# Instructions for Use

# Antibody to Vedolizumab ELISA

**Enzyme immunoassay for the semi-quantitative determination
of free antibodies to Vedolizumab in serum and plasma**

**REF: IG-BB116**

$\Sigma$ **12X8**          **2-8°C**

CE   IVD

1

**<u>Contents</u>**                                       **<u>Page</u>**

## 1. INTENDED USE

Enzyme immunoassay for the semi-quantitative determination of free antibodies to Vedolizumab in serum and plasma.

## 2. SUMMARY AND EXPLANATION

The drug Vedolizumab (trade name Entyvio®) is a humanised immunoglobulin G1 monoclonal antibody that binds exclusively to the lymphocyte integrin α4β7, is indicated for the treatment of adult patients with moderately to severely active ulcerative colitis [UC] or Crohn's disease [CD]. According to the prescribing information, the mean ± SD of Vedolizumab steady-state trough serum concentrations in patients with UC and CD are reported to be 11.2±7.2 µg/mL (n=77) and 13.0±9.1 µg/mL (n=72) respectively. It was also reported in another study that higher Vedolizumab trough concentrations were associated with greater efficacy in patients with both UC and CD. Higher Vedolizumab trough concentrations also were associated with numerically higher rates of clinical response and mucosal healing at week 6 in patients with UC. As with all therapeutic proteins, there is potential for immunogenicity. According to the prescribing information, in patients who received Vedolizumab, the frequency of antibodies detected in patients was 13% at 24 weeks after the last dose of the drug. During treatment, 56 of 1434 (4%) of patients treated with Vedolizumab had detectable anti-Vedolizumab antibody at any time during the 52 weeks of continuous treatment. Nine of 56 patients were persistently positive for anti-Vedolizumab antibody and 33 of 56 patients developed neutralizing antibodies to Vedolizumab. The presence of persistent anti-Vedolizumab antibody was observed to substantially reduce serum concentrations of Vedolizumab, either to undetectable or negligible levels at weeks 6 and 52.

The *ImmunoGuide* Antibody to Vedolizumab ELISA kit has been designed for the measurement of free antibodies against this drug. It does not detect such antibodies which already are bound to the drug.

## 3. PRINCIPLE OF THE TEST

This *ImmunoGuide* anti-drug antibody(ies) (ADA) kit is a bridging type ELISA for the determination of free antibodies against the drug Vedolizumab in serum and plasma samples. During the first incubation period, ADA in serum or plasma samples are captured by the drug coated to the microtiter wells. After washing away the unbound components from samples, a peroxidase-labelled drug conjugate is added and then incubated. ADA, if present in the sample, will make a bridge, with its identical Fab arms, between the drug coated on the well and the other drug molecule labelled with peroxidase. After a second washing step, the bound enzymatic activity is detected by addition of tetramethylbenzidine (TMB) chromogen-substrate. Finally, the reaction is terminated by adding a stop solution. The positive reaction is expected to be related to the presence of ADA in the sample.

## 4. WARNINGS AND PRECAUTIONS

1. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood. For further information (clinical background, test performance, automation protocols, alternative applications, literature, etc.) please refer to the local distributor.

2. In case of severe damage of the kit package, please contact *Tani Medikal* or your supplier in writing, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.

3. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.

4. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.

5. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details.

6. Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guidelines or regulations.

7. Avoid contact with Stop solution. It may cause skin irritations and burns.

8. If any component of this kit contains human serum or plasma it is indicated and if so, it have been tested and were found to be negative for HIV I/II, HBsAg and HCV. However, the presence of these or other infectious agents cannot be excluded absolutely and therefore reagents should be $_{treated}$ as potential biohazards in use and for disposal.

9. Some reagents contain sodium azide ($NaN_3$) as preservatives. In case of contact with eyes or skin, flush immediately with water. $NaN_3$ may react with lead and copper plumbing to form explosive metal azides. When disposing reagents, flush with large volume of water to avoid azide build-up

## 5. STORAGE AND STABILITY OF THE KIT

The kit is shipped at ambient temperature and should be stored at 2-8°C. Keep away from heat or direct sun light. The storage and stability of specimen and prepared reagents is stated in the corresponding chapters. The microtiter strips are stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2–8°C.

## 6. SPECIMEN COLLECTION, HANDLING AND STORAGE
**Serum, Plasma (EDTA, Heparin)***

The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

| Storage: | 2-8°C | ≤-20°C (Aliquots) | Keep away from heat or direct sun light |
|----------|-------|-------------------|------------------------------------------|
| Stability: | 3 d | 6 mon | Avoid repeated freeze-thaw cycles |

\* Drug administration/infusion may camouflage/mask the presence of anti-drug antibodies (ADA) in serum/plasma samples. Therefore, blood sampling time is also critical for detection of ADA. It is proposed to obtain blood sample just before the next administration of the drug.

## 7. CONTENTS OF THE KIT

| QUANTITY | COMPONENT |
|---|---|
| **1 x 12 x 8** | **Microtiter Plate**<br>Break apart strips pre-coated with the drug Vedolizumab. |
| **1 x 1 mL** | **Negative Control**<br>Ready to use. Contains serum and <15 mM $NaN_3$. |
| **1 x 1 mL** | **Positive Control**<br>Ready to use. Contains Vedolizumab-specific antibody and <15 mM $NaN_3$. |
| **1 x 15 mL** | **Dilution Buffer**<br>Ready to use. Contains orange dye, proteins and <15 mM $NaN_3$. |
| **1 x 12 mL** | **Assay Buffer**<br>Blue colored. Ready to use. Contains proteins and <15 mM $NaN_3$. |
| **1 x 12 mL** | **Enzyme Conjugate**<br>Red colored. Ready to use. Contains horseradish peroxidase(HRP)-conjugated Vedolizumab, Proclin® and stabilizers. |
| **1 x 12 mL** | **TMB Substrate Solution**<br>Ready to use. Contains 3,3',5,5'-Tetramethylbenzidine (TMB). |
| **1 x 12 mL** | **Stop Solution**<br>Ready to use. 1 N Hydrochloric acid (HCl). |
| **1 x 50 mL** | **Wash Buffer**, Concentrate (20x)<br>Contains buffer, Tween® 20 and Kathon™. |
| **2 x 1** | **Adhesive Seal**<br>For sealing microtiter plate during incubation. |

## 8. MATERIALS REQUIRED BUT NOT SUPPLIED
1. Micropipettes (< 3% CV) and tips to deliver 5-1000 μL.
2. Bidistilled or deionised water and calibrated glasswares (e.g. flasks or cylinders).
3. Wash bottle, automated or semi-automated microtiter plate washing system.
4. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength at 600-650 nm is optional).
5. Absorbent paper towels, standard laboratory glass or plastic vials, and a timer.

## 9. PROCEDURE NOTES
1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pre-treatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.

2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared readily at the appropriate time. Allow all reagents and specimens to reach room temperature (20-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.

3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each reagent, standard or specimen. Do not interchange the caps of vials. Always cap not used vials. Do not reuse wells or reagents.

4. Use a pipetting scheme to verify an appropriate plate layout.

5. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.

6. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.

7. Humidity affects the coated wells. Do not open the pouch until it reaches room temperature. Unused wells should be returned immediately to the resealed pouch including the desiccant.


## 10. PRE-TEST SETUP INSTRUCTIONS

### 10.1. Preparation of Components*

| Dilute/ dissolve | Component | | Diluent | Relation | Remarks | Storage | Stability |
|---|---|---|---|---|---|---|---|
| 10 mL | Wash Buffer | up to 200 mL | Distilled Water | 1:20 | Warm up at 37°C to dissolve crystals. Mix vigorously. | 2-8 °C | 4 w |

\* Prepare Wash Buffer before starting the assay procedure.

### 10.2. Dilution of Samples[a,b]

| Sample | To be diluted | With | Remarks |
|---|---|---|---|
| Serum/ Plasma | 1:10 | Dilution Buffer | For dilution at 1:10; 10 µL Sample + 90 µL Dilution Buffer |

[a] Negative and Positive Controls are ready-to-use and should NOT be diluted with the dilution buffer.

[b] Incubate 1:10 diluted serum/plasma samples for 15 min at room temperature (20-25°C) before pipetting 50 µL of each 1:10 diluted sample per well for analysis.


## 11. TEST PROCEDURE
### 11.1. GENERAL REMARKS

11.1.1. Before performing the assay, samples and assay kit should be brought to room temperature (about 30 minutes beforehand) and ensure the homogeneity of the solution.

11.1.2. All Standards should be run with each series of unknown samples.

11.1.3. Standards should be subject to the same manipulations and incubation times as the samples being tested.

11.1.4. All steps of the test should be completed without interruption.

11.1.5. Use new disposable plastic pipette tips for each reagent, standard or specimen in order to avoid cross contamination.

### 11.2. ASSAY PROCEDURE

| | |
|---|---|
| 1. | Pipette **100 µl** of **Assay Buffer** into each of the wells to be used. |
| 2. | Pipette **50 µL** of each **Ready-to-Use Negative Control, Ready-to-Use Positive Control, and 1:10 Diluted Samples (as described in section 10.2)** into the respective wells of the microtiter plate. Bubble formation during the pipetting of standards and samples must be avoided.<br><br>Wells<br>A1:                    Negative Control<br>B1:                    Negative Control<br>C1:                    Positive Control<br>D1 and so on:    Diluted samples (Serum/Plasma) |
| 3. | Cover the plate with adhesive seal. Shake plate carefully by tapping several times. **Incubate the plate on bench top for 60 min** at **room temperature (RT, 20-25°C)**. |
| 4. | Remove adhesive seal. Aspirate or decant the incubation solution. Wash the plate **3 X 300 µL** of **Diluted Wash Buffer** per well. Remove excess solution by tapping the inverted plate on a paper towel. |
| 5. | Pipette **100 µL** of **Enzyme Conjugate** (HRP-drug) into each well. |
| 6. | Cover the plate with adhesive seal. Shake plate carefully by tapping several times. **Incubate the plate on bench top for 60 min** at **RT**. |
| 7. | Remove adhesive seal. Aspirate or decant the incubation solution. Wash the plate **3 X 300 µL** of **Diluted Wash Buffer** per well. Remove excess solution by tapping the inverted plate on a paper towel. |
| 8. | Pipette **100 µL** of Ready-to-Use **TMB** Substrate Solution into each well. |
| 9. | **Incubate 15 min** at RT. Avoid exposure to direct sunlight**.**. |
| 10. | Stop the substrate reaction by adding **100 µL** of **Stop Solution** into each well. Briefly mix contents by gently shaking the plate. Color changes from blue to yellow. |
| 11. | Measure optical density (OD) with a photometer at **450 nm** (Reference at OD620 nm is optional) within **15 min** after pipetting the Stop Solution. |

### 11. 3. QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All standards/controls must be found within the acceptable ranges as stated below and/or label. If the criteria are not met, the run is not valid and should be repeated. In case of any deviation, the following technical issues should be reviewed: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

## 11. 4. CALCULATION OF RESULTS

For the run to be valid, the OD450 nm of the Positive Control should be ≥ 0.500 and the OD450 nm of each Negative Control should be ≤0.150. If not, improper technique or reagent deterioration may be suspected and the run should be repeated.

**The results are evaluated by dividing each individual OD results by the Cut-off OD value. The results are expressed in arbitrary units (AU/mL).**

---

**Cut-off value = 2 x the mean OD450 nm of Negative Control = 3 AU/mL**

---

80 different naive samples have been measured for estimating the cut-off value. In order to avoid a sample from being reported as false positive the cut-off value was determined by 2 times of the mean of Negative Control. All 80 screened naive samples show ODs (ranged between 0.045 and 0.079) lower than the cut-off value.

Samples which have an equal and higher OD than the cut-off value are considered to be positive.

| Range | Interpretation |
|---|---|
| ≥ Cut-off (3 AU/mL) | POSITIVE |
| < Cut-off (3 AU/mL) | NEGATIVE |

---

**An example for semi-quantitative calculation for a positive sample**
OD of patient's sample = 0.620
The mean OD of Negative Control = 0.062
Cut-off value (3 AU/mL) = 2 x 0.062 = 0.124
Result for the patient's sample = 0.620/0.124 × 3 AU/mL = 15 AU/mL

---

The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations. In addition, the positive reaction should be clarified whether it is true or false positive following the confirmatory assay as mentioned in section "12.1. Specificity" below.

## 12. ASSAY CHARACTERISTICS

### 12.1. SPECIFICITY

The detection of ADA formation is highly dependent on the sensitivity and specificity of the assay used.

The initial screening assay should be sensitive to low and high-affinity ADA. Endogenous and exogenous components in serum or plasma may influence assay results. Measuring immune responses to therapeutic protein products that possess Ig tails, such as mAb and Fc-fusion proteins, may be particularly difficult when RF is present in serum or plasma. RF is generally an IgM antibody that recognizes IgG, although other Ig specificities have been noted. Therefore, there is frequently a need to dilute patient samples and to make approach for minimizing interference from RF to maintain a reasonable ability to detect ADA. However, dilution and/or addition of RF-blocking reagents may not solve all potential interference related with matrix components contributing to non-specific signal in samples.

If the serum/plasma sample of a patient, a candidate for a specific monoclonal drug treatment, is negative at the time before starting drug therapy and become positive during treatment, it could be concluded that the induction of the specific ADA is suspected in this individual. As shown in Figure 1, the true positive reaction of the sample is inhibited by pre-incubation of the sample with the specific drug itself (samples are spiked with certain amount of drug). However, inhibition was not observed in false positive reaction related with other components in sample (Figure 1).

Based on the approach used in calculating the assay cut-off value of anti-drug antibody (ADA) ELISAs, approximately 1-5% of tested samples are expected to generate false-positive ADA response during initial screening analysis. Therefore, further confirmation of the specificity of the ADA activity in the samples, identified as positive, is also recommended by recent publications. In addition, the FDA released a guidance paper on immunogenicity testing of therapeutic proteins: (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM192750.pdf)

In order to confirm the specificity of the ADA signal, a Confirmatory Assay, (competitive drug inhibition test), is proposed by *ImmunoGuide*. The Confirmation Test is performed with the Confirmatory Reagent. (coded IG-CR116) This Confirmatory Reagent is available as a separate product and can be obtained from *ImmunoGuide* upon request.

**Figure 1.**
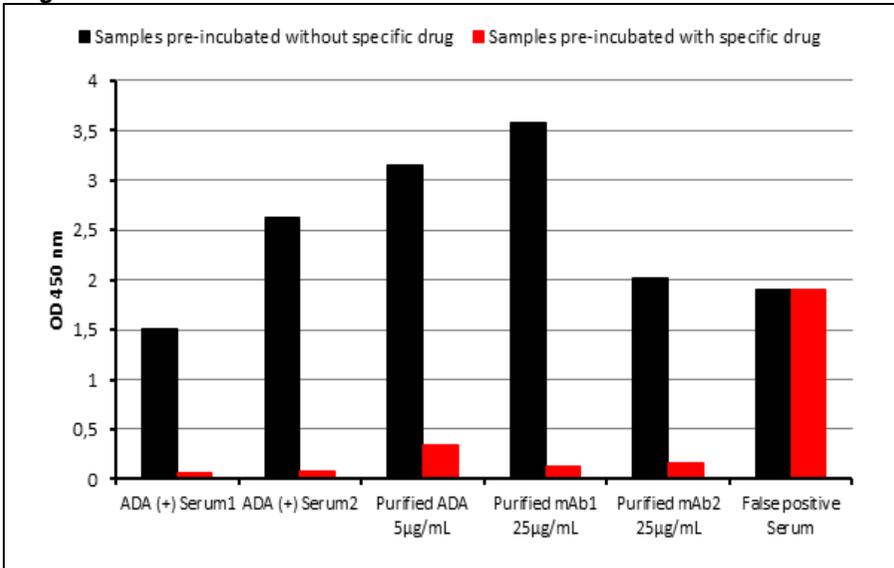


## 12.2. SENSITIVITY

Generally, the sensitivity of the ADA assay is calculated as the lowest concentration of the positive control that can consistently generate a positive signal. However, as shown in Figure 2 below, data obtained at the *ImmunoGuide* Laboratory, the assay sensitivity also differs significantly depending on the high vs low affinity antibody used for the construction of the standard curve in the assay system. For example, when the immunoaffinity-purified ADA was exogenously added into naive serum, it was observed that the lowest detectable level that can be clearly distinguished from the negative control value is 7ng/mL by the *ImmunoGuide* bridging ELISA. It is much lower when mAb2 is used instead. Therefore, it is clear that the assay sensitivity will be highly dependent on the properties of a particular positive control to be used in the assay (Figure 2).

**Figure 2.**
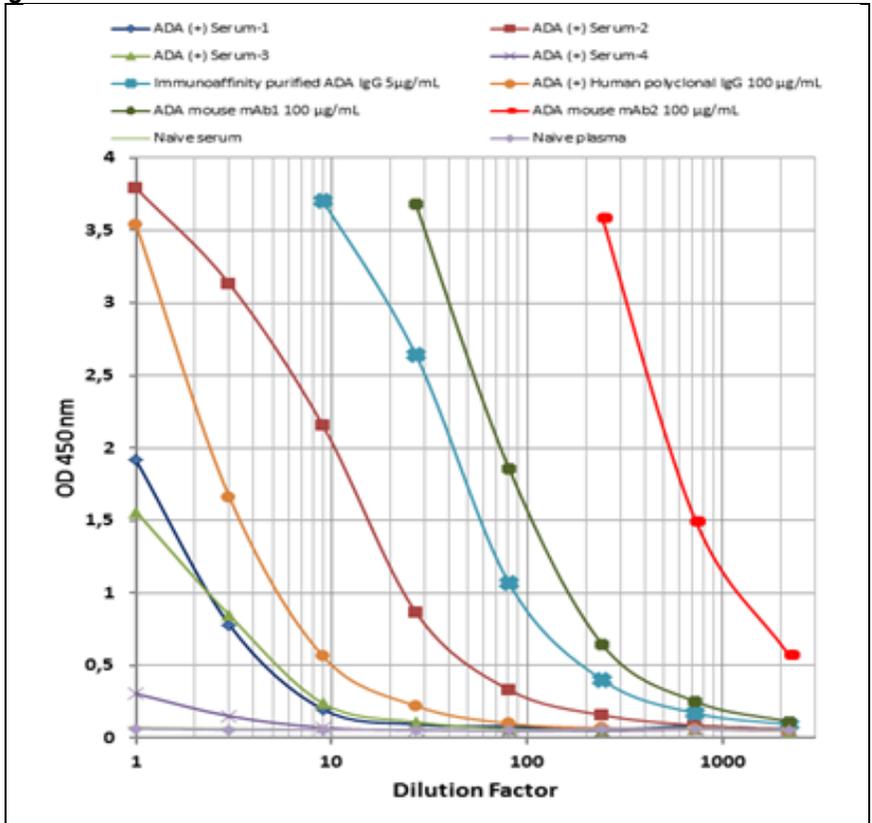


**12.3. PRECISION**
**Intra-assay CV:** <10%.
**Inter-assay CV:** <10%

**13. AUTOMATION**
The *ImmunoGuide* Anti Drug Antibody (ADA) ELISA kits are suitable also for being used by an automated ELISA processor.

## 14. REFERENCES

**1.** Williet N, Boschetti G, Fovet M, Di Bernado T, Claudez P, Del Tedesco E, Jarlot C, Rinaldi L, Berger A, Phelip JM, Flourie B, Nancey S, Paul S, Roblin X., Association Between Low Trough Levels of Vedolizumab During Induction Therapy for Inflammatory Bowel Diseases and Need for Additional Doses Within 6 Months., Clin Gastroenterol Hepatol. 2016: S1542-3565(16)31121-1. doi: 10.1016/j.cgh.2016.11.023.

**2.** Wyant T, Leach T, Sankoh S, Wang Y, Paolino J, Pasetti MF, et al., Vedolizumab affects antibody responses to immunisation selectively in the gastrointestinal tract: randomised controlled trial results., Gut. 2015;64(1):77-83.

**3.** Wang MC, Zhang LY, Han W, Shao Y, Chen M, Ni R, Wang GN, Wei FX, Zhang YW, Xu XD, Zhang YC., PRISMA--efficacy and safety of vedolizumab for inflammatory bowel diseases: a systematic review and meta-analysis of randomized controlled trials., Medicine (Baltimore). 2014; 93(28):e326. doi: 10.1097/MD.0000000000000326.

**4.** Poole RM., Vedolizumab: first global approval. Drugs. 2014;74(11):1293-303.

**5.** Milch C, Wyant T, Xu J, Parikh A, Kent W, Fox I, Berger J, Vedolizumab, a monoclonal antibody to the gut homing α4β7 integrin, does not affect cerebrospinal fluid T-lymphocyte immunophenotype, Journal of Neuroimmunology, 2013; 264(1–2): 123–126.

**6.** Bruce E. Sands, Brian G. Feagan, Paul Rutgeerts, Jean-Frédéric Colombel, William J. Sandborn, Richmond Sy, Geert D'Haens et al., Effects of Vedolizumab Induction Therapy for Patients With Crohn's Disease in Whom Tumor Necrosis Factor Antagonist Treatment Failed, Gastroenterology 2014;147:618–627.

**7.** Soler D, Chapman T, Yang LL, Wyant T, Egan R, Fedyk ER., The binding specificity and selective antagonism of vedolizumab, an anti-alpha4beta7 integrin therapeutic antibody in development for inflammatory bowel diseases., J Pharmacol Exp Ther. 2009;330(3):864-75.

**8.** Rosario M, French JL, Dirks NL, Sankoh S, Parikh A, Yang H, Danese S, Colombel JF, Smyth M, Sandborn WJ, Feagan BG, Reinisch W, Sands BE, Sans M, Fox I. Exposure-Efficacy Relationships for Vedolizumab Induction Therapy in Patients with Ulcerative Colitis or Crohn's Disease. Crohns Colitis. 2017 Feb 17. doi: 10.1093/ecco-jcc/jjx021. [Epub ahead of print]

**9.** Sands BE, Sandborn WJ, Van Assche G, Lukas M, Xu J, James A, Abhyankar B, Lasch K. Vedolizumab as Induction and Maintenance Therapy for Crohn's Disease in Patients Naïve to or Who Have Failed Tumor Necrosis Factor Antagonist Therapy. Inflamm Bowel Dis. 2017;23(1):97-106.

**10.** Cohen LB, Nanau RM, Delzor F, Neuman MG. Biologic therapies in inflammatory bowel disease., Transl Res. 2014;163(6):533-56.

**11.** Rosario M, Wyant T, Leach T, Sankoh S, Scholz C, Parikh A, Fox I, Feagan BG. Vedolizumab Pharmacokinetics, Pharmacodynamics, Safety, and Tolerability Following Administration of a Single, Ascending, Intravenous Dose to Healthy Volunteers. Clin Drug Investig. 2016;36(11):913-923.

**12.** Amiot A, Grimaud JC, Peyrin-Biroulet L, Filippi J, Pariente B, et al. Observatory on Efficacy and of Vedolizumab in Patients With Inflammatory Bowel Disease Study Group.; Groupe d'Etude Therapeutique des Affections

Inflammatoires du tube Digestif.. Effectiveness and Safety of Vedolizumab Induction Therapy for Patients With Inflammatory Bowel Disease. Clin Gastroenterol Hepatol. 2016;14(11):1593-1601.

**13.** Loftus EV Jr, Colombel JF, Feagan BG, Vermeire S, Sandborn WJ, Sands BE, Danese S, D'Haens GR, Kaser A, Panaccione R, Rubin DT, Shafran I, McAuliffe M, Kaviya A, Sankoh S, Mody R, Abhyankar B, Smyth M. Long-term Efficacy of Vedolizumab for Ulcerative Colitis. J Crohns Colitis. 2016 Sep 28. pii: jjw177. [Epub ahead of print].

**14.** Dulai PS, Singh S, Jiang X, Peerani F, Narula N, Chaudrey K, Whitehead D, Hudesman D, Lukin D, Swaminath A, Shmidt E, Wang S, Boland BS, Chang JT, Kane S, Siegel CA, Loftus EV, Sandborn WJ, Sands BE, Colombel JF. The Real-World Effectiveness and Safety of Vedolizumab for Moderate-Severe Crohn's Disease: Results From the US VICTORY Consortium. Am J Gastroenterol. 2016;111(8):1147-55.

**15.** Rosario M, Dirks NL, Gastonguay MR, Fasanmade AA, Wyant T, Parikh A, Sandborn WJ, Feagan BG, Reinisch W, Fox I. Population pharmacokinetics-pharmacodynamics of vedolizumab in patients with ulcerative colitis and Crohn's disease. Aliment Pharmacol Ther. 2015;42(2):188-202.

**16.** Colombel JF, Sands BE, Rutgeerts P, Sandborn W, Danese S, D'Haens G, Panaccione R, Loftus EV Jr, Sankoh S, Fox I, Parikh A, Milch C, Abhyankar B, Feagan BG. The safety of vedolizumab for ulcerative colitis and Crohn's disease. Gut. 2016 Feb 18. pii: gutjnl-2015-311079. doi: 10.1136/gutjnl-2015-311079. (Epub ahead of print)

**17.** Sandborn WJ, Feagan BG, Rutgeerts P, Hanauer S, Colombel JF, Sands BE, Lukas M, Fedorak RN, Lee S, Bressler B, Fox I, Rosario M, Sankoh S, et al.; GEMINI 2 Study Group. Vedolizumab as induction and maintenance therapy for Crohn's disease. N Engl J Med. 2013;369(8):711-21.

**18.** Fedyk ER, Wyant T, Yang LL, Csizmadia V, Burke K, Yang H, Kadambi VJ. Exclusive antagonism of the α4 β7 integrin by vedolizumab confirms the gut-selectivity of this pathway in primates. Inflamm Bowel Dis. 2012;18(11):2107-19.

**19.** Feagan BG, Greenberg GR, Wild G, Fedorak RN, Paré P, McDonald JW, Cohen A, Bitton A, Baker J, Dubé R, Landau SB, Vandervoort MK, Parikh A. Treatment of active Crohn's disease with MLN0002, a humanized antibody to the alpha4beta7 integrin. Clin Gastroenterol Hepatol. 2008;6(12):1370-7.

**20.** Yajnik V, Khan N, Dubinsky M, Axler J, James A, Abhyankar B, Lasch K. Efficacy and Safety of Vedolizumab in Ulcerative Colitis and Crohn's Disease Patients Stratified by Age. Adv Ther. 2017;34(2):542-559.