises one or more of TWEAK, CRP, ICAM, SAA, VCAM, IL-2, IL-8, IL-12p70, IL-1β, GMCSF, IFNγ, IL-6, TNFα, ASCA-A, ASCA-G, CBir1, Fla2, FlaX, OmpC, and an anti-drug antibody (ADA).

26. The method of claim 25, wherein the first set of markers comprises one or more of GMCSF, IL-2, and VCAM.

27. The method of any one of claims 22 to 26, wherein the second set of markers comprises one or more of AREG, EREG, HBEGF, HGF, HRGB, BTC, EGF, TGFA, FGF1, FGF2, FGF4, FGF7, FGF9, FGF19, SCF, PDGFA, PDGFB, PDGFC, VEGFA, VEGFB, VEGFC, VEGFD, TGFB1, IL-10, and an anti-TNFα antibody.

28. The method of claim 27, wherein the second set of markers comprises HGF.

29. The method of any one of claims 22 to 28, wherein each marker is assigned a value of from 0 to 6 based upon the concentration or level of the marker.

30. The method of claim 29, wherein the concentration or level of the marker is relative to the level of the same marker in a patient population without mucosal healing.

31. The method of any one of claims 22 to 30, wherein the value for each marker in the first set of markers is summed to form the inflammatory phase marker score.

32. The method of any one of claims 22 to 31, wherein the value for each marker in the second set of markers is summed to form the proliferation phase marker score.

33. The method of any one of claims 22 to 32, wherein the comparison in step (c) comprises applying an algorithm incorporating the inflammatory phase marker score and the proliferation phase marker score.

34. The method of claim 33, wherein the algorithm comprises subtracting the inflammatory phase marker score from the proliferation phase marker score to form a biomarker score of the subject at each time point.

35. The method of claim 34, wherein the subject is progressing through the phases of mucosal healing when the biomarker score of the subject increases at each time point over the plurality of time points.

36. The method of claim 35, wherein the subject is progressing from a phase of mucosal healing selected from an inflammatory phase and a proliferation phase onto the next phase of mucosal healing.

37. The method of any one of claims 33 to 36, wherein the algorithm monitors the progression of mucosal healing independent of clinical confounders.

38. The method of claim 37, wherein the clinical confounders comprise one or more selected from the group consisting of age of diagnosis, age of last sample, disease location, anal involvement, smoking, and surgery.

39. The method of any one of claims 33 to 38, wherein the algorithm monitors the progression of mucosal healing excluding serology markers.

40. The method of claim 39, wherein the excluded serology markers comprise one or more selected from the group consisting of ASCA-A, ASCA-G, CBir1, Fla2, FlaX, and OmpC.

41. The method of any one of claims 22 to 40, wherein the subject is receiving an anti-TNFα antibody.

42. The method of claim 41, wherein the anti-TNFα antibody comprises one or more of REMICADE™ (infliximab), ENBREL™ (etanercept), HUMIRA™ (adalimumab), and CIMZIA® (certolizumab pegol).

43. The method of any one of claims 22 to 42, wherein the marker at each time point is measured in a sample selected from the group consisting of serum, plasma, whole blood, stool, peripheral blood mononuclear cells (PBMC), polymorphonuclear (PMN) cells, and a tissue biopsy.

44. The method of any one of claims 22to 43, further comprising optimizing therapeutic efficacy of an anti-TNFα antibody therapy based upon the progression of mucosal healing in the subject.

45. The method of any one of claims 22 to 43, further comprising selecting an appropriate therapeutic regimen based upon the progression of mucosal healing in the subject.

Claims

1. A method for predicting the likelihood or monitoring the progression of mucosal healing in a subject having or suspected of having an inflammatory bowel disease (IBD), the method comprising:

(a) measuring a first set of markers to form an inflammatory phase marker score wherein the first set of markers comprises one or more of TWEAK, CRP, ICAM, SAA, VCAM, IL-2, IL-8, IL-12p70, IL-1ß, GMCSF, IFNγ, IL-6, TNFα, ASCA-A, ASCA-G, CBir1, Fla2, FlaX, OmpC, and an anti-drug antibody (ADA), and wherein each of the first set of markers is assigned a value based upon the concentration or level of the marker relative to a standard or a set of standards or based upon a quantile level of the marker;

(b) measuring a second set of markers to form a proliferation phase marker score wherein the second set of markers comprises one or more of AREG, EREG, HBEGF, HGF, HRGB, BTC, EGF, TGFA, FGF1, FGF2, FGF4, FGF7, FGF9, FGF19, SCF, PDGFA, PDGFB, PDGFC, VEGFA, VEGFB, VEGFC, VEGFD, TGFB1, IL-10, and an anti-TNFα antibody, and

wherein each of the second set of markers is assigned a value based upon the concentration or level of the marker relative to a standard or a set of standards or based upon a quantile level of the marker;

(c) comparing the inflammatory phase marker score to the proliferation phase marker score; and

(d) predicting the likelihood of mucosal healing or monitoring the progression based upon the comparison in step (c).

2. The method of claim 1, wherein the inflammatory bowel disease is Crohn’s disease or ulcerative colitis.

3. The method of claim 1, wherein the first set of markers comprises one or more of GMCSF, IL-2, and VCAM and/or wherein the second set of markers comprises HGF.

4. The method of any one of claims 1 to 3, wherein the value assigned to each marker is selected from 0 to 6 based upon the concentration or level of the marker and optionally wherein the concentration or level of the marker is relative to the level of the same marker in a patient population without mucosal healing.

5. The method of any one of claims 1 to 4, wherein

(i) the value for each marker in the first set of markers is summed to form the inflammatory phase marker score and/or

(ii) the value for each marker in the second set of markers is summed to form the proliferation phase marker score.

6. The method of any one of claims 1 to 5, wherein

(i) the comparison in step (c) comprises applying an algorithm incorporating the inflammatory phase marker score and the proliferation phase marker score; and/or

(ii) the algorithm comprises subtracting the inflammatory phase marker score from the proliferation phase marker score to form a biomarker score of the subject.

7. The method of claim 6, wherein the method comprises a method for predicting the likelihood of mucosal healing in a subject, wherein the subject has an increased likelihood of having complete improvement of mucosal healing without relapse when the biomarker score of the subject is higher than the biomarker score of a patient population without mucosal healing.

8. The method of claim 6, wherein the method comprises a method for monitoring the progression of mucosal healing in a subject, wherein the subject is progressing through the phases of mucosal healing when the biomarker score of the subject increases at each time point over the plurality of time points and wherein the subject is progressing from a phase of mucosal healing selected from an inflammatory phase and a proliferation phase onto the next phase of mucosal healing.

9. The method of claim 6, wherein the algorithm monitors the progression or predicts the likelihood of mucosal healing independent of clinical confounders and, wherein the clinical confounders comprise one or more selected from the group consisting of age of diagnosis, age of last sample, disease location, anal involvement, smoking, and surgery.

10. The method of any one of claims 6 to 9, wherein the algorithm predicts the likelihood or monitors the progression of mucosal healing excluding serology markers and wherein the excluded serology markers comprise one or more selected from the group consisting of ASCA-A, ASCA-G, CBir1, Fla2, FlaX, and OmpC.

11. The method of any one of claims 1 to 10, wherein the subject is receiving an anti-TNFα antibody, wherein the anti-TNFα antibody comprises one or more of REMICADE™ (infliximab), ENBREL™ (etanercept), HUMIRA™ (adalimumab), and CIMZIA® (certolizumab pegol).

12. The method of any one of claims 1 to 11, wherein the marker is measured in a sample selected from the group consisting of serum, plasma, whole blood, stool, peripheral blood mononuclear cells (PBMC), polymorphonuclear (PMN) cells, and a tissue biopsy.

13. The method of any one of claims 1 to 12, wherein the method comprises a method for monitoring the progression of mucosal healing in a subject further comprising optimizing therapeutic efficacy of an anti-TNFα antibody therapy based upon the progression of mucosal healing in the subject.

14. The method of any one of claims 1 to 12, wherein the method comprises a method for monitoring the progression of mucosal healing in a subject further comprising selecting an appropriate therapeutic regimen based upon the progression of mucosal healing in the subject.