Biomarker, kit and method for predicting clinical responsiveness to therapy with an agent that targets alpha4beta7 integrin

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(54) Title: BIOMARKER, KIT AND METHOD FOR PREDICTING CLINICAL RESPONSIVENESS TO THERAPY WITH AN AGENT THAT TARGETS ALPHA$_4$BETA$_7$ INTEGRIN.

(57) Abstract: The invention relates generally to biomarkers, methods and kits to predict therapy response in patients, in particular in patients having inflammatory bowel disease (IBD). Provided is a method for predicting whether a human subject is likely to show a clinical response to therapy with an antibody or pharmacological antagonist that targets $\alpha_4\beta_7$ integrin, preferably vedolizumab induction therapy, comprising (i) determining the level of eotaxin-1 in a sample obtained from the subject, and; (ii) classifying the subject as a responder based on the level of eotaxin-1.

Title: Biomarker, kit and method for predicting clinical responsiveness to therapy with an agent that targets alpha$_4$beta$_7$ integrin.

The invention relates generally to the field of pharmacodynamics, and more specifically to biomarkers, methods and kits to predict therapy response in a patient, for example in patients having a bowel disease, such as inflammatory bowel disease (IBD).

In general, IBD encompasses two major forms of chronic intestinal inflammation: ulcerative colitis (UC) and Crohn's disease (CD), known also as Crohn's ileitis, regional enteritis, or granulomatous colitis. UC is a chronic inflammatory disease of unknown etiology afflicting the large intestine. The course of the disease may be continuous or relapsing, mild or severe.(1) The earliest lesion is an inflammatory infiltration with abscess formation at the base of the crypts of Lieberkühn. A separation of the overlying mucosa from its blood supply leading to ulceration is observed. Signs and symptoms of the disease include cramping, lower abdominal pain, rectal bleeding, and frequent, loose discharges consisting mainly of blood, pus, and mucus with scanty fecal particles. A total colectomy may be needed to treat acute severe or chronic ulcerative colitis.

CD is also a chronic inflammatory disease of unknown etiology but, unlike ulcerative colitis, it can affect any part of the gastro-intestinal tract. The most prominent feature of the disease is the granular, reddish-purple edematous thickening of the bowel wall. With the development of inflammation and granulomas that often lose their circumscribed borders and integrate with the surrounding tissue.(2) Diarrhea and obstruction of the bowel are the predominant clinical features. Most patients with Crohn's disease require surgery at some point, but subsequent relapse is common. Consequently, continuous medical treatment is necessary ever after the initial diagnosis.

Biological agents have become the most important treatment modality for patients with moderate to severe inflammatory bowel disease (IBD).(3) One of these agents, vedolizumab, is a humanized monoclonal IgG1 antibody targeting the $\alpha_4\beta_7$ integrin heterodimer that is capable of blocking leukocyte trafficking across the endothelium in the intestine and/or modulates macrophage function as potential therapeutic mechanisms.(4-5) In several phase II studies and phase III randomized controlled trials (GEMINI), vedolizumab has shown efficacy, safety and tolerability as treatment for IBD. Clinical response and remission rates after induction therapy (classically week 14) vary between 49-64% and 24-36% for CD, and 43-57% and 23-39% for UC, respectively.(6-9) As such, since October 2014, vedolizumab has been approved in the Netherlands for IBD patients having moderate to severe disease activity and which failed on conventional treatment modalities (e.g. corticosteroids, immunomodulators) and/or anti-TNFα therapy.

Vedolizumab is a very expensive treatment for IBD. The biosimilars of TNFα inhibitors, such as infliximab or adalimumab, are more cost-effective than vedolizumab because they are cheaper. However, more recently in a head-to-head comparison vedolizumab was more effective than adalimumab in UC. Therefore, vedolizumab is the second-line biologic drug after anti-TNFα failure in CD, but it is likely to become the first line biological therapy in UC.(10)

Although adverse events of vedolizumab treatment are generally mild, remission can only be achieved in 31% in 52 weeks therefore 69% of patients are exposed to discomfort without gaining any benefit to this drug treatment.(11)

Thus, it is of critical importance to identify reliable predictive factors for therapy response in order to be able to identify IBD patients who will most likely benefit from treatment with vedolizumab.

Several predictive factors for clinical response to vedolizumab have been reported to date, most of which can be categorized as individual and/or life style factors (e.g. age, BMI, smoking behavior), clinical factors (disease-specific characteristics, e.g. disease location, surgical history, disease duration) and medication-related factors (e.g. concomitant use of corticosteroids or immunomodulators). However, these factors appear to have limited value in clinical decision making as they lack sufficient sensitivity and specificity. Moreover, inconsistent predictive power/value of these factors have been reported (12). Besides clinical factors, indicators of disease activity, such as C-reactive protein (CRP) or fecal calprotectin, have also been evaluated for their predictive performance (13-16). However, since these inflammatory biomarkers are general markers of systemic and bowel inflammation, respectively, they lack sufficient sensitivity and specificity regarding vedolizumab treatment response. Consequently, they have little predictive value for vedolizumab treatment response.

The inventors therefore set out to identify more powerful, reliable, validated and objectively measurable biomarkers for predicting therapy response to agents targeting the $\alpha_4\beta_7$ integrin heterodimer, such as neutralizing anti-$\alpha_4\beta_7$ integrin antibodies like vedolizumab, as well as pharmacological antagonists inhibiting the $\alpha_4\beta_7$ integrin heterodimer. In particular, they aimed at providing a predictive biomarker that is readily detected in a serum/plasma sample of any human test subject, be it a healthy individual, an IBD patient, a responder or a non-responder to therapeutic interventions which target anti-$\alpha_4\beta_7$ integrin.

It was surprisingly found that elevated baseline serum levels of C-C motif chemokine 11 (CCL11, also known as eosinophil chemotactic protein or eotaxin-1) is significantly associated with increased odds of clinical response or remission to vedolizumab induction therapy. It was observed

that a responder has an increased eotaxin-1 level as compared to a non-responder. The average reference values of serum eotaxin-1 in healthy subjects is 0.28 ng/ml, which is slightly enhanced in vedolizumab responders and reduced in vedolizumab non-responders (0.31 vs. 0.21 ng/ml $P < 0.05$, see Figure 1).

Importantly, serum eotaxin-1 levels appear to be far more accurate in predicting clinical response or remission to vedolizumab than serum CRP levels. The optimal cut-off value for serum eotaxin-1 levels yielded a sensitivity of 75.0% and specificity of 76.7% in predicting clinical response in an IBD study cohort with very good discriminative capacity (as represented by a Youden's index of 0.52). By comparison, in the same cohort, the optimal cut-off for serum CRP levels showed a sensitivity of only 41.4% and specificity of 85.7% in predicting response to vedolizumab induction therapy, with a ~50% lower discriminative capacity (Youden's index 0.27) compared to serum eotaxin-1 levels. Hence, serum eotaxin-1 is the most accurate biomarker to date to predict whether an IBD patient will respond to therapy using vedolizumab or a similar (antibody/antagonist) agent targeting $\alpha_4\beta_7$ integrin. Furthermore, incorporating serum eotaxin-1 levels as biomarker into already existing –but less powerful- predictive factors for vedolizumab treatment response may greatly improve the precision and reliability of a personalized forecast of treatment success.

Accordingly, in one embodiment the invention provides a method for predicting whether a human subject is likely to show a clinical response or remission to anti-$\alpha_4\beta_7$ integrin therapy, comprising: (i) determining the level of eotaxin-1 in a sample obtained (before the onset of therapy) from the subject, and (ii) classifying the subject as a responder or a non-responder based on the level of eotaxin-1.

Human eotaxin-1 (CCL11), also known as small-inducible cytokine A11 (SCYA11), is a 10.7 kDa glycoprotein that binds to the cytokine receptor CCR3. Eotaxin-1 was originally implicated in the selective recruitment of eosinophils into inflammatory sites during allergic reactions. It has been thoroughly investigated in asthma, allergic rhinitis, and other eosinophil-related conditions.(17-19) Eotaxin-1/CCL11 is also associated with a skewed immune response toward a T helper type-2 (Th2) profile. In addition to its role in the immune response, recent studies have shown that eotaxin-1/CCL11 is also associated with aging, neurogenesis and neurodegeneration, being able to influence neural progenitor cells, and microglia. Increased circulating levels of eotaxin-1/CCL11 have been described in major psychiatric disorders (schizophrenia, bipolar disorder, major depression), sometimes correlating with the severity of psychopathological and cognitive parameters.

It is proposed that eotaxin-1 might also play a role in the disease pathogenesis of IBD, and that it may be an interesting target for the treatment of UC. (20) However, the use of eotaxin-1 as predictive marker for therapy response to agents targeting $\alpha_4\beta_7$ integrin is not disclosed or suggested in the art.

US2016/0209426 and WO2016/088068 relate to assessing therapy responsiveness to vedolizumab by measuring the level of one or more predictive markers. In some embodiments, the level of gene expression of one or more cytokines selected from IL-1ß, IL-6, IL-12-p40, IL-17A, IL-17-F, IL-23A, IFNγ and TNFα is measured. Importantly, these documents are silent about eotaxin-1/CCL-11.

Zwicker *et al.* measured a range of chemokines, including eotaxin-1/CCL11, in a small cohort of 11 IBD patients that were treated with vedolizumab.(21) CCL11 was not implicated as predictive biomarker for the response to vedolizumab (see figure 1A in that report). Instead, CCL13 was

proposed to be of possible prognostic value for response to vedolizumab treatment in IBD patients.

Verstockt *et al.*, 2018 aimed to identify immunological biomarkers in an attempt to identify serum markers that predict the outcome to ustekinumab in patients with refractory Crohn's disease.(22) Whereas the study does implicate CCL11 as potential marker for response to ustekinumab, this finding bears no relevance to the present invention since ustekinumab is a monoclonal antibody that targets the p40 subunit shared by two cytokines, interleukin (IL)-12 and 23, thereby preventing their interaction with the receptor. This molecular action is structurally and functionally distinct from $\alpha_4\beta_7$ integrin which is the target of vedolizumab.

WO2014/196841 discloses methods and kits for detecting serum and plasma levels of eotaxin, MIP1α and CRP which are reported to act as biomarkers useful for determining the feasibility in instigating immunotherapeutic treatment when immunizing with a specific peptide fragment derived from human telomerase.

WO2006/073682 discloses serum cytokine profiles (including eotaxin-1) that may discriminate IBD from healthy controls, as well as UC from CD. There is no information on whether the IBD patients included actually have active disease and/or its severity. Furthermore, nothing is taught or suggested about predicting therapy response, let alone the use of eotaxin-1 as predictive marker for therapy response to agents targeting $\alpha_4\beta_7$ integrin.

Bourgonje *et al.* (2019) *Gastroenterology* 156(6):S-848 demonstrates that a combined set of four serum inflammatory markers reliably predicts endoscopic disease activity in IBD, the biomarkers being SAA, IL-6, IL-8 and Eotaxin-1. However, as demonstrated herein below in Example 2,serum markers for IBD disease activity/progression are not necessarily also a priori predictive markers for therapy response in IBD. Notably, only

eotaxin-1 but none of SAA, IL-6 and IL-8 was found to be a predictive marker for therapy response. Moreover, eotaxin-1 was not useful to predict therapy response to Infliximab therapy, which is another commonly used therapy to treat IBD patients.

These data convincingly show that a clear distinction must be made between "disease activity marker" and "predictor of therapy response". Accordingly, the implication of eotaxin-1 being linked to IBD disease progression/activity in no way implies or suggests its use as predictive a priori marker for therapy response to agents targeting α4ß7 integrin.

A method of the invention generally relates to predicting whether a human subject is likely to show a clinical response or remission to therapy which targets $\alpha_4\beta_7$ integrin. As used herein, "therapy which targets $\alpha_4\beta_7$ integrin" refers to any treatment with an agent (or multiple agents) which selectively acts on, and blocks, antagonizes and/or inhibits the functionality of, $\alpha_4\beta_7$ integrin found on surface of T-cells. For example, its blocks the interaction of $\alpha_4\beta_7$ integrin with MAdCAM-1, which is expressed on endothelial surface of venules within the GI tract and associated lymphoid tissue. As a result, it prevents leukocyte binding to endothelial surface and its extravasation into affected tissue. In one embodiment, said therapy comprises treatment with a drug that targets $\alpha_4\beta_7$ integrin. In a preferred embodiment, the therapy comprises treatment with a (gut-specific) $\alpha_4\beta_7$ integrin-neutralizing monoclonal Ab or pharmacological antagonist.

In a specific aspect, the invention provides a method for predicting whether a human subject is likely to show a clinical response or remission to vedolizumab. The chemical name for vedolizumab is IgG1-κ, antihuman integrin lymphocyte Peyer's patch adhesion molecule 1 (human – *Mus musculus* heavy chain), disulfide with human – *Mus musculus* α-chain, dimer. Its molecular formula is $C_{6528}H_{10072}N_{1732}O_{2042}S_{42}$ and molecular

weight is 146.8 kDa (WHO. International Nonproprietary Names for Pharmaceutical Substances. WHO Drug Information. 2008;22:311–67).

As used herein, a clinical response is defined by a lowering of a disease activity score, such as Physicians Global Assessment (PGA), CDAI, HBI, SSCAI, partial MAYO, etc.). Clinical remission is defined as a disease activity score below the conventional threshold score of a given disease (e.g. GPA normal, CDA <150, HBI <5, SCCAI <2.5, MAYO <2). See Sandborn *et al.* for CD (Gastroenterology 2002;122:512-530) or D'Haens *et al.* for UC (Gastroenterology 2007;132:763-786).

Typically, a human subject receiving or intended to receive therapy which targets $\alpha_4\beta_7$ integrin, e.g. vedolizumab induction therapy, suffers from inflammatory bowel disease (IBD) or a related disease. In a specific embodiment of the invention, the subject is a UC or CD patient.

The predictive eotaxin-1 biomarker is readily detected and quantitated in a biological sample obtained from the subject. Preferred samples are those that can be obtained in a non-invasive or minimally invasive manner. For example, the sample is a urine sample, a whole blood sample, a serum sample, a plasma sample. In a preferred aspect, the sample is a serum sample. However, other types of samples including an intestinal biopsy are also within the scope of the invention.

A method of the invention typically comprises detecting the concentration/level of eotaxin-1, and optional further biomarkers, by using one or more detection reagents. By "detecting" is intended measuring, quantifying, scoring, or assaying the amount, concentration, or relative abundance of eotaxin-1. The invention also provides the use of eotaxin-1 as a biomarker for predicting clinical responsiveness to vedolizumab induction therapy.

Eotaxin-1 levels can be determined at the mRNA level or at the protein level. In some embodiments, the mRNA level of eotaxin-1 is determined. In other embodiments, the protein level of eotaxin-1 is determined. It is also encompassed to determine eotoxin-1 levels by assessing the functionality or activity of eotoxin-1 protein.

Detection of the eotaxin-1 mRNA can be performed by techniques known in the art allowing mRNA qualitative and/or a quantitative analysis. The presence of an mRNA marker / biomarker genes and their expression level is for example determined by means of sequence-based methods, such as serial analysis of gene expression (SAGE) (as supersage), real-time quantitative PCR (qPCR) (such as RT-qPCR), bead technology, blotting, RNA or next-generation sequencing (as IonTorrent) - hybridization-based methods, such as in situ hybridization, Northern blot, DNA micro and macro arrays, or combinations thereof. The expression level of eotaxin-1 mRNA can be determined relative to a reference standard, such as a housekeeping gene. Exemplary housekeeping genes include ß-actin (*ACTB*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and 18S ribosomal RNA.

Very good predictive results were obtained with detecting the level of eotaxin-1 protein. Methods of evaluating biological compounds, including biomarker proteins, are known in the art. It is recognized that methods of detecting a protein biomarker include direct measurements and indirect measurements. One skilled in the art would be able to select an appropriate method of assaying eotaxin-1 biomarker protein. The biomarker protein(s) of the invention can be detected in a sample by any means. Preferred methods for biomarker detection are antibody-based assay, a protein array assay and a mass spectrometry (MS) based assay. For example, immunoassays, include competitive and non-competitive assay systems using techniques

such as Western blotting, radioimmunoassays, ELISA (enzyme-linked immunosorbent assay), immunohistochemistry, "sandwich" immunoassays, immunoprecipitation assays, precipitation reactions, gel diffusion precipitin reactions, immunodiffusion assays, fluorescent immunoassays and the like. Such assays are routine and well known in the art.

A method of the invention typically uses an agent or compound that specifically (or selectively) binds to, interacts with or detects the eotaxin-1 biomarker of interest. Such detection reagents may include an antibody, polyclonal antibody, or monoclonal antibody that preferentially binds the eotaxin-1 protein. The phrase "specifically (or selectively) binds" or "specifically (or selectively) immunoreactive with," when referring to a detection reagent, refers to a binding reaction that is determinative of the presence of eotaxin-1 in a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified detection reagent (e.g. antibody) binds to a particular protein at least two times the background and does not substantially bind in a significant amount to other proteins present in the sample. Specific binding under such conditions may require an antibody that is selected for its specificity for a particular protein. For example, polyclonal antibodies raised against eotaxin-1 from specific species such as rat, mouse, or human can be selected to obtain only those polyclonal antibodies that are specifically immunoreactive with eotaxin-1 and not with other proteins, except for polymorphic variants and alleles of eotaxin-1. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein (see, e.g., Harlow & Lane, Antibodies, A Laboratory Manual (1988), for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity). Typically, a specific or selective

reaction will be at least twice background signal or noise and more typically more than 10 to 100 times background.

"Antibody" as used herein refers to a polypeptide ligand substantially encoded by an immunoglobulin gene or immunoglobulin genes, or fragments thereof, which specifically binds and recognizes an epitope (e.g., an antigen). The recognized immunoglobulin genes include the kappa and lambda light chain constant region genes, the alpha, gamma, delta, epsilon and mu heavy chain constant region genes, and the myriad immunoglobulin variable region genes. Antibodies exist, e.g., as intact immunoglobulins or as a number of well characterized fragments produced by digestion with various peptidases. This includes, e.g., Fab' and F(ab)'2 fragments. The term "antibody," as used herein, also includes antibody fragments either produced by the modification of whole antibodies or those synthesized *de novo* using recombinant DNA methodologies. It also includes polyclonal antibodies, monoclonal antibodies, chimeric antibodies, humanized antibodies, or single chain antibodies. "Fc" portion of an antibody refers to that portion of an immunoglobulin heavy chain that comprises one or more heavy chain constant region domains, CH1, CH2 and CH3, but does not include the heavy chain variable region.

In a method of the invention, a test subject can be classified as a responder or non-responder based on the detected level of eotaxin-1. For example, it is determined how the measured eotaxin-1 level compares to the (range of) eotaxin-1 levels in a population of healthy subjects. In one embodiment, a test subject, e.g. an IBD patient, is classified as a responder based on an increased level of eotaxin-1, e.g. as compared to a reference value or reference range. In another embodiment, a test subject is classified as a non-responder based on a reduced level of eotaxin-1, e.g. as compared to a reference value or reference range.

A method of the invention may suitably comprise comparing the eotaxin-1 level detected to a cut-off value determined by analyzing a set of values of (serum) concentrations of the eotaxin-1 marker from subjects who received anti-$\alpha_4\beta_7$ integrin therapy and were classified as a responder or a non-responder based on one or more clinical endpoints. For example, the method involves determining whether a subject is likely to respond to, or resist, the anti-$\alpha_4\beta_7$ integrin therapy by comparing the eotaxin-1 level detected in a sample obtained from the subject (prior to therapy onset) with a reference eotaxin-1 level characteristic of patients responsive to, or non-responsive (resistant) to, the anti-$\alpha_4\beta_7$ integrin therapy. In one embodiment, it comprises comparing said concentration of eotaxin-1 in the test sample to an eotaxin-1 cutoff value whereby if the concentration is determined to be greater than or equal to the cutoff value, the patient is predicted to be a responder to anti-$\alpha_4\beta_7$ integrin therapy.

In one embodiment, a higher amount of eotaxin-1 mRNA or protein in a sample from a test subject compared to a cut-off value, e.g. the amount in a control sample, is an indicator that the human subject is likely to show a clinical response to vedolizumab induction therapy and/or stays in remission upon vedolizumab maintenance therapy.

Depending on the type of detection method, the amount of eotaxin-1 protein can be expressed as a concentration in ng eotaxin-1 protein per milliliter (mL) or in ng eotaxin-1 protein per μg total protein. The absolute concentration can depend on various assay conditions, including sample type, sample pretreatment and the like. In one embodiment, a serum concentration of eotaxin-1 protein of at least 0.30 ng/mL, preferably at least 0.35 ng/mL, more preferably at least 0.40 ng/mL is indicative of a subject likely to respond to anti-$\alpha_4\beta_7$ integrin treatment, such as vedolizumab induction therapy. In a specific aspect, an eotaxin-1 protein level of at least

0.45 ng/ml, like 0.46, 0.47, 0.49 ng/ml or higher, in a serum sample is used as cut-off value to classify the subject as a likely responder.

A method of the invention can be combined with one or more conventional approaches to predict therapy responsiveness. It may comprise the testing of additional compounds (biomarkers, metabolites, etc.) that can positively contribute to the outcome of the predictive assay. For example, a method may comprise detecting the level of eotaxin-1 and CRP in a serum sample. In a specific aspect, a reduced serum level of CRP (e.g. below 100-200 μg/g) is used as a further indicator of the subject being a therapy responder.

The invention also provides a diagnostic kit for assessing responsiveness to therapy which targets $\alpha_4\beta_7$ integrin, such as vedolizumab induction therapy, in a human subject. The kit comprises reagents for carrying out the method according to the invention.

In one embodiment, the kit comprises (a) a substrate for holding a blood or blood-derived sample isolated from a human subject to be tested for vedolizumab therapy responsiveness, (b) at least one detection reagent that specifically detects eotaxin-1 mRNA/protein biomarker; and (c) written instructions for reacting the reagent with the sample or a portion of the sample to detect the amount of eotaxin-1 biomarker in the sample and/or how this amount is to be correlated with responsiveness to therapy that targets $\alpha_4\beta_7$ integrin. The detection reagent can be any compound that selectively binds to eotaxin-1 mRNA or protein. In a preferred embodiment, it is an anti-eotaxin-1 antibody. The substrate for holding the sample can be hydrophobic, hydrophilic, charged or polar.

In another embodiment, the kit comprises a first container comprising a reagent for detecting eotaxin-1 protein, preferably an eotaxin-1-specific antibody, and a second container comprising eotaxin-1 protein.

A kit as provided herein may contain further useful reagents, in particular purified human eotaxin-1 protein, which is suitably used as positive control and/or the construction of a standard/calibration curve. The kit may further comprise reagents for detecting one or more additional predictive marker(s). For example, a kit comprises a third container comprising a reagent for detecting CRP, preferably a CRP-specific antibody.

LEGEND TO THE FIGURES

Figure 1. Baseline serum eotaxin-1 levels (ng/ml) of IBD patients at the start of vedolizumab induction therapy and subdivided in clinical responders and primary non-responders. Serum eotaxin-1 levels (ng/ml) at baseline are significantly higher in IBD patients who respond to vedolizumab induction therapy, *P< 0.05.

Figure 2. Serum eotaxin-1 levels (ng/ml) in IBD patients during the course of vedolizumab induction therapy. Serum eotaxin-1 levels (ng/ml) at baseline are significantly higher in IBD patients eventually responding to vedolizumab induction therapy, *P< 0.05, while no significant difference in serum eotaxin-1 levels is detected between responders and non-responders during vedolizumab therapy.

Figure 3. Receiver operating characteristics (ROC) curves for (panel A) serum eotaxin-1 levels (ng/ml); (panel B) serum CRP levels (mg/l); (panel C) Harvey Bradshaw Index (HBI) or Simple Clinical Colitis Activity Index (SCCAI) and (panel D) adjusted serum eotaxin-1 levels (combined predicted probability of multivariable logistic regression model). The best discriminative performance to predict clinical response or remission to vedolizumab induction therapy is demonstrated by the multivariate model of serum eotaxin-1 levels (ng/ml).

Figure 4. Baseline serum levels of (A) Eotaxin-1, (B) IL-6, (C) IL-8 and (D) SAA (biomarkers associated with active endoscopic disease; Bourgonje et al., 2019) in IBD patients before starting vedolizumab therapy and subdivided according to the effectiveness of the therapy (responders *vs* non-responders) as established at week 14. Only pre-therapy serum levels of eotaxin-1 predict therapy response to vedolizumab in IBD.

Figure 5. Baseline serum levels of Eotaxin-1 in IBD patients before starting Infliximab (anti-TNF) therapy and subdivided according to the effectiveness of the therapy (responders vs non-responders) as established at week 14. Pre-therapy serum levels of eotaxin-1 do not predict therapy success of Infliximab in IBD.

EXPERIMENTAL SECTION

EXAMPLE 1: Eotaxin-1 is a predictive marker for clinical responsiveness to anti-α4β7 integrin treatment.

Methods

Study population and data collection

This retrospective cohort study included patients from October 2014 to October 2017 at the IBD center of the University Medical Center Groningen (UMCG). All patients (n = 76) had an established diagnosis of IBD existing for at least 1 year, either Crohn's disease (CD) (n = 33) or ulcerative colitis (UC) (n = 43) and were treated with vedolizumab induction therapy. All recruited patients were treated with 300 mg vedolizumab intravenously at standardized clinical visits at weeks 0, 2, 6 and 14, the latter being considered the primary end of induction therapy. Patients aged < 18 years or patients with comorbidities causing significant changes in blood

leukocyte distributions (e.g. HIV or lymphoproliferative disorders) were excluded from the study. Clinically relevant data were retrieved from medical records: age, gender, body-mass index (BMI), smoking status, Montreal classification, current medication use (aminosalicylates, thiopurines, methotrexate, TNFα-antagonists), previous anti-TNFα therapy and surgical history. At each clinical visit, routine laboratory measurements were performed, including hemoglobin levels, C-reactive protein (CRP), erythrocyte sedimentation rates (ESR), white blood cell count (WBC), thrombocyte count, eosinophil count and fecal calprotectin levels (for selected patients). Fecal calprotectin levels were quantified by enzyme-linked immunosorbent assays (ELISA) (BÜHLMANN Laboratories AG, Switzerland). Serum samples were obtained after patients gave written informed consent (study approved by the Institutional Review Board of the UMCG, IRB no. 2008/338).

Definition of study outcomes

The primary study outcome was defined as a clinical response or remission after vedolizumab induction therapy at week 14. Clinical response was defined as a decrease of at least 3 points in the Harvey Bradshaw Index (HBI) for CD or Simple Clinical Colitis Activity Index (SCCAI) for UC from baseline or by global assessment of the treating physician.(23, 24) Clinical remission was defined as a HBI $\leq$ 3 for CD and SCCAI $\leq$ 2.5 for UC or by assessment of the treating physician. Primary non-responders were defined as patients that did not meet the above mentioned clinical response or remission criteria or patients whose therapy was ceased before the end of induction therapy.

The secondary study outcome measure was defined as a biochemical response after vedolizumab induction therapy at week 14. Biochemical response was defined as a CRP concentration $\leq$ 2.87 mg/L, based on the cut-

off from the GEMINI II trial.(8) Participants with CRP concentrations > 2.87 mg/L at week 14 were considered biochemical non-responders.

Measurement of eotaxin-1 and high-sensitive C-reactive protein (hsCRP)

Measurements of serum eotaxin-1 and high-sensitive CRP levels were performed as previously described.(25) In short, serum samples from all IBD patients at different time points were collected and stored in 1 mL aliquots at -80°C. Prior to analysis, samples were thawed and quickly centrifuged to remove remaining particulates. Serum eotaxin-1 and CRP were quantified using an electrochemiluminescence (ECL) multiplex assay (Meso Scale Discovery (MSD®), Meso Scale Diagnostics, Rockville, MD). ECL signals were fitted to a 4-parameter logistic model with $1/y^2$ weighting, ensuring a broad and dynamic range of molecule detection. Serum concentrations of eotaxin-1 and CRP were determined by using calibration curves to which the ECL signals were back-fitted. Final concentrations were calculated using the MSD Discovery Workbench analysis software®. All concentrations were above the lower limit of detection (LLOD).

Statistics

Baseline demographic and clinical characteristics were presented as means ± standards errors (SEM) or proportions with corresponding percentages (*n*, %). Serum eotaxin-1 and CRP levels were presented as median ± interquartile ranges (IQR). Assessing normality of continuous variables was performed using normal probability plots. Continuous variables were compared using Student's *t*-tests or Mann-Whitney *U*-tests, as appropriate. Categorical variables were compared using chi-square tests or Fisher's exact test, as appropriate. Univariable logistic regression analysis was performed to identify predictors for clinical response or remission to vedolizumab induction therapy at week 14. Non-normally distributed predictor variables were ^{2}log-transformed to facilitate results interpretation. All odds ratios

(ORs) with corresponding 95% confidence intervals (CI) are associated with a two-fold increase (i.e. doubling) of the predictor variable value. Multivariable logistic regression analysis was then performed using forced entry of covariates to allow for correction for confounding. Covariate selection was based on univariable logistic regression results and clinical relevance. All logistic regression analyses were performed for the total IBD cohort, and for CD and UC patients separately. To evaluate goodness-of-fit, predicted probabilities were put into receiver operating characteristics (ROC) curves using the area under the ROC curve (AuROC) as overall measure of fit. ROC curves were created using a non-parametric estimation method. Subsequently, binary classifiers were developed from continuous probabilities while associated thresholds were determined by equally maximizing sensitivity and specificity to compute the Youden's index (*J* statistic). Optimal thresholds or cut-off points (*c*) were established by selecting the highest Youden's index, defined as $J = \max_c \{\text{sensitivity}(c) + \text{specificity}(c) - 1\}$. Data were analyzed using SPSS Statistics 23.0 software package and R version 3.4.3. (R package 'pROC') and visualized using GraphPad Prism version 5.0. Two-tailed *P*-values ≤ 0.05 were considered as statistically significant.

Results

An overview and comparison of baseline demographic and clinical characteristics of the IBD patient cohort is presented in **Table 1.** In total, 76 IBD patients received vedolizumab induction therapy, of which 34 patients (44.7%) showed either response (13.1%) or remission (31.6%) at week 14, while 42 patients (55.3%) were considered as primary non-responder. There was no significant difference in response or remission rates between patients with CD or UC (64.7 vs. 35.3%, respectively, $P = 0.198$). Prior biological therapy failure occurred in 70 patients (92.1%). Patients who initially showed either clinical response or remission had a

mean age of 43.3 ± 2.6 years and consisted of 20 males (58.8%) and 14 females (41.2%), whilst patients who were primary non-responder had a mean age of 44.3 ± 2.4 years and consisted of 13 males (31.0%) and 29 females (69.0%) (age, $P = 0.653$; sex, $P < 0.05$). In the total IBD cohort, and for CD separately, serum levels of C-reactive protein (CRP) (mg/L) were significantly lower ($P < 0.05$) at baseline in patients who eventually responded to vedolizumab induction therapy, as compared to primary non-responders (IBD, 6.3 vs. 12.2 mg/L; CD, 6.8 vs. 17.0 mg/L, respectively).

Serum levels of eotaxin-1 among clinical responders and primary non-responders are presented in **Table 2** and displayed in **Figure 1**. In the total IBD cohort, baseline serum eotaxin-1 levels were significantly higher in patients eventually responding to vedolizumab induction therapy as compared to primary non-responders (0.31 vs. 0.20 ng/ml, $P < 0.05$, **Figure 1**). Already after 2 weeks of vedolizumab induction therapy, serum eotaxin-1 levels significantly increased, both in CD and UC and irrespective of clinical response at week 14 ($P < 0.01$, Wilcoxon's signed-rank test). Henceforth, serum eotaxin-1 levels remain relatively constant throughout the course of vedolizumab induction therapy (**Figure 2**).

Table 1. Baseline cohort demographic and clinical characteristics of IBD patients receiving vedolizumab induction therapy and either demonstrated clinical response or remission at week 14 or showed primary non-response.

IBD (*n* = 76)	**Response or remission (*n* = 34)**	**Primary non-response (*n* = 42)**	***P*-value**
Age (years)	43.3 ± 2.6	44.3 ± 2.4	0.653
Male sex	20 (58.8)	13 (31.0)	**<0.05***
BMI (kg/m^2)	24.7 ± 1.1	24.4 ± 0.7	0.794
Current smoking	7 (25.0)	10 (25.6)	0.647
Concomitant medication			0.481
– None	15 (51.7)	13 (37.1)	
– Mesalamine	9 (31.0)	11 (31.4)	
– Thiopurines/MTX	5 (17.2)	10 (28.6)	
– Combination therapy	-	1 (2.9)	
Prior biological therapy (no.)	1.4 ± 0.1	1.5 ± 0.1	0.682
Surgical history (ileocecal resection/colectomy)	7 (23.3)	15 (36.5)	0.093
HBI/SCCAI score	7.3 ± 0.6	7.9 ± 0.6	0.629
HBI/SCCAI score at week 14	2.7 ± 0.3	8.1 ± 0.7	**<0.001*****
Hemoglobin (mmol/L)	7.7 ± 0.2	7.4 ± 0.2	0.290
Hemoglobin (mmol/L) at week 14	7.9 ± 0.2	7.5 ± 0.2	0.188
CRP (mg/L)	6.3 ± 1.4	12.2 ± 2.2	**<0.05***
CRP (mg/L) at week 14	4.7 ± 0.9	12.5 ± 3.1	0.067
ESR (mm/h)	26.1 ± 3.8	29.8 ± 4.0	0.647
ESR (mm/h) at week 14	20.7 ± 3.8	26.4 ± 3.8	0.298
WBC (x10^9/l)	13.1 ± 4.4	8.2 ± 0.5	0.323
WBC (x10^9/l) at week 14	8.1 ± 0.8	8.1 ± 0.4	0.284
Thrombocytes (x10^9/l)	322 ± 16	342 ± 16	0.391
Thrombocytes (x10^9/l) at week 14	293 ± 14	348 ± 21	**< 0.05***
Eosinophils (x10^9/l)	0.14 ± 0.03	0.13 ± 0.02	0.868
Eosinophils (x10^9/l) at week 14	0.27 ± 0.03	0.25 ± 0.05	0.730
CD (*n* = 33)	**Response or remission (*n* = 12)**	**Primary non-response (*n* = 21)**	***P*-value**
Age (years)	42.4 ± 4.3	46.4 ± 3.6	0.600
Male sex	5 (41.7)	5 (23.8)	0.433
BMI (kg/m^2)	22.7 ± 1.2	22.9 ± 0.8	0.874
Current smoking	5 (41.7)	8 (40.0)	0.659
Concomitant medication			0.270
– None	8 (66.7)	8 (47.1)	

– Mesalamine	-	3 (17.6)	
– Thiopurines/MTX	4 (33.3)	6 (35.3)	
– Combination therapy	-	-	
Prior biological therapy (no.)	1.9 ± 0.2	2.0 ± 0.1	0.915
Surgical history (ileocecal resection)	7 (58.3)	14 (66.7)	0.716
Montreal classification, Location of disease			0.543
– L1 (ileal disease)	2 (16.7)	3 (14.3)	
– L2 (colonic)	-	2 (9.5)	
– L3 (ileocolonic)	10 (83.3)	16 (76.2)	
Montreal classification, Behavior of disease			0.656
– B1 (non-stricturing, non-penetrating)	5 (41.7)	6 (28.6)	
– B2 (stricturing)	5 (41.7)	9 (42.9)	
– B3 (penetrating)	2 (16.7)	6 (28.6)	
HBI score	7.8 ± 0.9	9.7 ± 1.0	0.209
HBI score at week 14	3.1 ± 0.4	9.2 ± 1.0	**< 0.001*** **
Hemoglobin (mmol/L)	7.7 ± 0.1	7.3 ± 0.2	0.165
Hemoglobin (mmol/L) at week 14	8.0 ± 0.3	7.5 ± 0.3	0.334
CRP (mg/L)	6.8 ± 2.2	17.0 ± 3.8	**<0.05***
CRP (mg/L) at week 14	4.9 ± 1.3	19.8 ± 5.9	**<0.05***
ESR (mm/h)	29.0 ± 7.0	38.8 ± 6.4	0.372
ESR (mm/h) at week 14	22.7 ± 5.8	34.1 ± 5.9	0.233
WBC ($x10^9$/l)	20.6 ± 12.1	9.0 ± 0.8	1.000
WBC ($x10^9$/l) at week 14	9.3 ± 2.0	9.1 ± 0.7	0.310
Thrombocytes ($x10^9$/l)	346 ± 30	359 ± 24	0.730
Thrombocytes ($x10^9$/l) at week 14	314 ± 25	358 ± 34	0.352
Eosinophils ($x10^9$/l)	0.20 ± 0.06	0.12 ± 0.02	0.243
Eosinophils ($x10^9$/l) at week 14	0.27 ± 0.05	0.17 ± 0.03	0.068

UC (*n* = 43)	**Response or remission (*n* = 22)**	**Primary non-response (*n* = 21)**	***P*-value**
Age (years)	43.8 ± 3.3	42.2 ± 3.1	0.846
Male sex	15 (68.2)	8 (38.1)	0.069
BMI (kg/m^2)	26.0 ± 1.6	26.1 ± 1.0	0.654
Current smoking	2 (12.5)	2 (10.5)	0.909
Concomitant medication			0.367
– None	7 (41.2)	5 (27.8)	
– Mesalamine	9 (52.9)	8 (44.4)	
– Thiopurines/MTX	1 (5.9)	4 (22.2)	
– Combination therapy	-	1 (5.6)	

Prior biological therapy (no.)	1.1 ± 0.1	1.0 ± 0.1	0.443
Surgical history (colectomy)	-	1 (5.0)	1.000
Montreal classification, Location of disease			0.736
– L1 (proctitis)	-	-	
– L2 (left-sided colitis)	5 (22.7)	6 (28.6)	
– L3 (pancolitis)	17 (77.3)	15 (71.4)	
Montreal classification, Severity of disease			0.507
– S1	-	-	
– S2	2 (9.1)	3 (14.3)	
– S3	10 (45.5)	12 (57.1)	
– S4	10 (45.5)	6 (28.6)	
SCCAI score	6.9 ± 0.8	6.1 ± 0.6	0.534
SCCAI at week 14	2.4 ± 0.4	7.1 ± 0.8	**< 0.001*** **
Hemoglobin (mmol/L)	7.7 ± 0.3	7.4 ± 0.2	0.635
Hemoglobin (mmol/L) at week 14	7.8 ± 0.2	7.5 ± 0.3	0.397
CRP (mg/L)	6.1 ± 1.8	7.3 ± 1.8	0.279
CRP (mg/L) at week 14	4.6 ± 1.2	5.8 ± 1.7	0.734
ESR (mm/h)	24.5 ± 4.5	20.7 ± 4.0	0.546
ESR (mm/h) at week 14	19.5 ± 5.1	19.1 ± 4.4	0.930
WBC ($x10^9$/l)	8.9 ± 0.8	7.4 ± 0.5	0.166
WBC ($x10^9$/l) at week 14	7.4 ± 0.6	7.2 ± 0.4	0.764
Thrombocytes ($x10^9$/l)	309 ± 17	324 ± 21	0.569
Thrombocytes ($x10^9$/l) at week 14	280 ± 17	339 ± 25	0.063
Eosinophils ($x10^9$/l)	0.11 ± 0.03	0.15 ± 0.03	0.336
Eosinophils ($x10^9$/l) at week 14	0.27 ± 0.04	0.33 ± 0.09	0.540

*Data are presented as mean ± SEM or proportions with corresponding percentages (n, %). Differences between groups were tested according to normality using Student's t-test or Mann-Whitney U-test for continuous variables and chi-square test or Fisher's exact test for categorical variables, as appropriate. *Two sided P-values < 0.05 were considered statistically significant. **P < 0.01. ***P < 0.001. Abbreviations: IBD, inflammatory bowel disease; CD, Crohn's disease; UC, ulcerative colitis; BMI, body mass index; MTX, methotrexate; HBI, Harvey-Bradshaw index; SCCAI, Simple Clinical Colitis Activity Index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; WBC, white blood cell count.*

Table 2. Distribution of serum eotaxin-1 protein levels (ng/ml) in IBD patients during the course of vedolizumab induction therapy who either demonstrated a clinical response or remission at week 14, or showed primary non-response.

Eotaxin-1 (ng/ml)	Time point	Response or remission	Primary non-response	*P*-value
IBD	Baseline	0.31 (0.22-0.46)	0.20 (0.16-0.29)	**<0.05***
	Week 2	0.38 (0.28-0.49)	0.35 (0.18-0.45)	0.247
	Week 6	0.37 (0.28-0.48)	0.30 (0.18-0.44)	0.059
	Week 14	0.39 (0.28-0.51)	0.32 (0.25-0.49)	0.429

*Data are presented as medians with interquartile ranges (IQR). Differences between clinical responders and primary non-responders were non-parametrically tested for each time point using Mann-Whitney U-tests. *Two-sided P-value < 0.05 was considered statistically significant.*

In the univariable analysis of predictors in all IBD patients, 2-log transformed serum eotaxin-1 levels (ng/ml) were significantly associated with an increased odds of clinical response or remission to vedolizumab induction therapy at week 14 (OR 2.60, 95% CI: 1.19 – 5.65) (**Table 3**). Thus, each doubling in serum eotaxin-1 level is significantly associated with a 2.60-fold increased odds of attaining clinical response or remission at week 14. Furthermore, female sex (OR 0.31, 95% CI: 0.12 – 0.81), 2-log transformed C-reactive protein (CRP) levels (mg/l) at baseline (OR 0.78, 95% CI: 0.61 – 0.99) and at week 2 of induction therapy (OR 0.65, 95% CI: 0.48-0.89) were significantly associated with a decreased odds of clinical response or remission to vedolizumab induction therapy at week 14.

Table 3. Univariable logistic regression analysis of predictors of clinical response or remission to vedolizumab induction therapy at week 14. Odds ratios (ORs) (with 95% CI) were calculated for all IBD patients, and for CD and UC separately.

Variable	IBD		CD		UC	
	OR	95% CI	OR	95% CI	OR	95% CI
Age (years)	1.00	0.97-1.03	0.98	0.94-1.03	1.01	0.97-1.05
Female sex	**0.31***	**0.12-0.81**	0.44	0.10-2.01	0.29	0.08-1.01
BMI (kg/m^2)	1.01	0.92-1.12	0.98	0.81-1.19	1.00	0.88-1.14
Current smoking	0.79	0.24-2.61	0.75	0.15-3.83	1.10	0.13-9.34
Combination therapy	0.55	0.20-1.50	0.44	0.10-2.06	0.55	0.13-2.26
Prior biologic therapy (no.)	0.89	0.49-1.63	0.93	0.34-2.58	1.60	0.52-4.92
Prior surgery	0.59	0.20-1.70	0.70	0.16-3.02	-	-
HBI/SCCAI at baseline	0.95	0.83-1.85	0.86	0.69-1.07	1.11	0.88-1.39
CRP (mg/l) at baseline†	**0.78***	**0.61-0.99**	0.79	0.53-1.16	0.80	0.57-1.12
CRP (mg/l) at week 2†	**0.65****	**0.48-0.89**	0.70	0.45-1.08	**0.61***	**0.38-0.97**
CRP (mg/l) at week 6†	0.79	0.61-1.02	0.70	0.45-1.07	0.97	0.68-1.37

Eotaxin-1 (ng/ml) at baseline†	**2.60***	**1.19-5.65**	**4.48***	**1.03-19.41**	1.81	0.73-4.50
Eotaxin-1 (ng/ml) at week 2†	1.85	0.87-3.91	1.71	0.50-5.92	1.81	0.67-4.86
Eotaxin-1 (ng/ml) at week 6†	2.11	0.97-4.57	2.36	0.63-8.78	1.81	0.68-4.84

†2log(x)-transformed predictor variables. **P*-value < 0.05. ***P* < 0.01.

Subsequently, a multivariable logistic regression model was composed using forced entry of covariates (**Table 4**). Covariate selection was based on results derived from the univariable analysis (**Table 3**) and clinical relevance. Ultimately, all predictor-associated ORs were adjusted for age, sex, disease (in the total cohort analysis, either CD or UC), combination therapy or not and number of prior biologic therapies.

In the multivariable logistic regression analysis of all IBD patients, 2-log transformed serum eotaxin-1 levels (ng/ml) appear to be even more significantly associated with an increased odds of clinical response or remission to vedolizumab induction therapy at week 14 (OR 3.28, 95% CI: 1.05 – 10.25). Furthermore, 2-log transformed serum CRP levels (mg/l) at week 2 of vedolizumab induction therapy remained significantly associated with decreased odds of clinical response or remission at week 14 (OR 0.67, 95% CI: 0.46 – 0.99). However, 2-log transformed CRP levels (mg/l) at baseline lost its significance in predicting vedolizumab induction therapy response (OR 0.80, 95% CI: 0.58 – 1.12).

In the multivariate analyses of either CD or UC patients, no significant predictors for clinical response or remission were demonstrated, though in CD patients, the 2-log transformed serum eotaxin-1 level (ng/ml) was almost statistical significance (OR 44.73, 95% CI: 0.91 – 2190, $P = 0.056$).

Table 4. Multivariable logistic regression analysis of serum eotaxin-1 levels (ng/ml) and serum CRP levels (mg/l) as predictors of clinical response or remission to vedolizumab induction therapy at week 14. Adjusted odds ratios (ORs) (with 95% CI) were calculated for all IBD patients, and for CD and UC separately.

Variable	IBD		CD		UC	
	OR	95% CI	OR	95% CI	OR	95% CI
Age (years)	1.00	0.97-1.03	0.98	0.94-1.03	1.01	0.97-1.05
Female sex	**0.31***	**0.12-0.81**	0.44	0.10-2.01	0.29	0.08-1.01
BMI (kg/m^2)	1.01	0.92-1.12	0.98	0.81-1.19	1.00	0.88-1.14
Current smoking	0.79	0.24-2.61	0.75	0.15-3.83	1.10	0.13-9.34
Combination therapy	0.55	0.20-1.50	0.44	0.10-2.06	0.55	0.13-2.26
Prior biologic therapy (no.)	0.89	0.49-1.63	0.93	0.34-2.58	1.60	0.52-4.92
Prior surgery	0.59	0.20-1.70	0.70	0.16-3.02	-	-
HBI/SCCAI at baseline	0.95	0.83-1.85	0.86	0.69-1.07	1.11	0.88-1.39
CRP (mg/l) at baseline†	**0.78***	**0.61-0.99**	0.79	0.53-1.16	0.80	0.57-1.12
CRP (mg/l) at week 2†	**0.65****	**0.48-0.89**	0.70	0.45-1.08	**0.61***	**0.38-0.97**
CRP (mg/l) at week 6†	0.79	0.61-1.02	0.70	0.45-1.07	0.97	0.68-1.37

Eotaxin-1 (ng/ml) at baseline†	**2.60***	**1.19-5.65**	**4.48***	**1.03-19.41**	1.81	0.73-4.50
Eotaxin-1 (ng/ml) at week 2†	1.85	0.87-3.91	1.71	0.50-5.92	1.81	0.67-4.86
Eotaxin-1 (ng/ml) at week 6†	2.11	0.97-4.57	2.36	0.63-8.78	1.81	0.68-4.84

†2log(x)-transformed predictor variables. **P*-value < 0.05. ***P* < 0.01.

To further analyze the predictive accuracy of serum eotaxin-1 levels (ng/ml) with respect to clinical response or remission to vedolizumab induction therapy, receiver operating characteristics (ROC) analyses were performed. For all IBD patients (n=76), the "area under the ROC" (AuROC) for serum eotaxin-1 levels (ng/ml) at baseline was 0.687 (SE: 0.067, 95% CI: 0.555 – 0.819, $P < 0.05$), as compared to an AuROC of 0.624 (SE: 0.071, 95% CI: 0.484 – 0.764, $P = 0.089$) for serum CRP level at baseline (mg/l) and an AuROC of 0.535 (SE: 0.071, 95% CI: 0.395 – 0.675, $P = 0.631$) for HBI or SCCAI score at baseline (**Figure 3A-C**). To evaluate goodness-of-fit of the multivariable logistic regression model of serum eotaxin-1 levels (ng/ml), adjusted ROC statistics were calculated for achieving clinical response or remission to vedolizumab induction therapy, resulting in an AuROC of 0.808 (SE: 0.061, 95% CI: 0.689 – 0.927, $P < 0.001$) (**Figure 3D**).

Univariately, a serum eotaxin-1 level > 0.31 ng/ml had a sensitivity of 51.7% and specificity of 82.9% in predicting clinical response or remission to vedolizumab induction therapy at week 14 (Youden's index 0.35). In the multivariate model, the same threshold of 0.31 ng/ml had a sensitivity of 91.7% and specificity of 60.0% in predicting clinical response (Youden's index 0.52). Alternatively, a serum eotaxin-1 level > 0.49 ng/ml yielded a sensitivity of 75.0% and specificity of 76.7% in predicting clinical response while retaining the same discriminative capacity (Youden's index 0.52). By comparison, a serum CRP level < 1.54 mg/l had a sensitivity of 41.4% and specificity of 85.7% in predicting vedolizumab induction therapy response (Youden's index 0.27).

EXAMPLE 2: Specificity of Eotaxin-1 as predictive marker

Key to the present invention is the finding that serum eotaxin-1 levels before the start of therapy can predict the clinical response to vedolizumab in IBD (see Figure 1). This example shows that a clear differentiation must be made between "disease activity marker" and "predictor of therapy response". More in particular, the data below demonstrate that a (known) serum marker for IBD disease activity/progression does not necessarily imply that it also suitable as a priori predictive marker for therapy response in IBD.

Serum eotaxin-1 levels were determined in IBD patients before (at t=0/baseline) vedolizumab therapy was started. Only after 14 weeks of vedolizumab therapy, patients were subdivided in responders versus non-responders. Serum eotaxin-1 levels were significantly higher at t=0/baseline in vedolizumab-responders compared to non-responders (see Figure 1). In contrast, IBD disease activity was comparable at t=0/baseline in both patient groups (see Table 4). Furthermore, baseline disease activity was determined using similar indices (HBI and SCCAI scores) as were incorporated into our definition of clinical response to vedolizumab induction therapy (Table 4).

Table 4. Disease activity in IBD patients before and after vedolizumab-induction therapy, subdivided in group A) demonstrating clinical response or remission at week 14 or group B) showing primary non-response.

IBD (*n* = 76)	**A) Response or remission (*n* = 34)**	**B) Primary non-response (*n* = 42)**	***P*-value**
Age (years)	43.3 ± 2.6	44.3 ± 2.4	0.653
HBI/SCCAI score at week 0	7.3 ± 0.6	7.9 ± 0.6	0.629
HBI/SCCAI score at week 14	2.7 ± 0.3	8.1 ± 0.7	**<0.001*****

*Data are presented as mean ± SEM . Differences between groups were tested according to normality using Student's t-test or Mann-Whitney U-test for continuous variables, as appropriate. *Two-sided P-values < 0.05 were considered statistically significant.***P < 0.001. Abbreviations: IBD, inflammatory bowel disease; HBI, Harvey-Bradshaw index; SCCAI, Simple Clinical Colitis Activity Index.*

Thus, serum eotaxin-1 levels do not correlate with IBD disease activity/progression at t=0/baseline when patients are subdivided by their response to vedolizumab therapy at week 14. Interestingly however, as demonstrated in the present invention, the group with active disease (with on average a high serum eotaxin-1 level) can be subdivided in a group with high serum eotaxin-1 levels that are likely to respond to vedolizumab therapy, and a group with low eotaxin-1 levels that are likely non-responders.

The specificity of serum eotaxin-1 at t=0/baseline for predicting Vedolizumab therapy response is further demonstrated by the fact that from 4 biomarkers identified earlier to be associated to IBD disease activity/progression (e.g. Eotaxin-1, IL-6, IL-8 and SAA, Bourgonje *et al* 2019 *Gastroenterology* 156(6):S-848), only Eotaxin-1 levels at t=0/baseline can predict therapy response to vedolizumab at week 14 (**Figure 4**).

The finding that eotaxin-1 is a unique predictive marker with respect to drugs that target $\alpha_4\beta_7$ integrin rather than a universal biomarker for predicting a response to any type of therapy used in IBD patients is demonstrated in **Figure 5**. Anti-tumor necrosis factor α (TNFα) therapy has revolutionized the medical treatment of IBD. Twenty years ago, infliximab became the first anti-TNF agent approved for IBD. Data from randomized controlled trials, large observational cohort studies, postmarketing registries, and meta-analyses show that infliximab is a very effective treatment for moderate to severe IBD with a good safety profile.

Nevertheless, up to 30% of patients show no clinical benefit following induction and up to 50% lose response over time. Figure 5 shows that serum eotaxin-1 levels in IBD patients at baseline (pre-therapy) do not predict response to anti-TNF-alpha (Infliximab) therapy.

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Claims

1. A method for predicting whether a human subject is likely to show a clinical response to a therapy which targets $\alpha_4\beta_7$ integrin, comprising:

(i) determining the eotaxin-1 level before start of therapy in a sample obtained from the subject, and

(ii) classifying the subject as a responder or non-responder based on the level of eotaxin-1.

2. Method according to claim 1, wherein the subject has inflammatory bowel disease (IBD).

3. Method according to any one of the preceding claims, wherein the sample is whole blood, a serum, a plasma sample, or a biopsy.

4. Method according to any one of the preceding claims, wherein the eotaxin-1 level is determined at the mRNA or protein level, preferably at the protein level.

5. Method according to any one of the preceding claims, wherein said therapy which targets $\alpha_4\beta_7$ integrin comprises treatment with a gut-specific, $\alpha_4\beta_7$ integrin-neutralizing monoclonal Ab or a pharmacological antagonist targeting $\alpha_4\beta_7$ integrin.

6. Method according to claim 5, wherein said antibody is vedolizumab.

7. Method according to any one of the preceding claims, comprising classifying a subject as a responder based on a serum eotaxin-1 protein level of at least 0.31 ng/ml.

8. Method according to claim 7, comprising classifying a subject as a responder based on a serum eotaxin-1 protein level at least 0.45 ng/ml, preferably at least 0.49 ng/ml.

9. Method according to any one of the preceding claims, wherein the method further comprises detecting CRP and/or fecal calprotectin.

10. Method according to claim 9, wherein a serum threshold level of CRP below 100 μg/g is used to classify the subject as a responder.

11. The use of eotaxin-1 as a biomarker for predicting clinical responsiveness to therapy which targets $\alpha_4\beta_7$ integrin, preferably for predicting clinical responsiveness to an antibody that targets $\alpha_4\beta_7$ integrin.

12. The use of according to claim 11, for predicting clinical responsiveness to an antibody that targets α4ß7 integrin.

13. A diagnostic kit for predicting clinical responsiveness to therapy that targets $\alpha_4\beta_7$ integrin, comprising a first container comprising a reagent for detecting eotaxin-1 protein, preferably an eotaxin-1-specific antibody, and a second container comprising eotaxin-1 protein.

14. Diagnostic kit comprising (a) a substrate for holding a sample isolated from a human subject to be tested for responsiveness to therapy that targets $\alpha_4\beta_7$ integrin, (b) at least one detection reagent that specifically binds eotaxin-1 biomarker; and (c) instructions for reacting the reagent with

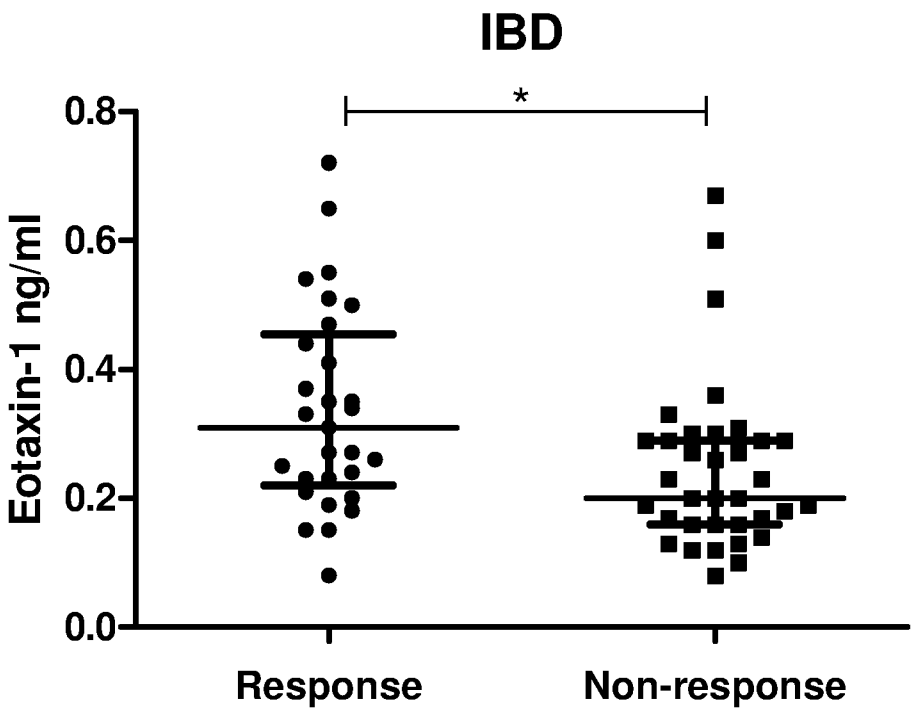
the sample or a portion of the sample to detect the amount of the eotaxin-1 biomarker in the sample and/or how this amount is to be correlated with responsiveness to therapy with an antibody that targets $\alpha_4\beta_7$ integrin.

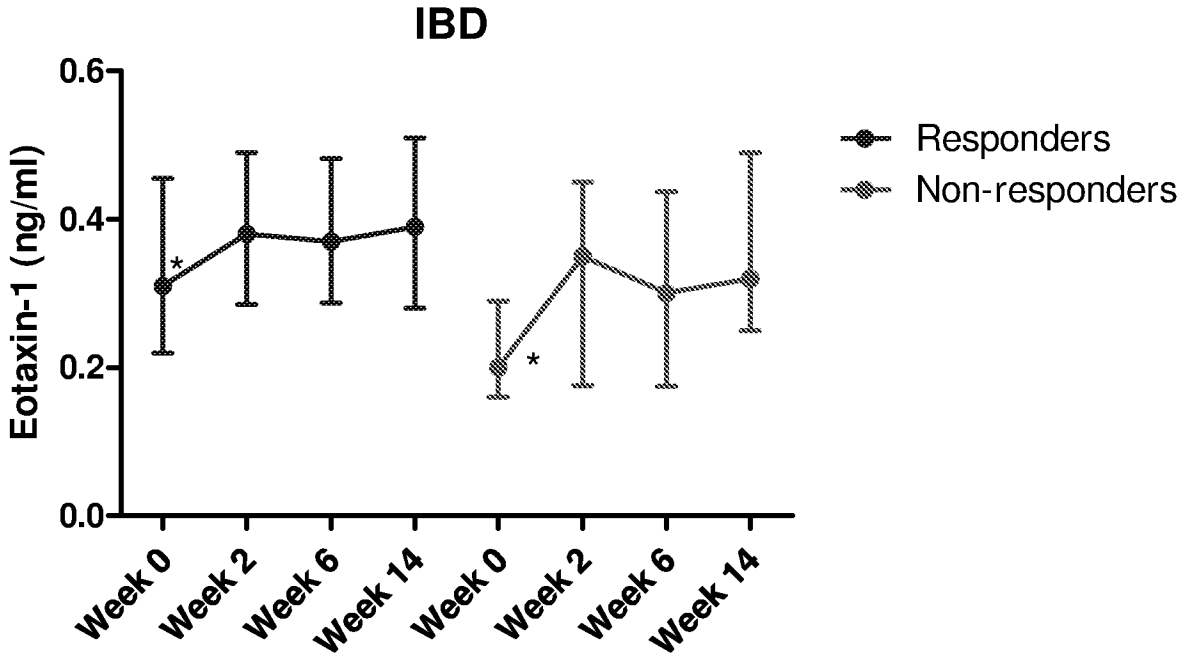
15. Kit according to claim 13 or 14, further comprising a container comprising a reagent for detecting CRP, preferably a CRP-specific antibody.

16. The use of a kit according to any one of claims 13-15, for predicting clinical responsiveness to treatment with an agent that targets $\alpha_4\beta_7$ integrin.

17. The use according to claim 16, for predicting clinical responsiveness to vedolizumab induction therapy.

Figure 1




IBD
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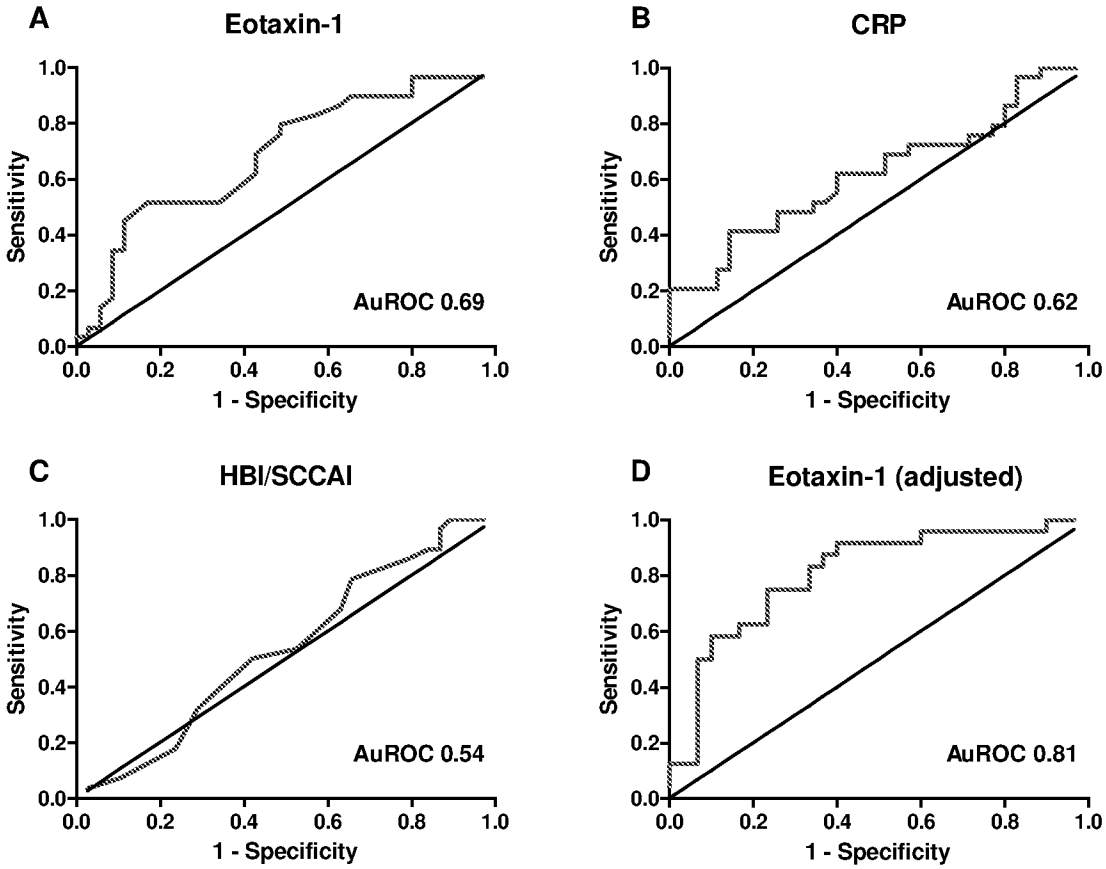
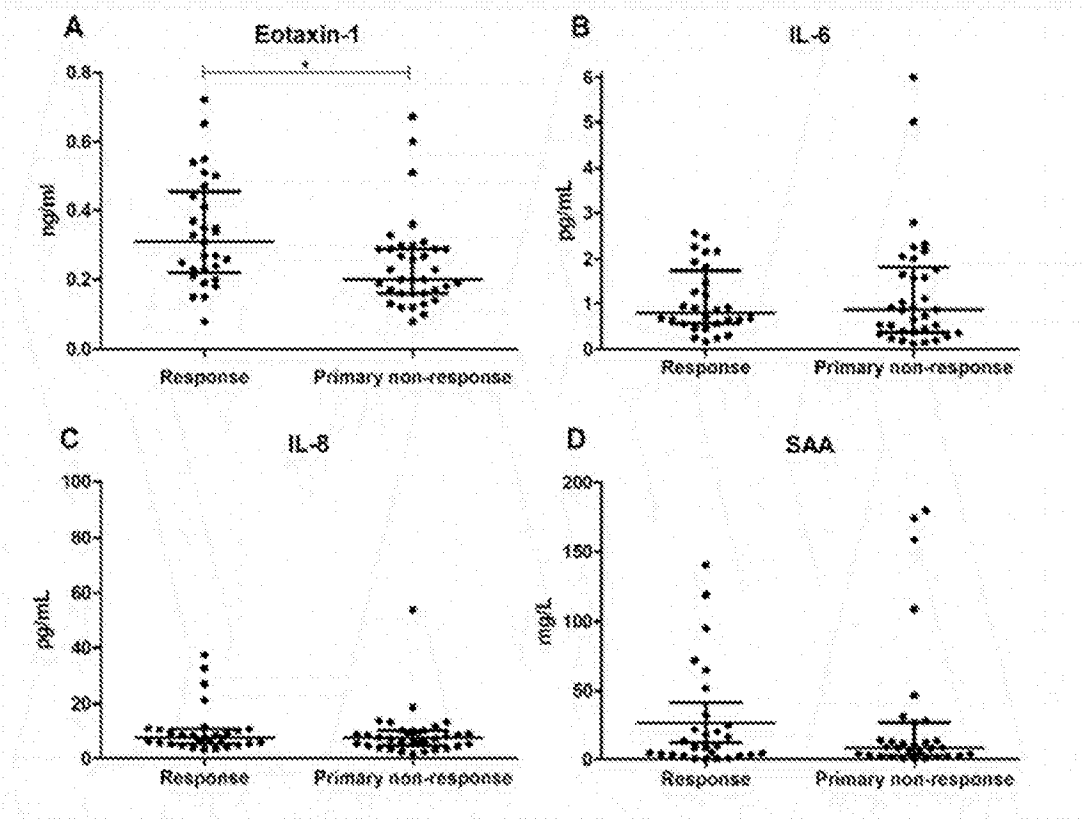
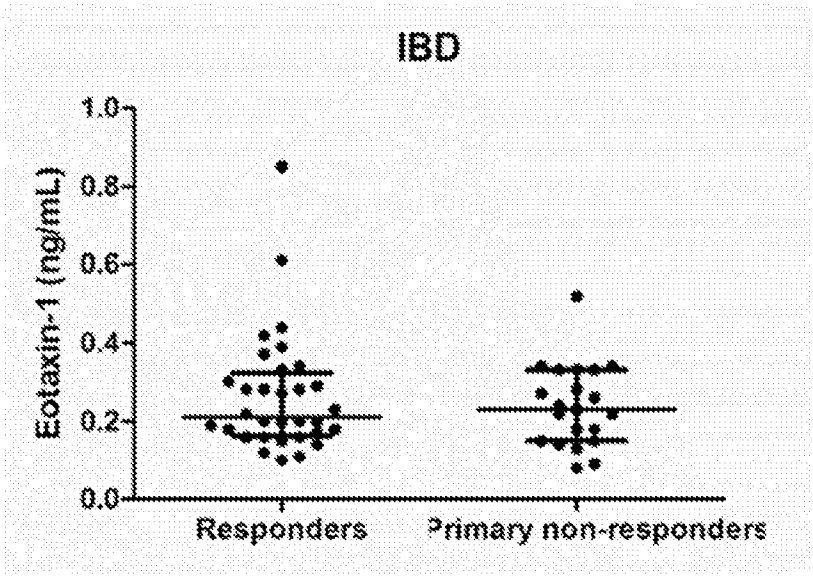
0.6
0.4
0.2
0.0
*
*
Week 0
Week 2
Week 6
Week 14
Week 0
Week 2
Week 6
Week 14
Responders
Non-responders


A
Eotaxin-1
Sensitivity
1 - Specificity
AuROC 0.69
B
CRP
Sensitivity
1 - Specificity
AuROC 0.62
C
HBI/SCCAI
Sensitivity
1 - Specificity
AuROC 0.54
D
Eotaxin-1 (adjusted)
Sensitivity
1 - Specificity
AuROC 0.81


A
Eotaxin-1
pg/ml
0.8
0.6
0.4
0.2
0.0
Response
Primary non-response
B
IL-6
pg/mL
6
5
4
3
2
1
0
Response
Primary non-response
C
IL-8
pg/mL
100
80
60
40
20
0
Response
Primary non-response
D
SAA
mg/L
200
150
100
50
0
Response
Primary non-response


IBD
Eotaxin-1 (ng/mL)
1.0
0.8
0.6
0.4
0.2
0.0
Responders
Primary non-responders

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2020/050294

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/68 A61P1/00 A61P37/06 C07K16/28
ADD. A61K39/00

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N C07K A61K A61P

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2016/088068 A1 (NESTEC S.A. [CH]) 9 June 2016 (2016-06-09) abstract paragraphs [0095] - [0114], [0131] - [0140] Examples claims 1-15	1-12
Y	WO 2006/073682 A2 (MESO SCALE TECHNOLOGIES, LLC [US]) 13 July 2006 (2006-07-13) abstract Examples claims 38-42	1-12

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
24 July 2020	07/08/2020

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Giry, Murielle

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	BOURGONJE A.R. ET AL.: "A combined set of four serum inflammatory biomarkers reliably predicts endoscopic disease activity in inflammatory bowel disease", GASTROENTEROLOGY, vol. 156, no. 6, Suppl. 1, MO1814, 1 May 2019 (2019-05-01), page S-848, XP085679100, abstract	1-12
X	WO 2014/196841 A1 (KAEL-GEMVAX CO., LTD. [KR]) 11 December 2014 (2014-12-11) abstract claims 6,15,17	13-17
X,P	BOURGONJE A.R. ET AL.: "A combined set of four serum inflammatory biomarkers reliably predicts endoscopic disease activity in inflammatory bowel disease", FRONT. MED., vol. 6, 251, 5 November 2019 (2019-11-05), pages 1-13, XP055717998, the whole document	1-17

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/NL2020/050294

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